VOLUME I

# Immunogenetics: A Molecular and Clinical Overview

*A molecular approach to immunogenetics*

Edited by
Muneeb U. Rehman
Azher Arafah
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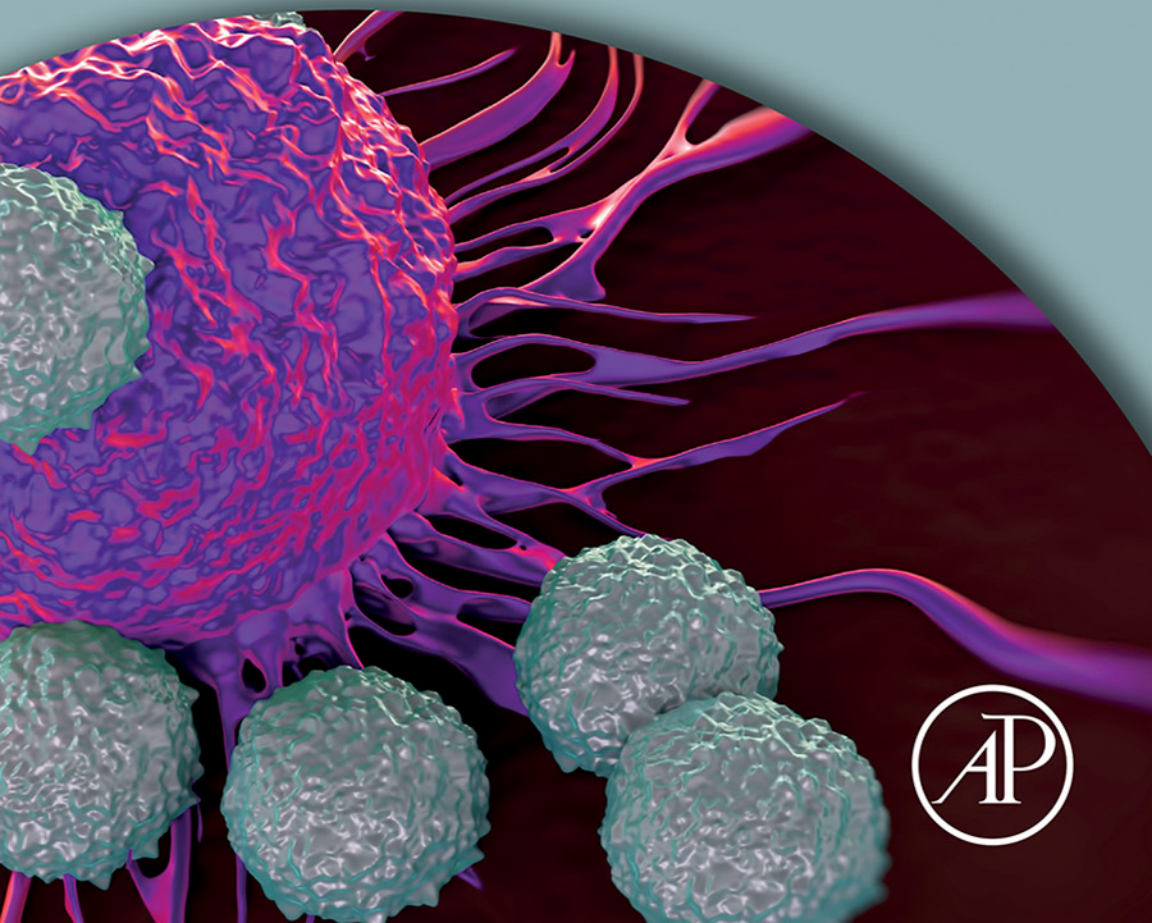
Shafat Ali



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A Molecular Approach to Immunogenetics

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## A Molecular Approach to Immunogenetics

**Volume I**

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ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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**British Library Cataloguing-in-Publication Data**
A catalogue record for this book is available from the British Library

**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-323-90053-9

For Information on all Academic Press publications
visit our website at https://www.elsevier.com/books-and-journals

*Publisher:* Andre Gerhard Wolff
*Acquisitions Editor:* Linda Versteeg-Buschman
*Editorial Project Manager:* Pat Gonzalez
*Production Project Manager:* Niranjan Bhaskaran
*Cover Designer:* Mark Rogers

Typeset by MPS Limited, Chennai, India

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# Preface

We are extremely delighted to edit this wonderful book entitled "Immunogenetics: A Molecular and Clinical Overview (Volume I): A Molecular Approach to Immunogenetics." Immunogenetics is the branch of science that describes the correlation between the immune system and genetics. It identifies genetic variants that dysregulate the normal immune pathways, thereby leading to immune disorders, which offer opportunities for the discovery of novel therapeutic targets for the treatment of immune defects. This book has a unique attribute of serving the purpose of a broader readership in the field of immunology, genetics, immunogenetics, pharmacology, immunopharmacology, and immunopharmacogenomics. It is expected to serve as a guiding torch and a single well-updated source of information to cater the needs of academicians, researchers, scientists, lecturers, clinicians, teachers, and students. This book consists of 15 chapters, each adorned with illustrations and tabulations for boosting the reading interest of readers. It discusses the origin, history, development, basic immunogenetics, immunogenetics as a tool for anthropological studies, immunogenetic surveillance to histocompatibility, gestational immunogenetics, role of immunogene polymorphisms in autoimmunity and infectious diseases, and immunogenetics causes of infertility. This book also discusses clinical applications, challenges, and future prospects of immunopharmacogenomics, immunopharmacology of Alzheimer's disease, hope of immunopharmacogenomics in the treatment of Carcinomas, and the future of immunopharmacogenomics in clinical medicine.

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Chapter 1

# Origin and history of immunogenetics

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## 1.1 Introduction

Immunogenetics consists of a series of actions within an organism, which on one side are run by the genes of an organism and on the other hand are remarkable with regard to their immunological defense system. In general, immunogenetics is a scientific discipline that uses the knowledge of immunology, molecular biology, and genetics to study the genetic factors affecting immunity, intraspecific diversity, inheritance of tissue antigen, and tissue compatibility (Encyclopedia.com, 2020). The Golden Era of Immunogenetics started when it was found that some inflammatory diseases have a genetic background. As genetic players have been found to have a remarkable impact on immune response, all the associations linked to immunogenetics serve as evocators of disease development and an important indicator of advancement of the genetic disease. So, the relation between individual's genetic makeup and its immune system forms the basis of this field of science. A genetic state that has influence on the development of immune system results in the inability to curb the infections and susceptibleness to autoimmunity and cancer (Allenspach and Torgerson, 2013).

The breakthrough that contributed to the emergence of immunogenetics as an independent discipline in immunology was the discovery of allograft reactions during a period of World War II and also by the theory of clonal selection by brunet in 1959. The medical history of immunology dates back to 19th century. Immunology is the science that deals with the defense mechanisms of body against various infections, and the failures of defense mechanisms. Understanding of genetic and molecular basis of immune response has increased with the advances in molecular genetics. As far as the

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00015-4**
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immune system is concerned it has two components both originating from same precursor stem cells. The bursa component produces a class of white cells called B lymphocytes which when properly stimulated produce plasma cells. These plasma cells later differentiate into antibodies or immunoglobulins. Antibodies are generally produced in response to foreign substances called antigens. These antibodies then elicit humoral immunity, where the antibodies recognize the specific antigen and cause its destruction. All these interactions are mediated by another group of cells called as T cells. Once the B cells comes in contact with a particular antigen it recognizes it in future encounter and the result of which is accelerated immune response. This is referred to as immunological memory. The most important problem in understanding the genetic basis of immunological response is to understand and explain the genetic regulation of immunoglobulin production. For example, the vast number of antibodies produced by a single gene or there are separate genes for each class of antibodies. These queries have recently been answered by recombinant technology has demonstrated how number of antibodies are encoded by limited number of genes. Each antibody consists of several different polypeptide chains, the heavy chain and the lighter chain. Both the chains are different in their protein parts. These chains have constant and variable regions in a given antibody. The constant regions have identical amino acid sequence while variable regions have different amino acid sequence in every antibody, which determine the specificity of antibody. (Corey, Jacob, & Wayne, 2017)

## 1.2 Immunogenetics and discovery of blood groups

The immunogenetics could be traced to demonstration of Mendelian inheritance of human blood groups in 1910 (KWOK & Janette, 2008). The importance of these groups of molecules is highlighted by their role in blood transfusion an organ transplantation. After that a large number of blood groups were discovered through antibodies. The Science of Immunogenetics started with the work of the two German scientists P. Ehrlich and J. Morgen Roth in the early 20th Century who discovered the blood groups in goats. The blood groups in humans called ABO blood types were discovered by Karl Landsteiner in 1901 (Landsteiner & Weiner, 1941). Karl Landsteiner was curious about the human response to different blood groups which resulted in the discovery of different blood groups. He started his experiment in his lab by collecting the blood samples from all his lab mates and separated the cellular contents from the plasma. The results after mixing the samples were very interesting; he observed that there was clumping of RBC when mixed with sera of different individuals, not only this agglutination was also seen on mixing human and animal blood. These results indicated to the fact that variation in blood groups really exist. Grounded on this experiment the human blood groups were characterized as group A, B; and

according to Landsteiner group A blood agglutinates with group B, but never with its own type. Likewise, group B blood agglutinates with group A, group C is distinct in that it agglutinates with both A and B. Sticking of red blood cells to form agglutins is because of the presence of antigens on the surface of these cells which react with antibodies present in plasma, which are the important constituent of immune response. Nobel Prize was awarded to Landsteiner in physiology in 1930 for discovery (Daruish & Marjan, 2013). This is how blood groups in humans were discovered through the existence of autoantibodies. This specific blood group interaction was called isoagglutination and the idea of agglutins (antibodies) which form the core of antibody antigen response in ABO complex was introduced. Through this experiment two antigens A and B and two antibodies anti-A and anti-B were discovered. Group (C) specified absence of both A and B antigen but contains anti-A and anti-B. Group C was then replaced by O. (Fig. 1.1) This is how understanding immunogenetics makes blood transfusion a success.

In humans these blood groups have evolved and the type of blood group one is having is always determined by the different set of genes one is inheriting from parents. (Thomas, Jochen, et al., 2019) The study of blood types and their interaction is just small part of the Immunogenetics.

## 1.3 Origin of immunogenetics

The origin of immunogenetics dates back to year 1976 when Edward Jenner found that small pox could be prevented when induced with cow pox, as the persons previously having cowpox were protected against small pox. (1) It was in 1980 when Baruj Benacerraf, Jean Dausset and George Snell demonstrated that genetically fixed structures present on the outer side of the cell balance the immunological processes. For this work they were jointly awarded the Nobel prize. George Snell discovered the genetic factors that depict the possibility of transplanting tissues from one person to another, and also introduced the concept of H antigen. The existence of H antigens in man was unraveled by Jean Dausset who also explained the genetic factors which are responsible for their regulation. Baruj Benacerraf showed that all

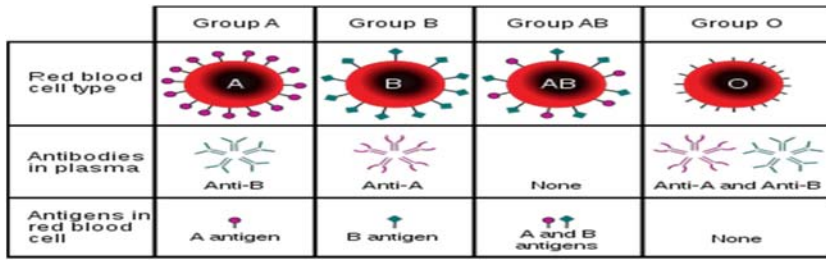


**FIGURE 1.1** Agglutinations of different blood groups.

the genes that control individual's distinctive composition of H antigens actually determine the interconnection among the various cells linked to the immunological system and are thereby foremost for the robustness of an immunological process.

As far as details of human body is concerned, each individual is unique with regard to these details. For example, Antigens (protein-carbohydrate complexes) present on the outer face of the cells are different. It was demonstrated that all these blood group antigens present on the outer face of red blood cells are entirely different. The information about these different antigens is regulated by the information found in genes. Thus the peculiarity of individual as well as of cells is genetically determined. In an individual when cells with contrasting exterior properties come in contact with each other, a reaction takes place which results for example, in tissue transplantation. Transplantation however, does not occur in nature and is an artificial situation. (An exception being pregnant women where fetal cells membranes have antigens fixed on by the genes from father). Therefore immunological response against foreign transplant is very important in order to see that cells do not modify their distinctive surface features, although this surface peculiarity could change in case of viral infection or when the normal cell is transfigured into a cancerous cell. It is at that point of time that body's capacity to differentiate between "self" and "nonself" holds a pronounced importance. It is very important that individuals defense system is well controlled otherwise autoimmune diseases would rise.

The immunological response against a foreign body has been found to be controlled by the genetic makeup of an individual. The body's defense mechanisms react differently in different individuals. It means the body's reaction is genetically determined Tumor transplants between different strains of mice were performed by, Little and Tyzzer in 1916 who demonstrated that tumors among some strains of mice, and among others. In 1927 Bover declared this rejection of tissues was not seen in identical twins. This is how it was demonstrated that tissue compatibility between donor and recipient is genetically determined. In 1933 Haldane suggested that immune reaction which leads to rejection of transplanted tumors was governed against normal cellular antigens which belongs to different strains rather than some unique antigens. In 1940 Medawar and his associates found that the rejection of tissues was because of body's immune response attacking the foreign tissue graft in rabbits (Dustin & Petteri, 2005)

Afterwards, it was George Snell who tried to explain these differences through his various experiments on rats, so as to explain the body's ability to distinguish between "self" and "nonself." In his experiments on mice, he performed continuous sibling mating to the extent that offspring were like homozygous twins. He was actually working on tumor cell lines and studying the likelihood of transmitting tumor cells from one strain to other. Later it was found that these rules of transplantation apply to normal tissues as

well. It was shown by Snell that transplantation is governed by small molecules present on the exterior of the cell. He named the structures as histocompatibility antigens. The genesis of these antigens was managed by a set of genes called as (H genes) present at a specific locus on chromosome called as major histocompatibility complex (MHC). In mice about 80 different genes within these loci have been discovered till now. This discovery laid the path for new field called as transplantation immunology. Though transplantation immunology was making a news in animals, nothing was known in humans till 1950. The experiments of Snell in mice were followed by Jean Dausset on humans, he was working on autoimmune diseases, and was trying to find the immunological reaction in patients undergoing repeated blood transfusions. Though the results were not important as far as autoimmune disease is concerned, but proved to be a very important benchmark of differences in cell structures of white blood cells between donor and recipients. When he later went on studying omen with multiple births, he was able to demonstrate that it is a single gene locus on chromosome which determine these antigens and were later called as human leukocyte antigens (HLA) and the corresponding genes as HLA genes. (Thorsby, 2009). Soon it was realized that the HLA system of humans have greater similarities with that of mice. After that similar genes were discovered in almost every species ranging from reptiles to all mammals. All these species have been found to have MHC. This system has made kidney transplantation much easier as both donor and recipient can be typed. The HLA system has number of applications like it can be used in case of disputed paternity, evolutionary studies. It has also been found that HLA system plays a very important role in disease predisposition which vary in humans depending upon the type of HLA. Although it needs further studies to substantiate. The loci on the chromosome which contains MHC genes have been found to be linked with some other genes which have been found to have some significant role. Thus this region has central role in immune response. This is why MHC is referred as super genes. Immunological self and nonself- recognition are partly controlled by major histocompatibility complex or MHC.

## 1.4 Major histocompatibility complex

MHC is a family of genes present in most of the vertebrates. The MHC consists of large portion of DNA covering about 4 million base pairs in humans or about 0.1% of human genome, and has over 200 coding loci (Caroline & Campbell, 2001). In mice MHC cluster is present on chromosome 17. In humans, chromosome 6 has a locus which consists of three subfamilies clustered near each other, in between MHC class I and II is present class III. Immunological self/nonself is influenced by MHC class I and II genes, which are highly polymorphic loci known in vertebrates. Glycoproteins encoded by class I are present on the exterior of all the nucleated somatic

cells, while MHC class II glycoproteins are a part of specialized antigen presenting cells (APCs), such as dendritic cells, macrophages, and B cells. MHC class I molecules are responsible for the presentation of peptide (antigen) which are intrinsic to CD8 + cytotoxic T lymphocytes (CTLs). As far as MHC class II are concerned, they are responsible for the presentation of peptide (antigen) which are extrinsic to CD4 + helper T (TH) cells. Both cell and antibody mediated specific immunological responses are controlled by MHC through antigen presentation. MHC were discovered due to their fascinating role in tissue transplantation, but today of all the genetic systems, MHC genes are exhaustively studied as they control the crucial traits, including resistance to infectious diseases. After the discovery of MHC, it almost took 20 long years to understand its biological role in peptide presentation and its response to T cells.

The main task of T cells is to protect the body against any invasion and to activate the macrophages and B lymphocytes. For this activation T cells need to interact with different cells like infected host cells, macrophages, dendritic cells, and B lymphocytes. T cells can recognize the antigen presented on other cells while as B cell receptor can recognize the antigens presented on cell surface. These cellular antigens are presented on protein molecules that are encoded by genes in a locus called MHC. The genes in these loci determine the fate of the tissue transplant based on the compatibility of genetic loci of the two individuals. The main feature of MHC loci is that it is polymorphic having alternate forms of genes. (Kim, Jennifer, et al., 2005)

## 1.5 MHC class I

Glycoproteins called MHC molecules are expressed on almost all nucleated cells by MHC class I. Each MHC class I codes for a 43 kDa transmembrane glycoprotein referred to as alpha or heavy chain. Each heavy chain consists of three extracellular domains: alpha 1, alpha 2, and alpha 3. It is the alpha 3 which is highly conserved and interacts with CD8 molecule present on cytotoxic T cells. The expression of MHC class I is possible only in association with small molecule called Beta 2 microglobulin, which is 12 kDa invariant polypeptide (Table 1.1). Class I are manifested on all the nucleated cells, though the expression varies, the highest being expressed by lymphocytes while hepatocytes express them at a very low level. (Somak, 2020) (Fig. 1.2)

## 1.6 MHC class II

Like MHC class I, class II MHC is transmembrane glycoprotein molecules with cytoplasmic tail and extracellular like domains which are called as alpha 1, alpha 2, beta 1, and beta 2. Class II MHC genes codes for alpha and Beta chains of approximately molecular weight 35,000 and 28,000 Da,

**TABLE 1.1** Characteristics of MHC class I and II.

| S. No. | Feature | MHC class I | MHC class II |
|---|---|---|---|
| 1. | Number of polypeptide chains | α chain (45KDa) β2 chain (12 KDa) | α chain (30–34 KDa) β chain (26–29 KDa) |
| 2. | Antigen binding site | α 1and α2 domains | α1 and β1 domains |
| 3. | Binding | 810 amino acids | 13–18 amino acids |
| 4. | Peptide binding | β sheets form the floor and helices form the sides. Helices are closed at both the ends | β sheets form the floor and helices form the sides, which are open at both the ends |
| 5. | Antigenic Presentation | CD + T cells | CD4 + T cells |

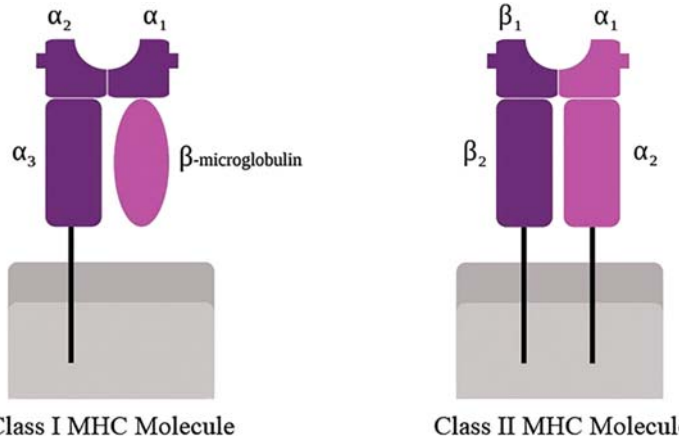


**FIGURE 1.2** Structure of MHC classes I and MHC class II.

respectively (Table 1.1). MHC class II molecules are also members of the Ig superfamily. Class II MHC genes express molecules only on APCs like dendritic cells macrophages and B lymphocytes. MHC class II molecules align antigens to the exterior of cell membrane, where TH cells stand equipped to bind and help with recognition of antigens. (Somak, 2020) (Fig. 1.2)

## 1.7 MHC class III

This class include all the genes that are associated with components of complement system, factors related to inflammation. Besides this class of genes

are also coding for various proteins involved in immune response. Antigen presentation is mediated by T cell receptors. These T cells recognize the antigen only when it is combined with MHC molecules. Both helper and cytotoxic T cells require MHC molecules for antigen presentation. This process is called as self MHC restriction Class I present processed endogenous antigen to CD8 T cells while class II present it to CD4 T cells. Both exogenous and endogenous antigens are processes differently. (Gruen & Weisman, 2001)

## 1.8 Immunogenetics and the spectrum of immune disorders

Autoimmune diseases mostly affect 3%−10% of the world population. (Kazuhiko & Yukinoro, 2019) The close relationship between autoimmune diseases and certain genetic factors was found in early 1970. This was considered as revolution in genetic epidemiology, as it helped to speculate how genetic markers can help unwind the genetics of complex diseases. The MHC loci on chromosome 6 is a crucial predisposing factor for number of auto immune diseases (Vasiliki, Vinod, et al., 2017). Predisposition to number of these autoimmune diseases is found to be associated with particular alleles of HLA-Dr, HL-ADQ genes. By fetching pattern recognition receptors (PRRs) for example, Toll like receptors (TLRs) and signal transducing particles, for example, interleukin-1 receptor associated kinase 4 (IRAK4), the innate immune system forms the middle of remembrance of molecular patterns and thereby the primary line of protection against foreign antigens. Unusually low activity of the system leads to under detection of foreign factors that will actually make the individual predisposed to infection. On the other hand, the undesired action of the system is reactive to self-components as seen in autoimmune diseases. Thus if something is hindering the absolute activity of the immune system that means the body will be more prone to infectious and autoimmune diseases. Immunogenetics focuses to cover HLA and non-HLA impact for all the mentioned groups with stress on autoimmune diseases and infections. (Yves, Celine, & Pascale, 2007)

## 1.9 Autoimmune diseases

### 1.9.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a very painful inflammatory disorder affecting many joints, involving hands and feet as well. In this disease the individual's immune system tend to attack its own tissues. The internal organs are also affected in most severe cases. In India alone the cases have reached to 1 million per year and approximately 5−7 million Indians are living with the disease (Ehtisham et al., 2011). There being no cure for this disease, medical treatment can help slow the progression of the disease.

The immunopathogenesis of rheumatoid arthritis compasses long time, starting with the formation of autoantibodies against post translationally modified proteins. This progressive immune system remodeling is then followed by erosion of tissue tolerance and their inflammation due to the formation of effector T cells and failure of macrophages to provide the effective protection. This transition of effector cells into more aggressive cells converts the disease from acute to chronic. It has been found that it is the defect in mitochondrial DNA repair system which results in telomere fragility and mitochondrial DNA instability (Cornelia & Jorg, 2021). In this disease cells like macrophages, fibroblasts monocytes and T cells make number of cytokines like IL-1, IL-6, and TNF-$\alpha$ and it is they who are responsible for irregular signaling pathways in this autoimmune disease. It has been found that MHC class II genes have a strong association with RA. This locus of MHC contains a typical amino acid sequence in the HLA-DBR1 region. In addition, to this there are some non-HLA loci which are found to be associated with RA as well (John & Kenneth, 2002).

### 1.9.2 Psoriasis

Psoriasis is a long lasting, noncontagious autoimmune skin condition which involves abnormal keratinocyte proliferation. Skin lesions of psoriasis are featured by percolation of inflammatory cells and also unusual differentiation of keratinocytes. This disease influences 2%−3% of global population and thereby affecting the quality of life of the patients and is responsible for chronic life impairment (Paolo & Giampiero, 2009). The pathogenesis of this disease is poorly understood. In more severe scenario it can predispose the patient to various cardiovascular, psychiatric and other problems. This disease involves both innate and adaptive immunities. T helper cells gets over activated and produce excess number of cytokines such as tumor necrosis factor, IL2, IL12, and interferon gamma (IFN-$\gamma$). It has been seen that plaque formation in psoriasis patients is also because of antigen presentation to T cells by dendritic cells. Almost 60 different gene loci have been found which are the risk genes for psoriasis. But at the same time, it has been observed that in about half of the psoriasis patient's allele at MHC class I gene is present. As far as HLA and non-HLA gene loci are concerned, they are considered to be the important mediators of antigen presentation and macrophage activation. A fundamental method for these genetic risk alleles in the pathological process of psoriasis is likely to be resolved through decreasing the threshold for stimulation of innate immunity (Shiju, Xin, & Xiaoxu, 2021).

### 1.9.3 Autoimmune thyroid diseases

Autoimmune thyroid diseases (AITDs) are another group of autoimmune diseases for example, Graves's diseases and Hashimotos thyroiditis where

immune system makes antibodies against body's own tissues. In Hashimotos thyroiditis immune system attacks the thyroid gland which leads to hyper or hypothyroidism. AITDs have exceptional significance because of their co-occurrence with other autoimmune disorders. It is a multifactorial disease where both environmental a genetic factors play their role, though the exact mechanism of interaction in not clear (Hanna, Chuek, et al., 2015). It is a T cell mediated disease designated by production of auto antibodies as well as cell infiltration in thyroid gland. A Large number of genetic loci within immune modulating and HLA genes have been found to be associated with AITDs that contribute to T-lymphocyte activation and antigen presentation. Some gene polymorphisms in cytokine Th2 have also been found to be associated with AITDs. Cytokine Th2 has a very important role in the development of the disease. In this way, a method of immunogenetic sensitivity to AITDs is conserved through intervention with central and peripheral tolerance and later stimulation of T cells (Souhir et al., 2020).

### 1.9.4 Primary biliary cholangitis

Primary biliary cholangitis (PBC) is an autoimmune disorder of liver (Fig. 1.3) with strong genetic component. It was also called as primary biliary cirrhosis (Ana et al., 2020). The frequency of this disease is more in women than in men. It is a progressive disorder which starts with slow obliteration of small bile ducts of the liver resulting in the collection of bile and other toxins in the liver, the condition called as cholethesis. It has been found that in this disease there is presence of antimitochondrial antibody (AMA) in 90% of the patients. In the liver of patients with PBC, there is manifold increase in CD4 + and CD8 + T cells specific to AMA. It is a disease with typical T cell signature. In the presence of AMA expression of TNF-related apoptosis is increased by macrophages. In addendum to humoral response, T cell responses including CD4 + and CD8 + T cells target the same antigen and are tangled up in the demolition of biliary epithelial cells (Fig. 1.4). The composition of the portal inflammation besides T cells include other immune

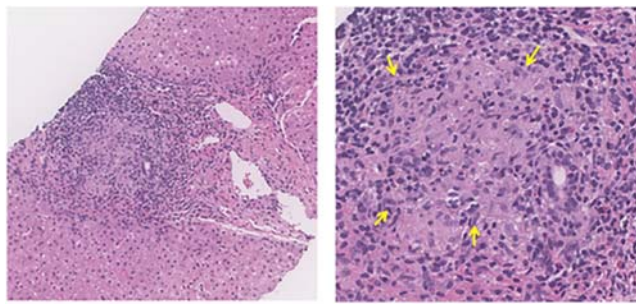

**FIGURE 1.3** Liver histology of PBC showing distortion of hepatic architecture.

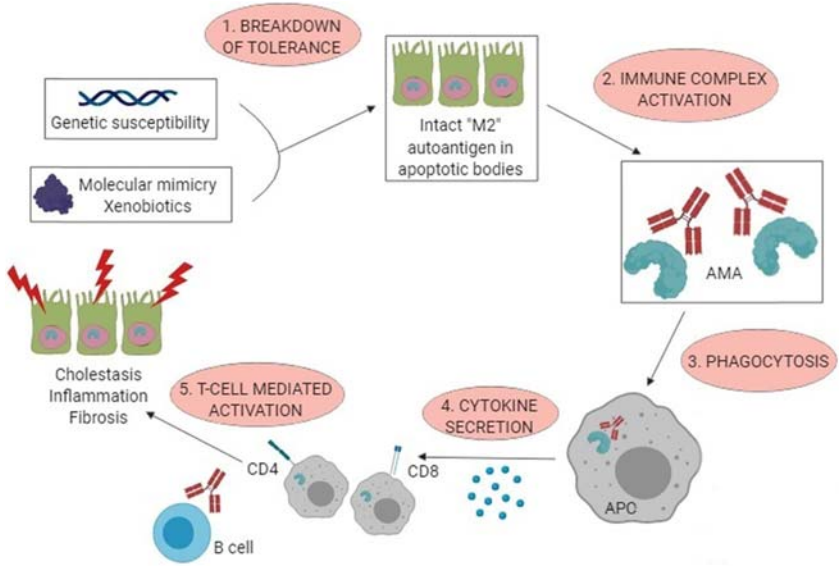


**FIGURE 1.4** Pathogenesis of PBC: in genetically vulnerable individuals environmental features are responsible for loss of tolerance to mitochondrial antigen via molecular mimicry. In the presence of AMA and macrophages mitochondrial antigens in cholangiocytes go through apoptosis resulting in innate immune response and increase in adaptive immune response.

cells as well like B cells, macrophages, and natural killer cells. In the long run this chronic inflammation results in loss of biliary epithelial cells. There are number of genetic studies which have shown HLA variants both susceptible and defensive against PBC. Non-HLA loci associated with PBC mostly involve genes allied with T cells (Justin, Srisha, et al., 2020)

### 1.9.5 Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) or insulin dependent diabetes is a complex condition manifested by body's lack of ability to form insulin because of autoimmune destruction of beta cells of pancreas. T1DM is a multifactorial disease (Fig. 1.5). T1DM is a T cell mediated autoimmune disease in which autoantibodies are formed against islet cells. It is genetic disorder where a large number of susceptibility genes are involved. Though polygenic HLA class II on chromosomes 6 genes are responsible for almost half of genetic susceptibility for T1DM. Positive association of this disease have been found with haplotypes HLA-DRB1*3 and negative association with DRB1*07,*11 (Neeraj, Narinder, et al., 2019). Although some loci with protective function have also been recognized. On HLA genes that have found to be associated with T1DM are variable number of tandem mini satellite repeats (VNTR) and CTLA-4, but they have much smaller effects than HLA. In future the

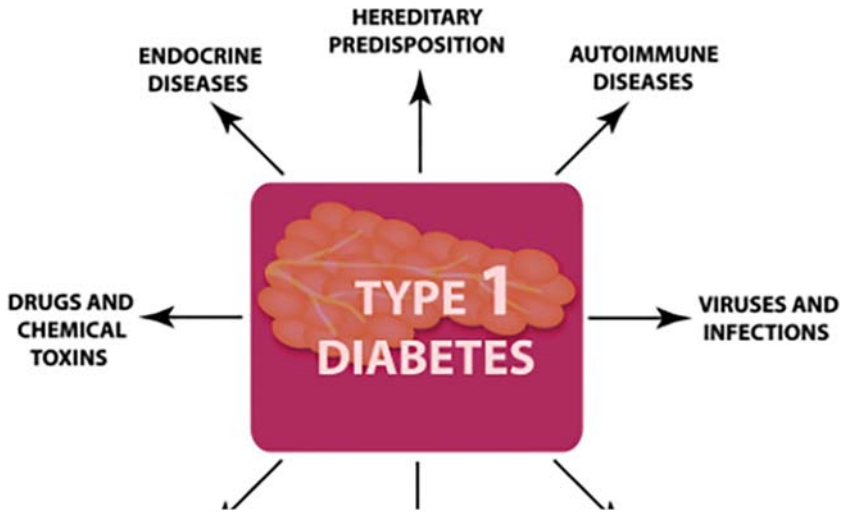


**FIGURE 1.5** Causes of type 1 diabetes.

identification of new loci and more analysis of new one will pave way for pathophysiological insights for the early intervention and prevention of the disease (Mimi & Constantin, 2005). In addition, to this it will also help in the identification of potential sub genotypes with different immune dysregulation patterns, but leading to common phenotypes of the disease.

### 1.9.6 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease influencing various organ systems and resulting in organ damage. It is a polygenic disease where number of gene loci are related with disease susceptibility. Genetic aspect plays a very important role in the pathogenesis of this disease. Almost 100 loci have been found to be associated with disease (Niklas, Christian, & Lars, 2020). The production of type I interferon and autoantibodies along with the stimulation of innate immune response favors pathogenesis of SLE. In SLE the process like apoptosis and clearance of neutrophil extracellular traps (NET) are defective. In this disease their higher levels of cytokine formation are attributed to the epigenetic changes in neutrophils which eventually lead to induction of T and B cell abnormalities. This epigenetic change in neutrophils directly influence the NET complexes which are increased in SLE and their clearance is defective. This intricate condition involves both HLA and non-HLA genes. Many HLA genes like HLA-DBR1, HLA-DQB2, HLA-DQA2, and HLA-Dr3 are related to SLE predisposition. In addition, to this they are also associated with autoantibody profile (anti-dsDNA, anti-Ro) in SLE patients. Genome wide associations

have found almost 100 loci are associated with SLE (Yong et al., 2021). However, it has been observed that these loci explains only about 30% of heritability of SLE as well as their biology in terms of effector genes and pathological pathways.

### 1.9.7 Systemic sclerosis

Systemic sclerosis (SSc) also known as scleroderma is a complex autoimmune inflammatory disorder. It is a connective tissue disorder characterized by structural and functional vascular abnormalities and affecting the multiple organ system. This complex disease is characterized by heterogeneous clinical manifestations. There is excessive accumulation of collagen in the skin leading to fibrosis. In this disease there is involvement of both adaptive and innate immune systems. MHC genes play very important role in the susceptibility of this disease. There are number of known HLA genes which confer susceptibility to SSc, in particular HLA-DRB1 and HPB1. As far as non-HLA loci associated with SSc are concerned about 28 loci are have been found to be linked to this disease by genome scan analysis. These non-HLA genes play a role in innate immunity, adaptive immune responses, and cytokine formation. In addition, to it B and T cell proliferation is also taken care of by these genes. The genome wide study has also shown that there are number of single nucleotide polymorphisms (SNPs) in non-HLA genes which are linked to SSc. Micro RNA (miRNA) expression has also been studied in SSc, and different mi RNA's have been found to be associated like epidermal growth factor (Bossini & Lopaz, 2015).

## 1.10 Neurological diseases

The proliferation of neurodegenerative diseases increases as the population ages. There are a number of neurological diseases which have immunogenetic basis like multiple sclerosis, Alzheimer's disease, Parkinson's Disease, neuromyelitis Optica, and myasthenia gravis. Multiple sclerosis and Parkinson's disease are used as prototypes to explain how immunogenetics influence the neurological disease.

### 1.10.1 Multiple sclerosis

Multiple sclerosis (Ms) is the inflammatory and potentially disabling disease of central nervous system and has been found to be one of the most common causes of non-traumatic neurological impairment in young population. Ethnicity and family history of an individual plays a very important role in the pathogenesis of this disease. Ms is a multifactorial disease where both environment and genetics play their role (Fig. 1.6). Ms happens to be one of the first diseases where the association of disease with HLA was found. The

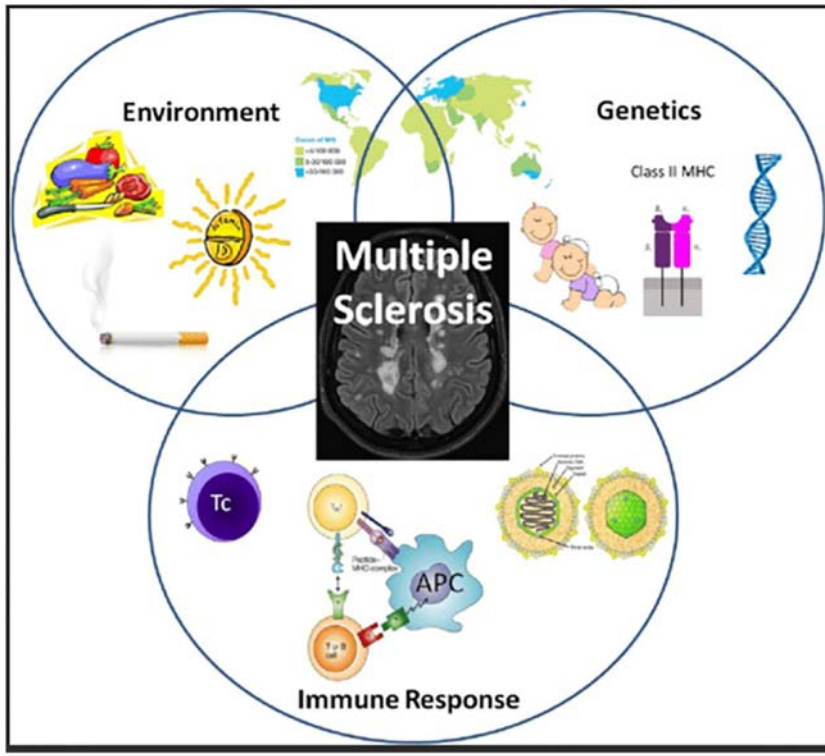


**FIGURE 1.6** Multiple sclerosis: a multifactorial disease. Genetic as well as environmental factors play a very important role in the pathogenesis of multiple sclerosis.

myelin sheath that wraps the axons and helps in communication between brain and rest of the body is attacked by the immune system. Ms is probably one of the first genetic disorders where HLA association has been seen, primarily with HLA class II factors. Ms is considered to be an autoimmune disorder where there is exhilaration of CD + autoreactive T cells, th1 cell polarization and CD8 + T cells which have the potential to damage the CNS tissues. So far more than 100 genetic loci have been found to be associated with multiple sclerosis. HLA contribute about 10% of the genetic variations (Jill & Jorge, 2015).

### 1.10.2 Parkinson's disease

Parkinson's disease (PD) is the most predominant and progressive neurodegenerative disease. Like other neurological diseases it is also shown to have immunogenetic basis. The main feature of Parkinson's disease is inflammation. In this disease there is enhanced activation of major histocompatibility

class II expression as analyzed by postmortem of Parkinson's brains. PD is not a single disease but is manifested has a range of clinical and genetic subtypes (Swanberg & Jimenez, 2018). It is a very complex disease where almost 10% of the cases are monogenic while 90% of cases are multifactorial with number of environmental factors leading to the pathogenesis of this disease. Up to 41 loci have been found to be associated with Parkinson's disease, among which two are within HLA locus coding for genes including MHCII. Combing both immunological findings and genetic analysis gives an idea that immunogenetics has major role to play in this disease (Thomas, Stoker, & Julia, 2018).

Thus immunogenetics plays a very important role in the etiology of neurological diseases. There is a hope that the immunogenetics of these neurological diseases may pave way for their better understanding and newer treatment options.

## 1.11 Infectious diseases

The other side of immunogenetics is its link to infectious diseases. Infectious diseases are the most common health problems worldwide. In addition, to environmental factors there are number of genetic factors that play a very important function in the etiology of these diseases for example, tuberculosis, HIV, HBV, and HCV infections. Chronic viral infections profoundly influence the immune system. It has been found that patients with infections like HCV/HIV have defective immune system with impaired CD4 + and CD8 + T cells that results to difficulty in warding off the infection (Jeol et al., 2018).

### 1.11.1 Tuberculosis

As a far as tuberculosis is concerned it is an infectious disease caused by Mycobacterium tuberculosis (MTb). Officially the only vaccine which gives protection against this disease is BCG. Long before it was found that interferons may have some anti-viral properties, but later they were considered as innate inflammatory cytokines and strong mediators between innate and adaptive immunity. It has been found that immunogenetics plays its function in the etiology of this disease in addition, to environmental factors. It has been revealed that the innate immune receptors which recognize MTb include TLR's, C type lectin receptors, CLR's, and NLR's. After identification there is engagement of different immune cells and cytokines which work by different mechanisms. Genetic factors include both HLA and non-HLA genes. HLA studies conducted in the Asian region, especially in India, indicated the association of HLA-Dr1 and HLA-DQ1 antigens with susceptibleness to TB (Aravindan & Sajitha, 2019).

### 1.11.2 human immunodeficiency virus

human immunodeficiency virus (HIV) is yet another infectious disease where immunogenetics has a very important role. It has been found that HIV has a phenomenal genetic diversity. Host genetic factors are an one of the most important committed factors to the inter-individual variation present in reaction to HIV infection and are connected to resistance to HIV infection among exposed individuals, and the likelihood of viral infection (Maureen & Marry, 2013). The genetic variants that have the central role in HIV infection include HLA class I genes. They exhibit the strongest association highlighting an important role for CD8 + T cells in resistance to the virus. HLA proteins play a crucial role in T cell mediated adaptive immunity by conferring immunodominant HIV epitopes to cytotoxic lymphocytes (CTLs and CD4 + cells). In addition, to this HLA also function in natural killer cell mediated innate immunity against HIV by communicating with killer cell immunoglobulin like receptors (Yongjian, Lei, et al., 2020).

### 1.11.3 HBV & HCV

HBV and HCV infections also have been found to have genetic origin. Again, immunogenetics plays a role in the pathogenesis of these infections. These infections result in impaired immune system. Both HLA and non-HLA genes have found to play their roles. The two genes that have found to be associated with these infections are MICA and DEPDC5 (Heiner, Tanvi, et al., 2020).

## 1.12 Atopic diseases

Recently it has been found that various atopic diseases have a strong connection with immunogenetics, for example, asthma allergic rhinitis and atopic dermatitis. These groups of diseases called atopic diseases are due to Th2 mediated process. The Th2 cytokines increase the formation of IgE by IgE + memory B cells and plasma cells. The genome wide scans have shown that most of these atopic diseases have genes located on chromosome 6 which also harbor MHC genes. These atopic diseases share their genetics with other autoimmune diseases. In atopic dermatitis there is a strong genetic predisposition and T cell driven inflammation. It has been found that this disease involves multiple immune pathways (Sinead, Alan, & Stephen, 2020).

## 1.13 Conclusion

In recent years a lot of progress has been made in the field of immunogenetics. The most important goal of immunogenetics is to determine the factors that are actually involved in the spread of new alleles in the population.

Immunogenetics has become a very important field and has made our understanding of autoimmune diseases easier. One of the most important applications of immunogenetics is HLA typing which has made our understanding of transplantation biology clearer. The main focus of immunogenetics has been to identify, characterize, and sequence the genes which are coding for multiple receptors that mediate the immune response. Immunogenetics has not only helped us to understand the mechanisms of autoimmune disorders but also has opened the doors for understanding the complex processes like immunosenescence, which actually refers to the decline in immune response with age leading to increased rate of infections and mortality in elderly. During the last few decades immunosenescence has received attention from geneticists all over the globe. Immunogenetics also plays a very important part in understanding the human reaction to vaccines, which vary from one individual to another. The main aim of vaccinomics is to find the answer in the genes that may explain this variation to large extent. It has been found that genetic factors have a very important contribution in deciding the safety of vaccine and the untoward effects as well. For almost the last thirty years, the database called international Immunogenetics was available on the site http://imgt.cines.fr.IMGT which consists of details related to immunogenetics on various factors like genes and their sequence polymorphisms, nucleotides and the proteins like immunoglobulins. It can be very helpful for therapeutic and diagnostic purposes. This can also pave the way for research in different fields particularly in various autoimmune and infectious diseases.

Computational Immunogenetics circumscribe utilization and execution of bioinformatics knowledge, mathematical work, and statical approach for the study of immune system. Computational methods are very essential to imbibe the repercussions of the treasure of gene expression data being collected from immune cells. Immune databases are very important in arranging the expansive quantities of experimental data gathered by modern high-throughput technologies. Thus computational immunology is making a crucial contribution in computational biomedicine which is an emerging field.

The other field where immunogenetics plays a great role is psychoneuroimmunology which actually is the study of association between immune system and human emotions. It is also called psychoneuroendoimmunology. These associations are always mediated by the endocrine and nervous system. It gives us an idea about the effect of the mind on health and resilience to diseases. In recent times it has been found that your emotions can directly influence your immune system or in broader terms your physical health, which was earlier considered to be a myth. Over a period of time researchers have proved that stress could really change the biochemistry of hormones and brain as well. Prolonged stress can also alter the immune function in the body, and hence affecting its efficiency. Both the immune and nervous system maintain a connection which includes communication with lymphoid organs from sympathetic and parasympathetic nerves. Neuroendocrine stress

responses and individual variation in emotional reactivity are mediated by genetic factors, which was shown by various earlier studies. Further it has been shown by various studies that the long-term effect of stress is being mediated by inherent variations in hypothalamic-pituitary-adrenal axis.

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Chapter 2

# Immunogenetics: the developmental course

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## 2.1 Introduction

Immunogenetics is a field of biology that incorporates immunology, molecular biology, and genetics to examine inherited factors that influence immunity, intraspecific heterogeneity, tissue receptor inheritance, genetic, and population dimensions of host-microbe interactions, and tissue incompatibility (Castiblanco & Anaya, 2015; Loker, 2012). The area of immunogenetics was founded on the work of German scientists P. Ehrlich and J. Morgenroth, who identified blood groups in goats in the early 20th century, and K. Landsteiner, who identified blood groups in humans (Bosch & Rosich, 2008). In 1930 an American scientist named M. Irwin invented the term "immunogenetics." Immunogenetics is the study of the mechanisms of autoimmune disorders and immunity in organ transplantation, with a focus on the role of genetics (Milinski, 2006). The immune system of a species develops to protect it from a variety of toxic substances such as fungi, viruses, bacteria, and other eukaryotic parasites. Immune system science, on the other hand, has influenced a wide variety of scientific fields, and it is now playing an increasingly significant role in the study and management of many human disorders, such as cancer and autoimmune diseases (Chaplin, 2010). Immune systems can be divided into two categories. Innate immune defenses rely on invariant receptors that recognize certain pathogen features but are not diverse enough just to recognize all forms of pathogens or accurate enough to prevent reinfection by the same pathogen (Janeway et al., 2001). This mechanism, while successful, lacks both specificity and the ability to obtain stronger receptors in the potential to deal with same contagious problem, a

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00002-6**
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phenomenon known as immunological memory. Specificity and memory are indeed the main elements of the second line of immune system, which is centered on antigen specific receptors and is known as the specific or adaptive immune system. Aside from these two families of molecules, that aid in immune detection of alien pathogens, of both innate and adaptive immune programs depend on soluble mediators such as cytokines and kemokines to coordinate between the various cells implicated in an immune response (Aspinall, Del Giudice, Effros, Grubeck-Loebenstein, & Sambhara, 2007; Silverstein, 2001) (Fig. 2.1). Immunogeneticists are primarily concerned with the discovery, characterization, and sequencing of genes that code for immune response receptors and mediators (Georgiou et al., 2014).

## 2.2 Genetic defects associated with immune deficiency

Complement, T cells, B cells, and phagocytes are the four main divisions of the immune system, which defend against pathogens (Fig. 2.2). They act through specific interaction as well as the processing of soluble mediators that trigger the immune response, such as cytokines and chemokines. Immunodeficiency diseases are classified into three categories: (1) genetic disturbances that consequences in a reduction in the amount of specific immune cell subsets; (2) defects that directly impact the basic "mechanisms" of some of these immune cell subsets; and (3) defects that hamper immune cells' potential to "communicate" with one another or with their surroundings by altering adequate signaling (Vazquez, Catalan-Dibene, & Zlotnik, 2015). In the study of patients vulnerable to specific kind of infections, the primary role of meticulous cytokines or cell types in human inflammation is

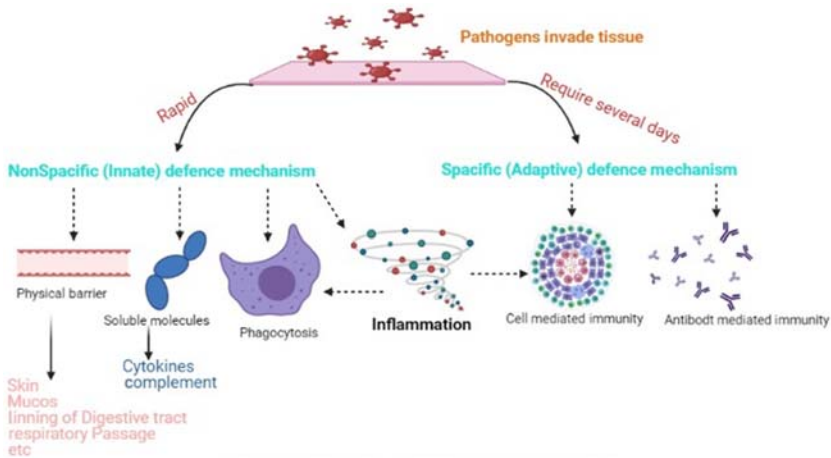


**FIGURE 2.1** Outline of innate and adaptive immunity.

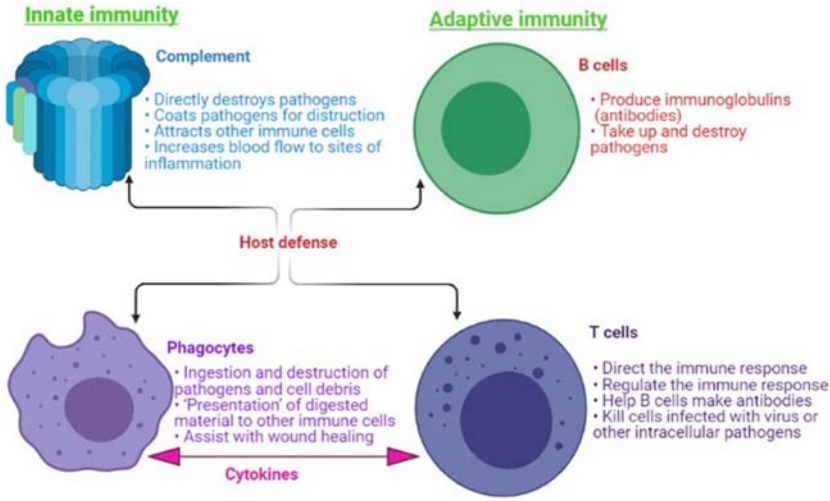


**FIGURE 2.2** The four major compartments of the immune system.

known. Approximately 180 individual gene abnormalities associated with immune dysfunction are reported (Grazia Roncarolo et al., 2006).

## 2.3 B cell deficiency

The primary part of B cells in disease or immunization is to produce antibodies. Patients with a lack of B cells are indeed not capable of producing adequate antibody volume, and therefore have recurring respiratory tract bacterial infects (bronchitis, pneumonias, ear, and sinus infections) (Slack, Balmer, & Macpherson, 2014). B cells have to pass through a trilogy of differentiation phases, in order to become adult plasma and memory cells that produce antibodies. This requires main events like: (1) pre-B cell receptor (BCR), (2) intracelled signaling mechanisms stimulation, and (3) an immunoglobulin (anticorps) reorganization of the gene in production (Janeway et al., 2001; Cano & Lopera, 2013). Alteration of each of these main events by mutations in essential molecules results in an absence of circulating B cells ($<1\%$), and a pro-B developmental arrest in the bone marrow before the B phase. Human B cell deficiency is caused by autosomal pre-BCR recessive proteins defects in the heavy chain immunoglobulin (IGHM), proxy light chain (V-preB and 5) and Ig-alpha (CD79) and Ig-beta (CD79) signaling chains (Cunningham-Rundles & Ponda, 2005; Vale & Schroeder, 2010). B cell deficiency may also be induced by defects in pre-BCR downstream signaling molecules, like the adapter molecule B cell linker protein (BLNK) and Bruton tyrosine kinase (BTK). Deficiency in B cell which is an X-linked antibody is caused by mutations in BTK, which accounts for 85% of initial

antibody deficit (Ponader & Burger, 2014; Singh, Dammeijer, & Hendriks, 2018). Finally, B cell deficiency is caused by alterations in the recombinase-activating-genes 1 and 2 (RAG1/2), Artemis (DCLRE1C), or DNA ligase 4 (LIG4), separates and rejoins DNA throughout the reorganization of the immunoglobulin gene loci. Patients with each of these mutations have deficit T cells, since such pathways are requisite for the alteration in gene loci of T cell receptors (Felgentreff et al., 2016).

## 2.4 T cell deficiency

The important function of T cells is to coordinate the inflammatory responses and management of intracellular pathogenic infection of viruses and fungi. Sever combination immunodeficiency (SCID), also defined as the "bubble-boy disease," is the prototypic T cell dearth (Goldman & Prabhakar, 1996). SCID patients are more likely to get Pneumocystis jirovecii pneumonia and get serious viral-infection. SCID at present is linked with 21 separate single gene defects. They differ significantly depending on the genetic mutation, but they both contribute to irregular T cell formation or function. Some SCID gene defects also impair B and natural killer (NK) cell growth and utility along with T cells. The IL-2 receptor gene (IL2RG) was mutated at X-Chromosome in 40% of SCID patients. The IL2RG is linked with IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors (Cook & Tangye, 2009; Tasher & Dalal, 2012). Patients with this X-linked SCID have normal B cells but low numbers of T and NK cells. But since IL-2 and IL-7 are important growth factors for T cells, and IL-15 is important for NK cells, mutations in the IL-7 receptor -chain (IL7RA) are another SCID-related genetic defect that causes T cell formation leading to a shortage of cytokine signaling (Rochman, Spolski, & Leonard, 2009; Uribe & Weinberg, 1998). To evolve beyond an early stage, T cells, like B cells, must adequately adjust their immunoglobulin gene and receptor gene. Mutations in SCID-associated genes or genes that induce nonhomologous end-joining and DNA repair, avert T cell progress in relation to T cell deficit (Hodges, Krishna, Pickard, & Smith, 2003; O'Driscoll, 2012). Most SCID-related defects influence T cell receptor complex molecules [CD3(CD3D), CD3(CD3E), CD3(CD3Z), CD3(CD3G), CD45(PTPRC), and TCR alpha-chain (TRAC)], intracellular kinases [ZAP70, p56lck(LCK), and (ITK), and intracellular pathways (TRAC)] (ORAI1, STIM1, and MAGT1). T cells can be found in these cases, but because they are not working correctly, they seem unable to manage infections (Morgan et al., 2011; Tasher & Dalal, 2012).

## 2.5 Regulatory T cell deficiency

Regulatory T cells (Tregs) are essential for keeping immune responses under control and preventing autoimmunity. The expression of specific proteins, such as CD25 and FOXP3, which are equally obligatory for Treg production

and process, is the most common way to identify them (Corthay, 2009; Taams et al., 2006). FOXP3 mutations induce IPPEX (immune-dysregulation-polyendocrinopathy-enteropathy-X-linked-syndrome), which is distinguished by the lack of Tregs (IPEX). IPEX patients have extreme, early-onset autoimmunity that influences the skin, intestines, and endocrine organs because of Treg deficiency (van der Vliet & Nieuwenhuis, 2007). Treg dysfunction is also caused by alterations in CD25, the -receptor chain for the cytokine interleukin-2 (IL-2), or in STAT5B, a transcription factor regulated by IL-2 (Agakidis et al., 2019). Patients who lack CD25 or STAT5B have a psychiatric condition similar to IPEX, with extreme autoimmunity and infections (Verbsky & Chatila, 2013).

## 2.6 Phagocyte deficiency

Immune cells such as phagocytes (neutrophils and monocytes) perform an important role in immune responses; it circulates and moves across tissues to take up and execute bacteria as well as cellular debris. The development of ROS within the phagocytes' phagolysosomes aids in the degradation of ingested material (Mak & Saunders, 2007). An increasing number of genetic disorders that cause extreme congenital absentee of neutrophils (ELANE), granulocyte (CSF3R, GFI1, HAX1, WAS, and G6PC3), as well as genetic abnormalities that cause congenital absence of dendritic cells, have recently been reported (GATA2 and IRF8) (Skokowa, Dale, Touw, Zeidler, & Welte, 2017). Other genetic abnormalities that impair phagocyte activity have been discovered in addition, to mutations that cause the absence of particular forms of phagocytes. Leukocyte adhesion deficiencies (LADs) are abnormalities that affect phagocytes' ability to move out of the bloodstream and into tissues (Andrews & Sullivan, 2003). They are affected by the lack of functioning adhesion receptors on the phagocyte cell surface (ITGB2, SLC35C1, and FERMT3). Chronic granulomatous disease (CGD) is the most widespread phagocyte-associated immunodeficiency, which is caused by phagocytic cells' failure to produce reactive oxygen species in the phagolysosomes following pathogen ingestion. Mutations in each of the six genes that code for subunits of the lysosomal NADPH oxidase complex trigger it (CYBB, CYBA, NCF1, NCF2, NCF4, and G6PD) (Roos, 2016; Lent-Schochet & Jialal, 2020). CYBB mutations affect the most common type of CGD, which is X-linked. The remaining diseases are autosomal recessive in nature. Skin and soft-tissue bacterial abscesses, as well as extreme, infectious bacterial infections, are the most frequent clinical consequences of missing or dysfunctional phagocytes (Song et al., 2011).

## 2.7 Defects in cytokine signaling

Any immune cell subset produces cytokines as a way of promoting development and modulating the immune response. Some cytokines/cytokine networks have emerged as being especially important for the treatment of

various types of infections (Vazquez et al., 2015). The following are some recent examples. Although certain mycobacterial species, such as Mycobacterium tuberculosis, are extremely pathogenic to humans, others, such as the Bacille Calmette−Guerin vaccine strain and Mycobacterium avium-intracellulare, are only slightly vitriolic and occasionally pathogenic to organisms with a natural immune system (Edwards et al., 1982; Johnson & Odell, 2014). Sufferers with a congenital susceptibility to invasive infections with even weakly virulent mycobacteria strains do occur. At least nine known genes have been related to mutations in Mendelian susceptibility to mycobacterial disease, including IL-12B, IL-12RB1, IFNGR1, IFNGR2, STAT1, NEMO/IKBKG, TYK2, and IRF8 (Rosain et al., 2019). These gene defects cluster the signaling pathways needed for responses to both the cytokines interleukin-12 (IL-12) and interferon-gamma (IFN). These cytokines have the final effect of activating IFN-responsive genes, which aid in the clearance of intracellular bacterial infections (such as mycobacteria). Besides their mutual resistance to atypical mycobacterial pathology, these syndromes exhibit clinical heterogeneity in aspects of phenotype complexity and often lead to other diseases, relying on the mutation and degree of expression (Lammas et al., 2002). For example, invasive salmonellosis is significantly more prevalent among patients with IL-12 receptor defects (44%) than those with IFNGR1/IFNGR2/STAT-1 pathway defects (7%), meaning that signaling pathways besides IFN-responsive genes are participating in the human host response to Salmonella (Ramirez-Alejo & Santos-Argumedo, 2014). Candida albicans is a naturally occurring commensal parasite that lives on the mucus membranes of animals and in their intestines. Candida rarely causes oral candidiasis (thrush) or allergic skin infections in healthy people after the first year of life. Candida, however, cause chronic, symptomatic mucocutaneous infections in certain conditions [chronic mucocutaneous candidiasis (CMC)] (Spampinato & Leonardi, 2013). CMC has been linked to defects in the role of the cytokine IL-17 [IL-17F(IL17F) and the IL-17 receptor -chain (IL17RA)] as well as the production of IL-17-producing Th17 cells in a number of recent studies (STAT1 and STAT3). The IL17F, STAT1, and STAT3 mutations are autosomal dominant, while the IL17RA mutations are autosomal recessive (Okada, Puel, Casanova, & Kobayashi, 2016).

## 2.8 Origin of immunogenetics

The origin of ABO blood group have sparked the field of immunogenetics. K. Landsteiner showed this in 1901 by demonstrating the presence of “natural” antibodies (isoantibodies). The presence of ABO blood groups in populations provided evidence of various-allele, one-locus theory instead of suggested two-locus (A, non-A and B, non-B) assumption in the 1920s by Felix Bernstein. Yamamoto, Clausen, White, Marken, and Hakomori (1990)

discovered the genetic variations amongst the blood groups O and A, along with A and B genes. MN was another blood group revealed by Landsteiner and Levine (1927). They infected rabbits having different red cell samples and then ingested the subsequent rabbit serum with some further red blood cell sections before discovering antibodies isolating ABO-type human blood. Antibodies (antisera) from maternal vaccinated towards red cell antigens were used to discover new blood group structures. One example is the Rh blood group scheme. In a brief but historic article, Race, Sanger, and Fisher (1968), Levine and Stetson (1939) mentioned that how parent of a premature baby fetus had a serious hemorrhagic reaction to her husband's blood transfusion. The husband cells and other ABO-compatible donors (80 of 104) were agglutinated by the mother's serum. The antigen in question was determined to be unrelated to the ABO, MN, or P blood classes. Since immunizing rabbits and guinea pigs with *Macacus rhesus* blood in 1940 antibodies are adhered to the red cells of approximately 85% of Rh positive white people in New York along with chimpanzees (Landsteiner & Wiener, 1940).

Anti-Rh antibodies were discovered in the blood of patients that had a response to ABO-matched blood transfusions, and the antibody discovered by Levine and Stetson (1939) in rabbits proved to be close of those generated by rhesus blood infusion. The process was called rhesus (Rh). Erythroblastosisfetalis caused by Rh incompatibility between the mother and the fetus, (Levine, Katzin, & Burnham, 1941). Lately, antirhesus and anti-Rh antibodies (named anti-LW) of rabbit and humans not establish similarity, therefore too old to fix the terminology. Later studies showed chromosome 19 genes, which are discrete from the RH gene on chromosome 1, encrypts the LW antigen. Notably blood transmission and maternal-fetal Rh inconsistency, offered some of the clearest evidence of Mendelism in the person before 1956, and significant cases of the implementation of genetic concepts in human health and infection.

## 2.9 History of immunogenetics

Landsteiner's discovery of antigenic variants in human blood in 1900 and Bernstein's discovery of genetic regulation of the A-B-O blood forms in 1924 developed the focuses of immunogenetic exploration. To explain his research on red cell antigens in dove hybrids, Irwin introduced the word immunogenetics in 1936. At the Department of Genetics in Madison, Wisconsin, Irwin has begun vital examination of immunogenetics in domestic animals. Ferguson, Stormont, and Irwin (1942) discovered the inheritance of 30 antigenic aspects in bovine red cells as early as 1942. These practices at Irwin's experiment in the 1940s sparked international attention in domestic animal blood group research and their use in animal breeding. Biologists became concerned with the roots of biological precision in 1930. Heidelberger and Avery's (1923) observe that carbohydrates were essential for immunological procedures' identification of

various forms of pneumococci was a watershed moment in our perception of the chemical existence of biological precision. Prior to this observation, it was commonly thought that proteins could only bestow biological specificity. The bulk of attempts to separate serum proteins from closely related organisms failed (Nuttall, Graham-Smith, & Pigg-Strangeways, 1904; Reichert, 1914). Jacques Loebin 1917, for example, wondered whether species-specific characteristics were Mendelian, theorizing that they might have originated in the eggs cytoplasm. In addition, Landsteiner and Van der Scheer stated in 1924 that distinguishing the horse, donkey, and their hybrid, the mule, was much simpler utilizing blood cells instead of sera. Todd and White (1910) published a paper clearly indicating significant uniqueness of cattle red blood cells, accompanied by Todd (1930) with confirmation that the chicken red blood cells distinctiveness was a heritable trait. Wiener studied these data in 1934 and 1939 and suggested limited number of antigenic disparities were prerequisite to justify the findings in cattle and chickens. Before the early observations on the Rh system by Levine and Stetson (1939), Levine and Katzin (1940), and Landsteiner and Wiener (1941), the three antigenic processes (ABO, MN, and P) of human cells were the best understood hereditarily within a group. Later, when Landsteiner and Wiener (1940) recognized the Rh element, Wiener and Peters (1940) wrote, "With the agglutinogens$A_1$, $A_2$, B, M, N, P, and Rh alone can identify up to 72 distinct kinds of human blood. In short, several blood group workers in 1930 were skeptical about the possibility of finding new blood groups outside those already established. Furthermore, various attempts to determine taxonomic relationships between animal species using protein similarities and variations were generally unsuccessful in distinguishing closely related species.

The area of immunogenetics was dominated by research on blood groups (red cell antigens) in humans and domestic animals. Some red cell antigens, on the other hand, were discovered early on to play a noteworthy part in the arena of transplantation in mice (Gorer, 1937). Snell (1948) coined the words histocompatibility genes and antigens to identify the genomes and pathogens, required for graft function during grafting of a skin or an organ. The idea of Major Histocompatibility Genes was developed for genes linked to severe tissues and cancer implantation reactions. Such genes are now considered to be members of gene cluster known as the Major Histocompatibility Gene Complex (MHC) or System of Histocompatibility Genes (SHG) (MHS). This division of immunogenetics grew in importance over time and is now a significant part of human medicine (Dausset, 1981). Antigenic markers can also be observed in soluble plasma compounds, because of serological techniques. In 1956 (Grubb, 2012), Grubb discovered Gm triggers in human immunoglobulins, which became the first results of this technique. Immunodiffusion and immunoelectrophoretic approaches helped to advance the understanding of antigenic variations in plasma proteins, and Oudin (1956) coined the word allotypes to describe certain genetic differences among organisms.

Allotype studies have also made significant contributions to understanding the genetic regulation of immunoglobulin chain formation and association. By using basic synthetic polypeptides with minimal structural heterogeneity as immunogens, Mcdevitt and Benacerraf (1969) pioneered an innovative area of immunogenetics. They discovered that particular immune response (It) genes mediated the identification of certain specific antigens. As a result of this, several important discoveries about the genetic control of immune regulation were published over the last era (Benacerraf & Katz, 1975). Furthermore, by genetic modification mice for elevated/low susceptibility to a dynamic multideterminant immunogen, Biozzi, Stiffel, Mouton, Bouthillier, and Decreusefond (1970) showed that general immune resistance was a polygenic trait (sheep red cells). Many genetic immunodeficiencies have been discovered in man, and the Xlinkedaggammaglobulinaemia discovered in 1952 was just a forerunner (Bergsma, Good, Finstad, & Paul, 1975). Nearly all animal cells have histocompatibility antigens on their surfaces. Lymphocytes are often used for serological identification. The mouse was the first animal to have histocompatibility antigens identified, and therefore inbred mouse strains became good model for studying histocompatibility antigens in further species (Klein, 1975). In mice, the main histocompatibility complex MHC is made up of antigens from the H-2 system. The H-2 system in humans, the B system in domestic fowl (Hala, Vilhelmova, & Hartmanova, 1977), the SLA system in pigs (Vaiman, Renard, Lafage, Ameteau, & Nizza, 1970), the BLA system in cattle (Amorena & Stone, 1978), the OLA system in sheep (Ford, 1974; Millot, 1974), and the GLA system in goats (Van Dam, Werkhoven, Van der Donk, & Goudswaard, 1976) all have homologous equivalents. Almost all animals have an MHC mechanism that is, similar to the H-2 system. For a long evolutionary period, the MHC seems to have remained as a knot of meticulously associated genetic factor. The MHC is an intricate hereditary system that spans around4 Mb and is found on chromosome 6 short arm. Human leukocyte antigen (HLA) loci, code primarily for antigens that express on the nucleated cell surface, important in transplantation compatibility. HLA molecules that recognize "self" and "nonself" control the immune response. Immunoglobulins, T cell receptors, CD4, CD8, and other molecules in the Immunoglobulin Supergene Family contain them.

HLA molecules present the antigen (its protein chain) to T lymphocytes, triggering a complex immune response. The HLA mechanism was discovered in the early 1950s when leukoagglutinating antibodies were discovered in the sera of polytransfused persons and multiparous women. Such sera's receptive structures revealed that they only recognized cells from unique people, with no autologous responses, implying that agglutinins were alloreactive and identified antigens from a polymorphic gene level. Jean Dausset identified the very first HLA antigen, HLA-A2, in 1958. The majority of

additional HLA antigens has grown, and the exchange of sera from different laboratories has made it easier to detect new molecules.

The first International Histocompatibility Workshop (IHWS) was held in 1964 to promote cooperation, altercation of well-described sera, assay assessment, and new data sharing. The workshop's significant results inspired imminent investigation after HLA system influenced kidney and skin compatibility (Petersdorf et al., 2013). Following the discovery of new HLA loci, a Nomenclature Committee was created to normalize the terms of existing HLA antigens and to organize the designation of novel ones. Progresses in science, the description of Class I and II HLA antigens, and, most recently, the implementation and standardization of molecular techniques that revealed the HLA system's strong polymorphism, have all contributed to the development of knowledge about the HLA system (Marsh et al., 2010; Torres & Moraes, 2011). DNA-based typing has certain benefits over serologic technique as it is specific since type-out reagents like primers and samples focuses on a precise nucleotide sequence. The novel reagents could be produced once the novel alleles can be found makes this technique more versatile (Franco-Duarte et al., 2019). A threat for grease refusals or failure is the existence of a donor-driven HLA Antibody for strong organ recipients such as kidneys and coronations. Standard protocol for antibody screening requires HLA laboratories to regularly extract and monitor the serum of prospective HLA antibody recipients (Schinstock, Gandhi, & Stegall, 2016). Lymphocyte objective in complementary cytotoxicity assays have been used in the past for antibody identification. New solid-phase strategies to resolve the reduced sensitivity and specificity of the dependent cytotoxicity test complement have only been implemented (Althaf, El Kossi, Jin, Sharma, & Halawa, 2017; Chowdhry et al., 2018).

## 2.10 Discovery of the major histocompatibility gene complex

Following his findings of rabbit immune sera agglutinating erythrocytes, Peter Gorer was the first to characterize a histocompatibility mechanism in mice in 1936. George Snell expanded on this research by showing that antigen incompatibility caused graft rejection in mice. The antigen II discovered by Gorer was assigned the name "H2" to the murine MHC (Snell, 1986). In humans, the HLA complex's history dates back to Jean Dausset's *princeps* discovery in 1952. He proposed that a specific antigenic mechanism to that found on the surface of mouse erythrocytes could occur on human leukocytes surface, by demonstrating gigantic leucoagglutination by serum from a polytransfused patient. Conversely, it was not until 1958 that the first human MHC antigen, MAC (HLA-A2), was definitively identified, subsequent a traditional separation population study with a serum that only reacted in a subset of the sample. The Jon van Rood, Rose Payne, and Walter Bodmer,

identified the antigens 4a and 4b (Bw4 and Bw6), and HLA-A2 and HLA-A3, respectively on multiparous women, and verified this polymorphic mechanism. The IHW identified gene cluster on chromosome 6 with serologically specified alleles, like HLA-A, HLA-B, and HLA-C. The accompaniment structure was found to be located in the identical genetic area as the complement system. HLA class II alleles were first described in the 1970s by searching for mixed lymphocyte reactions. The HLA system could then be analyzed directly at the gene level, rather than via the products of the genes.

## 2.11 Genetic organization of the human leukocyte antigen system

The MHC comprise a variety of manifold genes, and are classified into three groups (Classes I, II, and III) based on functional and structural variations. The so-called HLA code for Class I and Class II. Two very complex and polymorphic regions, each of which comprises many loci and alleles, dominate class I and II molecules (Cruz-Tapias, Castiblanco, & Anaya, 2013). The Class IIIMHC contains genes responsible for controlling the production of complement system proteins, cytokines, heat shock proteins, and the enzyme 21-OH hydroxilase, involved in the immune response (White, New, & Dupont, 1985). The new version 3.7.0 (2012−01−12) of the "IMGT/HLA" Database, identifies over 7000 allele containing a specialized directory of HLA sequences and incorporates these for the WHO Nomenclature Committee for HLAs (Robinson, Malik, Parham, Bodmer, & Marsh, 2000).

The HLA molecule is distributed into three sections: extracellular, intramembranous, and intracellular. The alpha 1 and 2 are peptide-binding domains representative of alleles in the gene's second and third exons, respectively, dividing the extracellular region into three parts (Robinson et al., 2017). HLA Class I antigen binds with these antigenic peptide domains and interact with a variety of T cell receptors. In a mixed lymphocyte culture response, lymphocytes from putative HLA-identical individuals activate one another, which led to the discovery of HLA Class II (van Drongelen & Holoshitz, 2017). Only immune cells like macrophages, activated T lymphocytes, and B lymphocytes, carry HLA Class II antigens. HLA-Dr, HLA-DQ, and HLA-DP are 3 kinds of human Class II molecules which are encoded by genes in different regions that have different numbers of alpha and beta chain alleles (Deshpande, 2017). Since the beta chain is even more pleiotropic than the alpha chain, HLA typing is currently done on the beta chain (HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQB1, and HLA-DPB1) (Choo, 2007).

The extracellular hydrophilic region of the alpha chain includes two extracellular domains, alpha 1 and 2, having oligosaccharide binding site to; the extracellular hydrophilic region of the beta chain also contains two

extracellular domains, beta 1 and 2. The amino-acid sequences indicates first extracellular domains of both chains is the most complex, while the second and third exons are connected to the first (alpha 1 and beta 1) and second (alpha 2 and beta 2) extracellular hydrophilic domains, respectively.

The International IMGT/HLA project database (release 3.7.0) lists 6959 alleles for HLA Class I and Class II, with 5399 for Class I and 1757, 2338, 1304 for HLA-A, HLA-B, and HLA-C, respectively. Nowadays, there are 1560 alleles for Class II and 1166, 162, 152 for HLA-DRB1, HLA-DQB1, and DPB1. Among populations and ethnic groups, the spread of alleles and discrete haplotypes fluctuates. The majority of novel alleles are discovered only once, and they are mostly found in exons representing antigen—presenting cells domains, with certain mutations found in introns or exons in further domains. In certain cases, the alteration is only in the nucleotide series, and the amino acid sequence is unaffected (Yang & Zheng, 2017). The number and specification of probes or primers used for the analysis determine the extent of allele description in HLA typing. Typing at the antigen level is possible with a small number of probes or primers, and the findings identify a large category of alleles which lead to a serologically specific antigen (Billard et al., 2012). To resolve the frequent HLA ambiguities, high resolution typing necessitates a mixture of primers or probes, as well as a variety of techniques. A computer programme is needed to associate the findings with the library data series of WHO-recognized alleles in order to interpret and assign HLA alleles. The patient and donor's histocompatibility is necessary for effective hematopoietic stem cell transplantation (Paunić, Gragert, Madbouly, Freeman, & Maiers, 2012).

Allele-level screening in haematopoietic stem cell transplantation must assess viability for the HLA-A, B, C Class I and DRB1 and DQB1 Class II loci in the standard graft program because inconsistencies at several HLA loci have been well established to increase the risk of transplant versus host disease (Tiercy, 2016). In solid organ transplantation, determination at an antigen level is generally sufficient and can be done using serological or molecular biology approaches. In this context, it is critical to assess the HLA antibody in patients using a variety of serological techniques (Fürst, Neuchel, Tsamadou, Schrezenmeier, & Mytilineos, 2019).

## 2.12 The most polymorphic human genomic region

The extraordinary existence of human genetic variation has been revealed after Landsteiner discovered the first human biological polymorphism, varies from single to wide-ranging structural genetic discrepancy covering thousands of base pairs. SNPs, substitutions, and deletion insertion polymorphisms (DIPs) are examples of single nucleotide polymorphisms (SNPs), as well as multibase variability like short tandem repeats (STRs) (microsatellites), massive insertion deletions (INDELs), inversions, and extra repeats. STRs and SNPs have

proven to be especially useful as genetic markers in producing the first physical and genetic map of the human genome. Despite the fact, that such variants have been discovered in almost every gene, no other region of the genome can match the exciting polymorphism observed in HLA genes. In 1980 8−39 codominant alleles were known for each four genes identified by Jean Dausset in his Nobel lecture, HLA-A, -B, -C, and -Dr, and the quantity of haplotypic variations was already in the thousands. Extra MHC-linked genes were highly polymorphic as well, and emphasize that almost each person carries a special grouping (Dausset, 1981). More than 15,000 HLA alleles had been identified by 2000 (MHC Sequencing Consortium, 1999). With 86 SNPs per kb and 1195 alleles, the HLA-B locus is the utmost polymorphic genetic factor in the human genome, with the continuous increase in alleles (Robinson et al., 2000). HLA-A is the second least polymorphic gene, with 733 alleles. For HLA loci, there are currently 3371 HLA alleles and 106 nonclassical HLA alleles (MICA, MICB, TAP1, TAP2). Polymorphic sites are frequently found in the second and third exons of class I loci, and also the second exon of HLA-DRB, -DQA, -DQB, -DPA, and -DPB genes in classical HLA genes. In this scenario, nonsynonymous single nucleotide replacements lead in amino acid variations in the antigenic peptide binding domain. HLA loci variant distribution differs from that of other functional genes whose variants are mostly present in introns (Cruz-Tapias et al., 2013).

Point mutations, recombination, and genic conversion are all responsible for the emergence of such extreme polymorphism. Thanks to "genetic hitchhiking," variations are more prevalent in noncoding sequences in regions adjacent to the most polymorphic genes. The heterozygous state's competitive benefit, which allows for a more complex immune reaction to potential diseases like HIV-1, is assumed to maintain polymorphism in class I and II genes. Polymorphic rare alleles also offer a population-wide benefit in terms of infection resistance. Both pathways are expected to be active at the same time. Because of the great polymorphism in the MHC, a new nomenclature was created, in which the locus term is trailed by an asterisk and four digits indicating the allele, the first two of which refer to the prior serological nomenclature. For synonymous improvements or noncoding variations, extra digits are added. This method is used for class I and class II genes, as well as TAP and PSMB genes. Microsatellites were commonly studied to identify infection associations especially in this modern era of genome-wide linkage maps and the use of SNP microarrays as genetic markers. Until the 13th IHW, which discussed aliases for 281 microsatellites with an optical thickness of one per 45 kb, there was no systematic nomenclature. Although since, the dbMHC database has grown significantly. (Vandiedonck & Knight, 2009) ad has been modified to include all microsatellites.

In the present genome assembly, the 14th IHW successfully mapped 664 microsatellite primer pairs. Submicroscopic structural genomic polymorphism is becoming more commonly accepted on a larger scale. It includes copy number

variants, segmental duplications, inversions, and translocations that are larger than 1 kb in size. Within the MHC (specifically chromosome 6p21.3), for instance, the database of genomic variants presently records two inversions, 63 indels (100−1 kb in size), and 181 copy number variants, many of which are of possible functional significance, like CNV-507 and CNV-505, that share the susceptibility loci for sarcoidosis and psoriasis. Segmental duplications affect two major regions of the MHC: the HLA-DRB1 region and the complement region, both of which are unique to certain allelic combinations or "haplotypes" (Vandiedonck & Knight, 2009).

## 2.13 Role of human leukocyte antigen in creating the first physical and genetic map of the human genome

Jean Dausset received a significant legacy from an art collector, HelèneAnavi, in 1984 for his laboratory, thanks to a joint Nobel Prize in 1980. With this unexpected gift, he founded the Center d'Etude du Polymoprhisme Humain (CEPH) with Howard Cann and Daniel Cohen, which later becomes a Foundation Jean Dausset-CEPH (Dausset et al., 1990). The MHC connotation work motivated Dausset and Cohen to search for polymorphisms through the entire genome and establish markers, which could isolate in families to explore linkage in various ailments. The gathering featured many big families, including Ray White's extensive Utah pedigrees. The human genome's first microsatellite genetic and physical maps were reported because of the CEPH consortium, paving the way for the Human Genome Project. This priceless reserve ushered in a novel age in genetic science, enabling researchers to classify various disease vulnerability genes. The CEPH samples are still being researched extensively today, and they have played an important part in recent genomic breakthroughs like International Hap Map project and the ongoing '1000 genomes' sequencing project (Zhang & Dolan, 2010).

## 2.14 Conclusion

Our understanding of immunogenetics has exploded in the last 15 years. The discovery of gene mutations in patients with acute immunodeficiency conditions has revolutionized our knowledge of human immunity. We believe that our knowledge of complex gene defects and vulnerability to outbreaks and autoimmunity will constantly evolve as new acquaintances are created, and therefore this awareness would have an ever-increasing effect about how these and many other diseases are addressed. Consequently, the discovery of the genetic regulation of histocompatibility antigens and immune tolerance, as well as the correlation of such immunogenetic factors with disease susceptibility in humans and animal studies, is quite encouraging. The MHC will certainly attempt to investigate everyone, and it holds more mysteries that

will have consequences for the remaining genome. Nevertheless, an inclusive strategy focusing on opportunities for improving should keep MHC progress at the forefront of human genomics.

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Chapter 3

# Basics of immunogenetics: application and future perspectives

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## 3.1 Introduction

Immunity is an individual's ability in which an individual recognizes their self-molecules that makeup their own body and which have the potential to differentiate from other molecules which are found in infectious microorganisms and toxic substances. This process has an important genetic component. Information about the genetic and molecular basis of immune system of mammals has improved autonomously with the volatile advances that are made in somatic cell and molecular genetics. Human health is greatly affected by altered immune functions. Because, immune responses are affected by genetic factors, and association between the immune system and genetics serves as an interpreter of the development of disease and a biological indicator of progression of disease. The genetics and immunology both are important in the study of immunogenetics. Impairment in immune regulation causes an enormous number of autoimmune disorders including "rheumatoid arthritis," "inflammatory bowel disease," "psoriasis," and are distinguished by dysregulation of different aspects of normal immunity and inflammation. Explanation of the genomic foundation of unusual genetic disorders including deficits in the inborn and acquired immune reaction is really helpful in understanding the mechanisms of disorders and the basic developments sustaining immunity (Aberg et al., 2012; Casanova, Holland, & Notarangelo, 2012; Cunningham-Rundles, 2012). Typical genomic strategies are not very efficient against genetic disorders. Therefore development of advanced technology has been needed. These advanced technologies

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00001-4**
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including genotyping and DNA sequencing are efficiently resolving the unusual genetic diseases and importantly report the hereditary origin of other common disorders including impairment in immune functions. Furthermore, with the help of these advance technologies we can easily examine the usual deviation concerning human health to evaluate chromosomal regulations of immune functions and also control the amount of the particular influences on immune utility reliant on tissue nature and ecological aspects (Long et al., 2019). Genome-wide association study (GWAS) is one of the genomic approaches used to recognize proteins, genes, genomic loci, and pathways that take place in that process. The perspective of GWAS reached toward remarkable attainment in relation of autoimmune and other immune-related illnesses. Though, significant contest persists: even if GWAS have delivered a genomic direction plot of possible suitable locus/loci, in paramount illustrations we do not recognize what the particular fundamental practical alternatives are, which genes are being modified, and how they communicate to sickness. One more level of complication is added through the statement that, in maximum cases, nontranslated segment variations have been randomly associated by GWAS, and immune disorder is not an irregularity in this development (Maynard & Ackert-Bicknell, 2019; Sullivan & Geschwind, 2019).

Also, proclamation assessable feature plotting compromises a significant corresponding method for under taking monitoring genomic variations and the protein sequence exaggerated. Remarkably, all these learnings have exposed substantial connection amid illness and expression-linked hereditary indicators (Majewski & Pastinen, 2010; Montgomery & Dermitzakis, 2011; Nicolae et al., 2010), and also pointed toward resemblance amid ailments traditionally thought unconnected. The classification range and fundamental genomic variety discovered by sequencing readings that is 1000 Genomes Project, joined with the unexpected complication of the genomic monitoring back ground existence discovered by developments in efficient genomics such as the ENCODE Project, highpoint the trials and prospects that lie onward in recognizing functionally significant hereditary deviation in antisense areas and its significances for immune function. Furthermore, to considering hereditary deviation in both coding and controlling areas, advancement in accepting ailment pathogenesis and chances for management of immune infections will have need of the assimilation of inherited and ecological hazard in a much more complete way than has been accomplished to date, taking into description of epigenetic procedures and situation-particular properties in function of immune system (Consortium, 2012; Fernández-Morera, Calvanese, Rodríguez-Rodero, Menéndez-Torre, & Fraga, 2010; Rakyan, Down, Balding, & Beck, 2011). Major histocompatibility complex (MHC) is located at chromosome number 6 and its hereditary material is about 4 Mb in length. At fixed position on a chromosome human leukocyte antigen (HLA) present at chromosome number 6 and plays an important role

in coding expressing genes, helps in organ or tissue transplantation. It controls the immune responses through the identification of self or nonself and belongs to immunoglobulin includes T cell (receptors), CD4 and CD8 etc. These immunoglobulin (HLA molecules) initiating the particular immune response and is showing the antigen to the T lymphocytes (Tiercy, 2002). Agglutination involving antibodies were initiate in serum of polytrans fused patients (Dausset, 1958). Examination of serum reactive pattern identified of individual cells was observed, which implied agglutinins reactive antigens and hereditary material (polymorphic locus). The first HLA was named as MAC but now, it is commonly known as HLA-A2 (Dausset, 1958). Later, the identification of new molecules by interchanging serum increased the sequence of HLA antigens (new) in different laboratories (Van Rood & Van Leeuwen, 1963). Various studies reported that the early observation shows HLA system pretentious liver, kidney, and dermis compatibility, especially the involvement of histocompatibility tests in organ transplantation (Thorsby, 2009). For the last 20 years, technology has evolved standard molecular techniques which show polymorphism in antigen system (class I or class II antigens). In serological technique, DNA-based typing has major advantages, that is primers and probes are more distinct and based on a particular base pair sequences, more specific, more flexible, more vigorous. It does not require a precise cell type and it is low impact on human health (Cano et al., 2007; Erlich, Opelz, & Hansen, 2001). The standard repetition needs to regulate the HLA, collection and serum regulation to transplant the HLA antibodies. Antibody detection was performed with lymphocyte mark in accompaniment reliant "cytotoxicity assay."

## 3.2 Application of genomic procedures to major immunodeficiency syndromes

In history, genomic exploration of special Mendelian principal immunodeficiency diseases has been extremely illuminating in accepting immune purpose (Abbas, Lichtman, & Pillai, 2019;Kubes & Jenne, 2018; Notarangelo, 2010). Such "experiments of nature" (Boisson-Dupuis et al., 2012) demonstrated in several cases to be manageable to traditional connection investigation and memory representing. Specific individual Intisar rayon or after unadorned joint immunodeficiency diseases, along with particular deficiencies comprises humoral and cellular immune system, to deficiencies in newborn, that is decreased Toll like receptors (TLR) signaling. Interpretation of the straightforward lymphocyte disorders for instance was tremendously significant in determining utilizations of immune instruction, remarkably the nature and role of the HLA is CIITAwhich means MHC class II transactivator (De Preval, Lisowska-Grospierre, Loche, Griscelli, & Mach, 1985) and the RFX complex in regulating class II gene developers (Reith & Mach, 2001). Principal immunodeficiencies categorized by substantial autoimmune

disorder have conveyed significant comprehensions into the fundamental acceptance as of research of the unusual ailment autoimmune polyendocrine syndrome 1 that is (APS1) and the recognition of transmutations in the autoimmune regulator, that is, (AIRE), it is a transcript element modifiable appearance of self-antigens inside the thymus gland (Metzger & Anderson, 2011); and of negligible acceptance from characterization of the function of the transcript feature FOXP3 (forkhead box P3), in which transmutation reasons immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX), then it marks in autoimmunity by way of dysregulation of supervisory T cells (Bennett, Li, Harvey, & Gorassini, 2001; Wildin et al., 2001). Infrequent single-gene deficiencies restraining B-cell employment and construction of antibodies that give rise in immune deficiency have also been helpful. A number of pattern series occur from examination of X-linked gammaglobulinemia, enlightening the important function for the tyrosine kinase BTK that is Bruton's tyrosine kinase in B cell receptor indicating and development, to alterations fundamental X-linked hyper IgM condition representing the importance of CD40 for class interchanging and enlargement of B cell memory (Cunningham-Rundles, 2012). In spite of these achievements, the documents of subsidizing alleles for nearly key immune-deficiencies by way of traditional methods have been hindered via the lesser amounts of instructive belongings. The latest improvements in genetic tools including whole-exome sequencing (WES), that is whole-exome sequencing have solved these kind of unusual illustrations and are undertaking to progress the genomic field considerably. These kind of methodologies have emphasized in what way mutually loss-of-function and gain-of-function genes of Signal transducer and activator of transcription 1 (STAT1), which is a key transcriptional factor participating in IFN signaling and vulnerability to bacterial and viral contaminations, also which may discompose standard immune purpose, prominent toward unblemished disease phenotypes (Boisson-Dupuis et al., 2012), in this case, long lasting CMC that is mucocutaneous candidiasis, a determined or persistent contamination with *Candida albicans* concerning the different parts like nails, skin, oral, or genital mucosa of body. Even though a variability of genetic etiologies has been acknowledged, here is the cause of autosomal prevailing CMC that is mucocutaneous candidiasis was definitely considered. The premeditated methodology of consuming WES for analyzing a lesser amount of entities and then and there categorize the translated segment of the concerned protein sequence into a bigger amount of people was greatly dynamic. In the main, 36 patients of 20 families' existed to be heterozygous for one of twelve recognized transmutations comprising the helical-loop sphere of STAT1 protein (Liu et al., 2011). This effort similarly demonstrates an important improvement in reviewing immune system characters, specifically that purposeful description is comparatively manageable agreed that maximum of the appropriate cell categories and tissues are reachable. In the STAT1, review a strong function for the notorious

increase-of-function genes was establish in positions of diminished IL-17 immunity (Liu et al., 2011). Even though, the asking prices of WES and whole-genome sequencing is declining, it leftover a reduced amount of communal genomic procedure than GWAS. The frequency of principal immune-deficiency syndromes creates complications, and in around cases, it is difficult to categorize appropriate belongings for the GWAS to be effectively mechanical for the reason that this methodology necessitates enormous amounts of circumstances and gear stick to assess for overtone spending a genetic-extensive section of hereditary indicators. In the case of CVID (common variable immunodeficiency), a GWAS comprising 363 patients established the part of the MHC, but also associated other loci for instance the disintegrin and metalloprotease genes as well as many unusual organizational deviations (additions and removals) involving such as ORC4L, a gene significant for beginning of DNA replication process that was up to that time concerned in B cell lympho-proliferative disorders (Radojkovic et al., 2009). The hereditary foundation of CVID residues uncertain (Cunningham-Rundles, 2012), however, it is anticipated that done better-quality scientific phenotyping (Chapel, 2012) and genomic tactics the presently heterogeneous diseases of CVID possibly will be resolute into different ailments which will support in the expansion of extra surround rehabilitations and developed quantifiable upkeep. A current correspondingly sized GWAS (common variable immunodeficiency) examining IgA deficiency also established the former MHC class II relationship with this ailment (Fernández-Morera et al., 2010). Consequent adequate charting and assertion of HLA type determined the principal suggestion indicator to HLA-DQB1*02, which is associated with type 1 diabetes (T1D) and celiac ailment (CA). Interestingly, the GWAS demonstrated that the additional non-MHC association involving two loci causes autoimmune disease (Fernández-Morera et al., 2010). These GWAS are including as IFIH1, which encodes interferon inducible RNAs stimulating viral infections. Later, other type of IFIH1 variants were found to shows autonomous association with T1D (Nejentsev, Walker, Riches, Egholm, & Todd, 2009). Also, CLEC16A is the second loci, which encodes a C-type lectin, is also associated with T1D (Type 1 diabetes mellitus), Ms (multiple sclerosis), SLE (systemic lupus erythematosus), and RA (rheumatoid arthritis) (Wang, McPherson, Marsh, Gortmaker, & Brown, 2011), signifying some correspondence in disease pathogenesis (Fernández-Morera et al., 2010; Mboowa, Sserwadda, Amujal, & Namatovu, 2018; Turro et al., 2020).

## 3.3 Biogenetic variation, functional genomics, and the immune system

Biogenetic tools modifying genetic factor assertion, including methylation of DNA molecule, alterations in histone proteins and nontranslating RNAs, illustrate a specific function in the immune system of organisms

(Fernández-Morera et al., 2010; Suarez-Alvarez, Rodriguez, Fraga, & López-Larrea, 2012). It is noticeable in the multipart and necessary management of immune-cell characteristics and its commitment throughout growth and discrepancy to create multicellular ancestries, along with transformation of the epigenotype performing to control or stimulate genetic factor assertion and also regulate body cell fate. Epigenetic management has established itself as greatly active and cordial technique, by providing an important crossing point by way of environmental surrounding and memorial of past experiences. Numerous ecological threat meant for immunological disorder have been interconnected by biogenetics comprising hand-rolled cigarette smoking, toxicities for instance "Epstein−Barr virus," reproductive hormones induction and deficiency of vitamin D (Costenbader, Gay, Alarcón-Riquelme, Iaccarino, & Doria, 2012). Interactions between gene and environment have been determined such as in RA between cigarette smoking and HLA-DRB1 (Human Leukocyte Antigen-Dr isotype) risk genes (Karlson et al., 2010) collected by further protein sequence loci for example, PTPN22 (Protein tyrosine phosphatase, nonreceptor type 22) (Costenbader, Chang, De Vivo, Plenge, & Karlson, 2008). Epigenomic tactics can be instructive and meant for particular environmental hazard factors including deficiency of vitamin D. VDR or vitamin D receptor is a ligand stimulated transcript element, also current investigation of VDR genome-extensive tenancy by means of ChIP-seq or ChIP sequencing, discovered that loci related by GWAS in a number of immunological conditions containing IRF8 (Interferon regulatory factor 8) and PTPN22 (Crohn's disease and T1D) were secured by VDR (Ramagopalan et al., 2010). On behalf of particular illness threat genes in the MHC, here is the indication signifying gene-particular enrollment of VDR at the promoter of HLA-DRB1, which demonstrates exactly how ecological and genomic threat factors may possibly be connected (Ramagopalan et al., 2009). Genomic methodologies including GWAS and WES have established themselves as an actual influential in describing the amount and behavior of genomic deviation into the perspective of immune property and its diseases. Yet, substantial contests persist, not minimum in forming fundamental purposeful alternatives. The proposal of efficient trainings involves suspicious deliberation of perspective-particular properties, along with aggregate prominence on investigation of principal cells of body in a sickness situation wherever corresponding efficient genetic, biogenomic, and proteomic methods can be functional organized with immunological examines and analysis of suppositions in typical animals. Explanation will be simplified by implementation of an additional organizations established tactic and the investigation and assimilation of inherited, epigenetic, and ecological type of disease. Appeared measurable-feature representation has proven a cherished apparatus to resolute monitoring variants, and future studies in specific situations help to defining the genetic nature which involving trans-acting variants. Noncoding RNAs and splicing transcripts has specify expression of

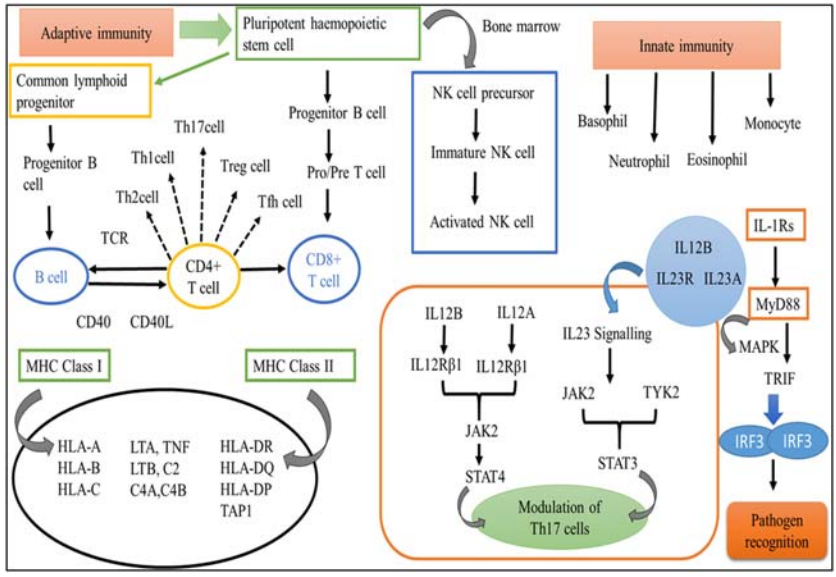


**FIGURE 3.1** Showing mechanism of immunogenetics modulation of adaptive and innate immunity during pathogenic attack.

genes involved in immune dysfunction. RNA sequencing should supplementary upsurge the formativeness of such studies involved in immune dysfunction (Fig. 3.1).

## 3.4 Histocompatibility complex region of humans and neurological disease

The balance between inborn and acquired immune system is nowadays esteemed as a significant group in the purpose of neural illness consequence. There are molecules which coded via the key histocompatibility compound (MHC) segments standardize the inborn and acquired supports of humanoid immune reaction over antigen exhibition, annoyance guideline and the counterpart coordination and also the influence of this province in a number of immune-facilitated circumstances, comprising neurological disorders, has elongated stayed acknowledged (Hamza et al., 2010; Shiina, Hosomichi, Inoko, & Kulski, 2009; Song, Yan, Zhao, & Liu, 2016; Steele et al., 2017). The human MHC (histocompatibility compound) genetic factor domestic charts to chromosome 6. MHC (histocompatibility compound) has a mass of closely five Mega base pairs Mbp and it translates around "165 protein-coding genes," many of them related to immune system, and includes just about 13% of the human genome (Shiina et al., 2009).

Therefore the HLA section developed itself as the supreme considered area of vertebrate genetic material. HLA section is deliberated as the impenetrable area of the human's genetic material and along with the determination of the MHC sequencing association through the structure and in year 1999 the gene map of this region was produced firstly. The HLA area is considered by means of a life-threatening zone of polymorphism and wide-ranging outlines of "linkage disequilibrium" (LD), which fluctuates amid populaces. The genes of this specific region are distributed into five different subtypes: the prolonged class I, class I, class III, class II and prolonged class II (Shiina et al., 2009). Comprehensive MHC area encompasses larger than 400 interpreted genetic factor and pseudogenes or false genes (Horton et al., 2004). The HLA class I segment comprises of three traditional loci, "HLA-A," "HLA-B", and "HLA-C," laterally with three nontraditional loci: "HLA-G," "HLA-E" and "HLA-F." The nonconventional HLA class I particles are considered by way of extra restricted point of fluctuation accompanying to their traditional complements (Shiina et al., 2009). HLA class I particles stay communicated on wholly nucleated cells of body and also their key purpose is demonstration of nonself-antigens initiated from intracellular that is within the cell, causes to "cytotoxic (CD8 + ) T cells" meant for elimination of the "antigen-presenting cells" that is (APCs). Correspondingly, the class II segment of "HLA" encompasses three traditional loci, "HLA-DP," "HLA-DQ" and "HLA-Dr," alongside with two nontraditional loci, "HLA-DO" and "HLA-DM" (Shiina et al., 2009). Genes including traditional HLA belongs to class II loci are communicated onto the external portion of specialized antigen-presenting cells, which are commonly existing antigens of extracellular starting point, (Holling et al., 2004) including those resulting from nutrition (metabolites) or bacteria for the demonstration to coworker "(CD4 + ) T cells." Another HLA class III segment comprises of inflammatory monitoring genes. Central nervous system is a well thought-out immune system fortunate site, also it was time-consuming deliberated that usual neurons did not communicate HLA class I. Nevertheless, this concept was disallowed subsequent the detection of HLA class I mRNA or protein communication in different neuronal inhabitants, containing motor nucleous, substantia nigra pars compacta (Huh et al., 2000; Lidman, Olsson, & Piehl, 1999), dorsal root ganglia neurons (Lindå, Hammarberg, Piehl, Khademi, & Olsson, 1999; Neumann et al., 1997), "dopaminergic nigral cells" (Lindå et al., 1999), developing and "adult hippocampal pyramidal cells" (Corriveau, Huh, & Shatz, 1998; Neumann et al., 1997), vomeronasal organ's sensory neurons (Loconto et al., 2003), brainstem (Lidman et al., 1999) and spinal (Lidman et al., 1999; Lindå et al., 1998), motor neurons and "cortical pyramidal cells" (Corriveau et al., 1998; Huh et al., 2000). In current times, a straight connection has been recognized for HLA class I in purposeful and operational synapse trimming in the central nervous system (Lee, Cole, Palmiter, &

Koh, 2000; Shatz, 2009). Additional, the capability of microglia, the brain's inhabitant macrophage cells, to present antigen over the "class II MHC" to T-cells authorities these usually inactive cells to accomplish a significant role in defining the therapeutic results of numerous nervous system disorders. Usefulness of microglia in numerous nervous system disorders is well acknowledged (Holtman et al., 2015; Keren-Shaul et al., 2017; Misra, Damotte, & Hollenbach, 2018; Noristani et al., 2015) (Table 3.1).

## 3.5 Killer-immunoglobulin-like receptor network is a novel range of neural infection in genetics of immune system

Killer-immunoglobulin-like receptor (KIR) compound was identified and in present is known as KIR obstructer. According to Vilches and Parham (2002), all members of KIR proteins can be indicated on NK (natural killer cells) and some amount of "T cells" (Björkström et al., 2012). "KIR" compound plot on the in "chromosome 19q134" is responsible for the critical constituents of natural and advance immune response. While "HLA" and "KIR" belongs to distinct groups. The interconnection in between "KIR" and "HLA" (class I), they helps in manage NK uses and also regulates stability of immune response. KIR is known to be a surface obstructer which helps in maintenance of NK uses and receptiveness (Parham, 2004). Above aspartate contains 2D and 3D external "immunoglobulin domain." The killer cell contains lengthy cellular chain differentiating ITIMs (immune-receptor tyrosine-based inhibitory motifs). And the activated receptor having small cellular chains can initiate "DAP12" via activated silt in transmembrane domain. Although, "killer- immunoglobulin-like receptor 2DL4" is rarely found in having elongated cellular chain with immune-receptor tyrosine-based inhibitory motifs when interlinked with "FceR1c" (Kikuchi-Maki, Catina, & Campbell, 2005; Kikuchi-Maki, Yusa, Catina, & Campbell, 2003). KIR considers more than one determinant of "human leukocyte antigen (class I)." "Killer- immunoglobulin like receptor" is usually attached with the HLA (class I) which makes the connection with "N-terminal part of the α-helix," the "C-terminal part of the α-helix," and "the bound peptide" (Saunders, Granger, & Sabatini, 2015). Genetic variability in class I helix governs the three important determinant recognized through killer- immunoglobulin-like receptor, "HLA class I," Bw4. Prohibit "Killer- immunoglobulin like receptor 2DL1" and Killer- immunoglobulin like receptor 2DL2/3 or initiating "killer-immunoglobulin-like receptor 2DS1," "killer-immunoglobulin-like receptor" 2DS2 and killer-immunoglobulin-like receptor 2DS4 interlinked adversely with mutually different C1& C2 determinant bear by various HLA class shapes and short HLA-B fragment (Colonna, Nakajima, & Cella, 2000; Moretta, Biassoni, Bottino, Mingari, & Moretta, 2000; Parham & Moffett, 2013). However, HLA-A and -B with "Bw6" determinant do not attached with killer- immunoglobulin like receptor. Additionally all the receptor are

**TABLE 3.1** Shows the involvement of immunogenetics in neurological diseases.

| Sr. no. | Neurological diseases | MHC region (on chromosome 6) | | KIR (killer immunoglobulin-like receptors) associations | References |
|---|---|---|---|---|---|
| | | Class I | Class II | | |
| 1. | Ms (Multiple sclerosis) | HLA-A | DQA1 | KIR3DL1S1KIR2DL1 | Hamza et al. (2010), Song et al. (2016), Misra et al. (2018) |
| 2. | PD (Parkinson's disease) | HLA-B | DQA1DRB5 | KIR2DS4KIR3DL2/DS2 | Shiina et al. (2009), Steele et al. (2017), Misra et al. (2018) |
| 3. | AD (Alzheimer's disease) | HLA-AHLA-B | DPB1DRB1DRA | KIR3DL2KIR2DS4 | Neumann et al. (1997), Lindå et al. (1999), Misra et al. (2018) |
| 4. | Schizophrenia | HLA-AHLA-BHLA-C | DQA1DRB1 | KIR2DS2KIR3DL2KIR2DS4 | Noristani et al. (2015), Misra et al. (2018) |
| 5. | MG (Myasthenia gravis) | HLA-BHLA-C | DQA1DQB1DRB1 | KIR2DL2/3KIR2DS4 | Holtman et al. (2015), Misra et al. (2018) |
| 6. | ALS (Amyotrophic lateral sclerosis) | HLA-AHLA-BHLA-CHLA-F | DPB1 | KIR3DL1S1KIR2DS4KIR2DL1/S1 | Misra et al. (2018), Keren-Shaul et al. (2017) |

interlinked with the "allelic variations" of "killer-immunoglobulin-like receptor" and "HLA class1" through the series of compelled peptide (Cassidy et al., 2014; Moesta et al., 2008; Thananchai et al., 2007). KIR compound shows a large diverseness in genetic material at intra population and intra population range. Killer- immunoglobulin-like receptor genetics contains 4−14 genes on the bases of its gene appearance forms in A and B groups (Parham, 2005). There are considered as A and B haplotype in which seven genes are exposed inhabitation of KIR and remaining formed "B-haplotypes." In this B-haplotypes maximum functioning of KIR (Hsu et al., 2002; Middleton, Tran, & Craig, 2007; Uhrberg, Parham, & Wernet, 2002). Genotype may occur by the mixtures of different "centromeric and telomeric" genetic material motifs, and also forms in A group and B group. So, there are many genotypes defined some related genotype continuously amount more than 90% of killer- immunoglobulin like receptor genetic difference formed in a particular population detected in many "ethnic groups" (Hollenbach et al., 2013; Hollenbach, Nocedal, Ladner, Single, & Trachtenberg, 2012). Recently reported work considered that predominance of "killer- immunoglobulin like receptor (KIR) genotype" and a particular relation between "KIR" and "HLA genotype" are connect in "autoimmune" (Hollenbach et al., 2009; Khakoo & Carrington, 2006) and some disease like HIV and HCV "hepatitis C" (Bashirova, Thomas, & Carrington, 2011; Khakoo et al., 2004; Li et al., 2004), tumor (Boudreau et al., 2017) and censorious to HCT (haematopoietic stem cell transplant) and gestation (Hiby et al., 2004; Hiby et al., 2014; Moffett & Hiby, 2007; Moffett-King, 2002). Evidences shows the important role for natural killer cells (NK) in different infectious diseases like multiple sclerosis, neuromyelitis optica (Ratelade et al., 2012), Parkinson's disease (Solerte et al., 2000), Alzheimer's disease (Solerte et al., 1998) schizophrenia, myasthenia gravis (Shi, Forsberg, Torne's, Østensen, & Goldberg, 2000) and amyotrophic lateral sclerosis build up that killer- immunoglobulin like receptor differentiation useful in growth. The essential role of "natural killer cells" is to boost immune strength and also considered to protect from various diseases. In "Ms murine model," "EAE (experimental autoimmune encephalomyelitis)" play a significant role in natural killer cells in negative regulation of infection disorders. The regulation of seriousness of infection in experimental autoimmune encephalomyelitis which can connect with natural killer cells acceptance with particular necrosis (Shi et al., 2000; Vollmer et al., 2005). There are many work in which man indicate an "immune-regulatory role" for "natural killer cells" in multiple sclerosis and cause reduction of mitigating track (Takahashi, Kitajima, Abe, & Mishiro, 2004). Distinctly, in artificial work it is indicated that natural killer cells (NK) directly break the nerve cells and cause damage in multiple sclerosis (Bäckström et al., 2003; Backström, Chambers, Kristensson, & Ljunggren, 2000; Haspels, Rahman, Joseph, Gras Navarro, & Chekenya, 2018).

## 3.6 Human leukocyte antigen antibody screening by ELISA

The first assay to use antigenic determinants was enzyme-linked immunosorbent assay (ELISA)-based technique that gathered from blood cells including platelets for "human leukocyte antigens Class I assay" and from LCLs (lymphoblastoid cell lines) for "human leukocyte antigens (HLA Class II)" to object the antibody. Antigens or foreign particles that are soluble tied with compact substance and may be the source of a microtiter plate. The blood serum of patient is incubated with inactivate HLA that aimed to consent the binding of antibodies (Tait & Green, 2010). The antigen-antibody tie complex is identified through "fluorochrome-conjugated antihuman IgG." The test serum reactivity is stabilized contrary to adverse panels as well as the back ground of the target. These kinds of analyses have various remunerations above cellular assay, including no require aggregation or parting of prominent cells, and they also spot balance linkage as well as imbalance linkage antibodies, quicker experiment timer, outcomes are hemiquantifiable and solitary HLA are distinguished. There are binary altered outcomes provided by ELISA that is one is screening assay that determine the occurrence of HLA antibody and another is identification assay which determines specification of antibody. Hence, screening of large number of patients sample in brief period of time is the main ability of ELISA. However, another significance of ELISA identification is the capability to individually identify HLA class II particularity in the presence of "high-panel reactive class I." Additionally, a study revealed that ELISA is more subtle than complement-dependent cytotoxicity, but are less consequently than flow cytometric assays. Glass microchips and microtiter plate wells are used for antibody testing platform. There is a greater sensitivity in microchip system and has a higher number of HLA phenotypes than it could be involved in a usual ELISA microtiter plate. The HLA are spotted on a microchip that fixes in the microtiter tray wells, thus both HLA Class I along with Class II antibody could be examined in a single well.

## 3.7 Flow cytometry and luminex techniques for the screening of human leukocyte antigen antibody

These techniques are dependent on soluble or recombinant HLA molecules that are bound to polystyrene units and are presently believed as primary or suitable standard for antibody assessment and recognition (Zeevi, Girnita, & Duquesnoy, 2006). However, the Luminex analyses utilize globules that are segregated by progressions in their "primary fluorescent dyes." Specific groups of gobbets are treated using HLA or with a phenotype. These gobbets groups are assembled inside a same duct to conduct various testing. The antigen-antibody complex reaction onto the gobbets is perceived along with antiglobulin reagents characterized with fluorochrome. The analysis of test

are depends on products used even through the "conventional flow cytometer" or via "communal Luminex Fluoroanalyzer." There are two uses of Luminex platform: one is laser that senses variances in hundred individual polystyrene units, individually stained with variable amount of two unusual flurochromes, providing a distinctive but reproducible gated position. Every set of beads is then bound to either a mixture of HLA or only HLA. Then, beads are integrated with the serum of patient to allow antibody binding. The second antihuman antibody associated with a correspondent particle is then included to the feedback combination and the correspondent fluorescence dignified using one more laser, providing a semi-quantifiable antibodies concentration in the sample of a patient. Therefore for screening as well as identification, Luminex and flow cytometric assay could be utilized as equally "complement fixing" and "noncomplement fixing" antibodies can be perceived in the methods of "flow cytometric screening." "Luminex assay" can identify individually HLA, class II specificities in the existence of HLA Class I specificities. The use of "single antigen Luminex test" determines the antigen reaction in high panel reactive sample, permitting determination of suitable HLA mismatches and this increases the chance of successful transplantation (Bingaman, Murphey, Palma-Vargas, & Wright, 2008; Heinemann, 2009; Zachary, Lucas, Detrick, & Leffell, 2009). Antibody-facilitated elimination is an active procedure prejudiced via the stability in the middle of the destruction produced as a result of giver antibody, also has the capability of soft tissue to overhaul damage and the efficiency of "immune-suppression therapy." Although, there is still a controversy in the number of HLA-antibodies detected by "Luminex technology" and the "Class I and II specific antibody" contrary to the transplant are related by an initial or durable prolonged elimination. In the overview of gobbets, it contains HLA-DQA1, DQB1, DPB1 molecules that authorized additional consideration in which these antibodies play an active role in the compact tissue replacement. Therefore this one is now probable to customarily monitor patients by an extensive beads section comprising HLA Class II loci, which were problematic to have CDC (The Centers for Disease Control) statistics. The experimental utility of this varied conduction is emphasized by the fact that all HLA Class II specificities can be located in relocated patients and every so often associated in allograft dismissal.

## 3.8 Human leukocyte antigen antibody identification

Patel et al. (2020) documented the deleterious effects of antibodies in the kidney transplant hyper acute rejection. Almost 80% of kidneys were transferred into cross counterpart progressive beneficiaries were nonserviceable as reported by Patel et al. (2020). Contact to foreign HLA through pregnancy, transfusion of blood, and transplantation provokes "human leukocyte antigen-specific antibodies." The harmful function of antibodies in contrast

to HLA was almost immediately documented and in the past few years has been getting superficial with the expansion of subtle new analyses recognizing earlier untraceable antibody specificities and "intragraft C4d deposition" (Terasaki & Cai, 2005). Formerly, the transfer of antibody was carried out with lymphocyte objects in "complement-dependent cytotoxicity assays." Although, such types of assays were nonparticular and less sensitive as compared to other modern practices. The "solid-phase antibody analyses" and purpose of the "flow cytometric technology" have transformed the compassion and accuracy of HLA.

## 3.9 Polymerase chain reaction sequence-based typing

Accuracy is the main importance of sequence-based typing (SBT). The only technique that exactly identifies the base sequences of an allele is "PCR-sequence-based typing (PCR-SBT)," hence enabling accurate assignment (Rozemuller & Tilanus, 2000). PCR-sequence-based typing that is (PCR-SBT) is the present method for accomplishing advance determination of HLA genes and there is method via Sanger which is used in bleach terminator interaction and is furthermost used in automated apparatus for monotonous HLA typing. Sangar developed sequencing of DNA that takings benefit of consuming DdNTP (20, 30-di-deoxynucleotides) as substratum when it is combined at the 30 termination end of the developing sequence and extension of chain is clogged particularly at A, C, G, and T nitrogen bases as the sequence dearth a 30-hydroxyl assembly. There are four dissimilar bleaches that are utilized to recognize the A, C, G, and T nitrogen bases addition feedback and every single bleach releases brightest discrete wavelength when it provoked by laser. Due to random assimilation of considered dNTP, chain elongation stops, and various deoxyribose nucleic acid fragments of different size are produced. These DNA fragments are detached by capillary duct electrophoresis by following PCR sequencing. Additionally, all the four nitrogen bases at the fragment ends could be identified and differentiated by the definite fluorescence released. The generation of nucleotide sequence by sequence analysis software is evaluated with a catalog comprising all the arrangements of well-known identified genes (Voorter et al., 2007). Sequencing for HLA resolutions is inhibited into various magnitudes of gene exhibiting distinctive sites including exon 2 for HLA class II and Exon 2 & 3 for HLA Class I. The sequence-based typing (SBT) is an influential approach for expressing HLA genes and could too classify unknown genes, but it is controlled via obscurities. Meanwhile, both the alleles are intensified as well as classified together, the period of distinctive categorization topics is not recognized and this takes two or more than two allele arrangements as the arrangement ideas could be communal by many HLA alleles (Sharon & Lis, 2004). In such case, more amplification with certain primer with suggests the modification requisite to be implemented. These basal primers are

intended to increase one gene or gene group in an uncertain arrangement (Abbott, Rönnbäck, & Hansson, 2006).

## 3.10 Polymerase chain reaction-sequence specific primers or (PCR-SSP)

An innovative molecular biology procedure was explained to achieve HLA typing as a substitute to serological Dr entering in experimental repetition during early 90s. This method is depending upon the principal of totally complemented primer will be much competently used in a reaction called polymerase chain reaction (PCR) than a primer with one or more disparities. Basis of the procedure are the particularity of primers including a 30 distinct-base mismatch prevents the instructing of a nonparticular reaction. Due to lacking 30−50 exonuclease activity of Taq polymerase, even when the primer couple does not harden nonparticularly, they do not intensify competently. A profitable SSP tray of HLA Class I and II comprises many sets of PCR primers that are intended to galvanize within DNA nucleic acid regions existing in specific genes or sets of genes. However, if the consequent genes exist, the existence of their exact amplicons could be noticed by gel electrophoresis technique. There is a need for interpretation in the size of PCR products and electrophoresis movement need to be run prolonged to detach all PCR (polymerase chain reaction) fragments. There is a pattern to check for polymerase chain reaction restriction or absence of intensification, a distinct nondistinctive switch is comprised in every portion of the tray. Thus evaluation along with molecular magnitude hierarchies approves that applicably sized amplicons were attained. The technique used is best for entering single sections because of an elevated numeral of primer blends in the equipment are desired to concealment of all HLA particularities (Moalic, Mercier, & Ferec, 2004; Ferrer et al., 2005).

## 3.11 Future perspectives of immunogenetics

Bearing in mind the direction of the upcoming future of immunogenetics, (Misra et al., 2018) commended attention upon elevation of genetic constitution for both "*HLA*" and "*KIR*". Examining both translating and nontranslating segments distinction in these immune-genetic loci via in elevation quantity high-determination expertise in assemblages with miscellaneous lineages drive nearly unquestionably is essential to entirely raise the value of their function in neurological disorders. There are only restricted assessments of *HLA* and *KIR* discrepancies on transcriptomics that is complete set of RNA transcripts and proteomics, that is study of protein structures levels; consequently, the purposeful valuation of both allelic and monitoring provincial discrepancy is extremely required. The influence of micro-RNA on dissimilar *HLA* and *KIR* alleles in monitoring segments also desires to be

assessed in neurological disorders in direction to distinguish the consequence of biogenetic aspects in sickness/disorder pathophysiology. To end with, our current surveillance, that assured human metabolites in body inhabits the P4 summarizing of Ms-vulnerable *DRB1*15:01* haplotype in most populaces and possibly will be associated in autoimmunity, (Misra et al., 2018) put forward that analogous inquiries of both *HLA* class I and class II particles in a gene precise fashion could be commenced. This strategy might be beneficial to weightiness or cluster composed different *HLA* genes and genes that are convoluted in susceptible transversely diseases. The practical valuation of obligations of human metabolites with *HLA* class I and class II molecules, and examination of their influence onto T cell propagation and approachability, may possibly lay concrete on the way for scheming innovative treatments, prominent to a step nearer toward the goal of a modified treatment.

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Chapter 4

# Immunogenetics: a tool for anthropological studies

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## 4.1 Introduction

The starting of immunogenetics was reported around 1900 when Karl Landsteiner discovered the ABO blood group by haemagglutination analyses (Marbut, Hamdi, Jumaa, & Salman, 2018; Sukumaran, Padma, Rajani, Vanitha, & Padma, 2015). After this discovery, a lot of studies used immunogenetics to study variations in human genetics, and during the start of the 20th century more development in this field took place by studying the human population for antigens of red blood cells with allozyme using electrophoresis technique (Metgud, Khajuria, & Mamta, 2016). Immunogenetics also helped to reveal individual differences in immunoglobulins (Paridar et al., 2016), HLA (human leukocyte antigen) molecules with a high rate of polymorphism at their peptide bond region (Middleton & Gonzelez, 2010; Sanchez-Mazas, Lemaître, & Currat, 2012). Using this technique, researchers also discovered complex polymorphism in KIR (killer cell immunoglobulin like receptor), which show variation in both a number of genes and alleles (Hsu, Chida, Geraghty, & Dupont, 2002; Marsh et al., 2003). A lot of diseases like seasonal influenza and colds due to viruses, each year a large number of people get infected due to the high turnover rate of viruses. The reason for high level of polymorphism in human is due to their exposer to diverse infectious agents and specific environmental pressure (Fumagalli et al., 2011). Variation in HLA allele was found to be maintained by heterozygotic advantage, due to which it is associated with resistance to many deadly diseases. Some of the HLA heterozygote associated with diseases

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00008-7**
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resistance are HLA-B-27 (Cauli et al., 2013), HLA-B 51 (Gul & Ohno, 2012), HLA-B 57 (Bailey, Williams, Siliciano, & Blankson, 2006).

The use of population genetics to study polymorphisms showed the genetic diversity around the world display geographical patter of structure (Lao, van Duijn, Kersbergen, de Knijff, & Kayser, 2006). The studies until now show discrimination in populations of different continents, one of these controversies is the position of African; as a separate occidental group from Europeans (Mesa et al., 2000), as oriental groups from Asian and Amerindian (Tishkoff & Verrelli, 2003), and as an oceanian population from other countries (Watkins et al., 2003). Many studies concluded that the reason for evolution of polymorphism in genetic diversity patterns is also affected by patterns of human immigration and recommends using immunogenetics as a tool to rebuild human inhibiting history (Uitterlinden, Fang, van Meurs, Pols, & van Leeuwen, 2004).

## 4.2 Human genetic diversity

The study of human genetic diversity is always a topic of interest as it has great importance to understand human evolution and distribution of genetic disorders in a population (Dunning et al., 1999). In comparison to other species of plants and animals diversity in the human population is low (Gao, Starmer, & Martin, 2008). As per a study based on protein polymorphism showed between group diversity to be 15% (Eloranta et al., 2011), whereas another study based blood groups, protein polymorphism (Ketto et al., 2017), mitochondrial survey, and Y- chromosome (Mamuris, Moutou, Stamatis, Sarafidou, & Suchentrunk, 2010) showed that the diversity of the human population is more within than between population (Al-Zahery et al., 2011). The biggest study of human genome known as the "human genome project" started in 1990 and was completed in 2003 (McElheny, 2012; Wilson & Nicholls, 2015). The human genome consists of six billion DNA nucleotides as set of 22 autosomes and 1 pair of sex chromosome (Mali et al., 2013). The high rate of polymorphism in human DNA is due to large size of genome (Howe et al., 2013).

The use of genetic marker (GM) in immunogenetic system was first discovered in human serum agglutination (Aiello et al., 2019) by Grupp and defined this system by serological heavy chain immunoglobulins like IgG1, IgG2, andIgG3 (Aiello et al., 2019; Oxelius & Pandey, 2013). By 1950 around 15 different GM allotypes were identified and reported as IgG1: G1M1-G1M3 and 17 (Simon et al., 2016), IgG2: G2M 23 (Simon et al., 2016), IgG3: G3M5,6,10, 11, 13, 14, 15, 16, 21, AND 24 (Butler et al., 2011). Many studies found the allotypes associated with exact exchanges at the DNA level, whereas other studies only partly associate them (Calonga-Solís et al., 2019). Studies suggest that the frequencies of some GM haplotypes are highest in most of the border areas of each country including

Northwest Europe (Kusza et al., 2014), Southeast part of Asia (Bhatti et al., 2017), and sub-Saharan African areas (Secher et al., 2014), whereas the frequency in central regions like East Africa, were found medium. These observations support centrifugal movement of human population (Hawley, 1972; Lee & McCracken, 2012).

Studies on African regional level GM polymorphism states that using linguistics predictor is most suitable method to study comparison of population GM to geographical haplotype variation and its more in population whose language belongs to different etymological of the continent (Weiner et al., 2010). The different linguistic phyla of African population are Nilo- Saharan (NS) (de Filippo et al., 2011), Khoisan (KH) (Hewlett, DeSilvestri, & Guglielmino, 2002), Niger-Congo (Blench, 2017), and Afro- Asiatic (AA) (Fan et al., 2019). With the expansion of one language speaking population the polymorphism of GM also increases as in case of NC- speaking African population (Sands, 2009), the demographic expansion occurred around 3000 years ago when NC pushed KH population from northeast to southern part of Africa. The comparison on GMs shows that the KH retain their ancestral genetic profile and moderate frequency for a single haplotype GM1; 17 and 21, which are rarely found in Sub-Saharan population but common in East African people (Sands, 2009).

GM variations are also a mean to study anthropology of East Asian countries. A high frequency of GM 1, 2, 17 and 21 are found in north-south region (Blench, Sagart, & Sanchez-Mazas, 2005). A continuous differentiation in genetic frequency is observed in population of Tibet to Burma, from Japan to Korea, from north China to South China, between Austro and Asiatic (Chen, 2006).

## 4.3 HLA and KIR polymorphism

Natural Killer (NK) cells are well defined as innate immune cells (Bernareggi, Pouyanfard, & Kaufman, 2019; Sun & Lanier, 2009), they are adaptive lymphocytes having the power to destroy tumor cells and/or infected cells (Gao et al., 2017; Prestwich et al., 2008; Quigley & Kristensen, 2015).

NK cells are the main initiator of the innate immunity through their sophisticated multifunction, they are able to recognize infected cells via pattern recognition receptors and they can interact with infectious particles (Habif, Crinier, André, Vivier, & Narni-Mancinelli, 2019; Prestwich et al., 2008; Vivier et al., 2011), define the infected cells through expressed cell surface receptors (O'Shea & Hogan, 2019; Storset et al., 2004), interact with the adaptive immune system directly and via producing cytokines (Tailleux et al., 2005; Yeaman et al., 2004). Such cytokines including either of immune-regulatory influence in some cases (Bertzbach, van Haarlem, Härtle, Kaufer, & Jansen, 2019; Shida, Nanno, & Nagata, 2011), or have

predominant pro-inflammatory activity such as interferon—Gamma (IFN-γ) and Tissue Growth Factor—Beta (TGF-β) in other cases (Fabregat & Caballero-Díaz, 2018; Taube et al., 2015).

This power of NK cells is regulated by the expression of HLA (Gardiner, 2008; Laird et al., 2017), where HLA—class I molecules are regulated by polymorphic KIRs (Parham, 2005; Saunders et al., 2016; Wan et al., 2017). Thus NK cell activation is this complex that is expressing the balance between inhibitory and activating receptors (Averdam et al., 2009). Depending on the net signal derived from KIRs (Haspels, Rahman, Joseph, Gras Navarro, & Chekenya, 2018; Yawata et al., 2006), NK cell effector function can be initiated and whether or not their activation or inhibitory activity can be defined (Bernson et al., 2017; Boyington & Sun, 2002) (Fig. 4.1).

There are thirteen (13) distinct KIR genes located on chromosomal region 19q13.4 (Deng et al., 2020; Middleton & Gonzelez, 2010; Single et al., 2007), these genes can be combined in more than one pattern, creating high level of diversity, this high diversity of KIRs and their alleles is strongly associated with the influence of HLA and many diseases, either positively or negatively (Lu et al., 2008; Singh, Rajak, Kadam, & Rajadhyaksha, 2019; Wang et al., 2012).

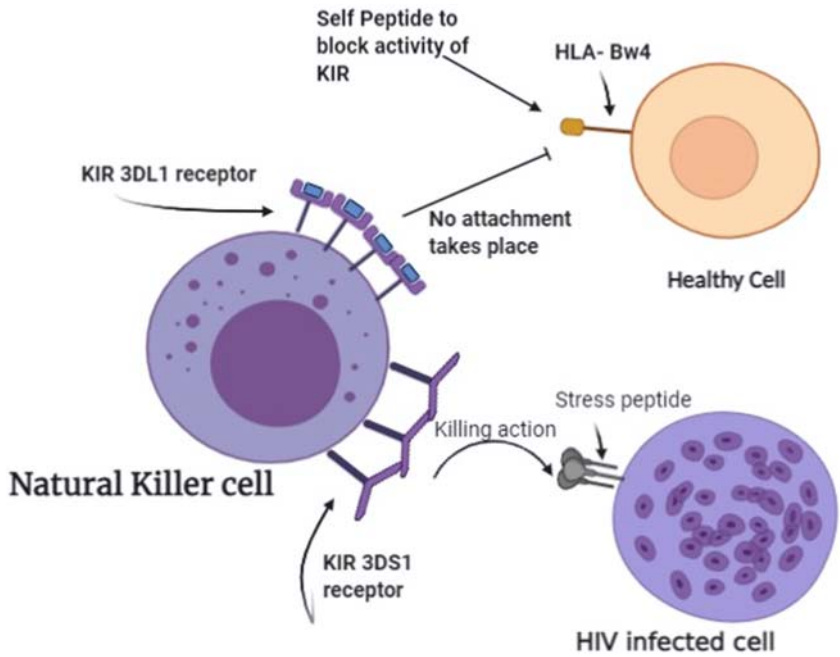


**FIGURE 4.1** Association between HLA, KIRs and NK for killing infected cells (HIV cells).

HLA class I molecules' main function begins inside the cell (Muhlethaler-Mottet, Otten, Steimle, & Mach, 1997; Oystreck, 2019), where it binds to a peptide; usually a nonamer (Emami, 2018; Parham & Ohta, 1996); carrying this peptide to the cell surface thus this complex of HLA—peptide is then conjugated to KIRs (Borrego et al., 2002; Naiyer et al., 2017) and other lymphocytes (Kärre, 2002; Spies & DeMars, 1991) as the complex legend. This can be seen in the case of healthy cells, protein do not stimulate any immune response (Cuadros, Lopez-Hernandez, Dominguez, McClelland, & Lustgarten, 2004), while in the case of abnormal cells, infected cells or transformed tumor cells surfaces (Perez et al., 1990; Wilson et al., 2019); pathogen specific or tumor specific peptide binds to HLA I molecule and as a result (Ma et al., 2019; Raz, Meromsky, & Lotan, 1986), some changes will be induced leading to stimulation of lymphocytes and immune response will be initiated (Lyford-Pike et al., 2013; Ma et al., 2019). Such an effective reaction between HLA class I molecule and KIRs takes place rapidly under the effect of wide diversity of tumors (Adler, Glorioso, Cossman, & Levine, 1978; Daly, Carlsten, & O'Dwyer, 2019) and microorganisms (Donatelli et al., 2014). Beside the diversity of the immune response depending on the nature of the complex of HLA—KIRs and their legend; the response is also varies within each individual, more than this, the diversity is an individual's diversity (Fan et al., 2012) (i.e., differs from person to person) and this individual variation leads to variety in the pattern of epidemiology and spread of infection within the same family and between families in the same community (Stoletov et al., 2010).

Although both KIRs and HLA class I families are important to induce the immune response against certain pathogens (Ashiru et al., 2010); KIRs are more likely to be more responsible for creating the diversity of this immune response within human populations (Koch, Steinle, Watzl, & Mandelboim, 2013). This prominent diversity feature of KIRs is originated from their fast and continuous evolution (Jamil & Khakoo, 2011) and their scarcity of conservation between species (Pietra, Romagnani, Manzini, Moretta, & Mingari, 2010), which cannot be referred just only to the divergence in HLA—I molecules (Iwasaki & Medzhitov, 2010). Many studies disclosed the influence of the interaction between HLA class I and KIR genes on disease progression (Augusto & Petzl-Erler, 2015) (Table 4.1).

Function and activity of NK cells are controlled by KIRs (Augusto & Petzl-Erler, 2015), where there are 14 different KIR (Leung, 2011) controlling the activity of NK either activating (3DS1, 2DS1−5) (Björkström et al., 2010) or inhibiting (3DL1−3, 2DL1−3, 2DL5) (Della Chiesa, Sivori, Carlomagno, Moretta, & Moretta, 2015) or regulating their function via both activation and inhibition via 2DL4 (Benson Jr et al., 2011). The interaction between HLA—Bw4 allele and KIR 3DS1 can lead delay the progression of immune status of AIDS patients (Chagoury, 2009), while in the case of

**TABLE 4.1** Disease prognosis as a reflection of different HLA—KIRs complexes.

| Disease | KIR | HLA | Disease progression | Proposed contribution by KIRs | Population/Country | References |
|---|---|---|---|---|---|---|
| Cervical neoplasia | 3DS1 | HLA-C1 homozygous | Increased | Less inhibitory effect | Eastern United States Costa Rica Portland | |
| | KIR other than 3DS1 | HLA-C2 and/or HLA-Bw4 | Decreased | More inhibition | | |
| Acquired Immunodeficiency Syndrome | 3DS1 | HLA-Bw4 $^{(Ile80)}$ | Decreased | Less inhibitory effect | Undefined | |
| | 3DS1homozygous | Other than HLA-Bw4 $^{(Ile80)}$ | Increased | More inhibitory effect | | |
| Hepatitis C | 2DL3homozygous | HLA-C1 homozygous | Decreased | Less inhibitory effect | Caucasians African Americans | Rajagopalan and Long (2005) |
| Malignant melanoma | 2DL2 and/or 2DL3 | HLA-C1 | Increased | More inhibitory effect | Bulgarian | |
| Diabetes (Type 1) | 2DS2 | HLA-C1 | Increased | Less inhibitory effect | Type I Diabetic Cohort/ Netherlands | |

infection with Hepatitis C Virus (HCV) (Raimondo et al., 2006); interaction between HLA—C1 and KIR2DL3 leads to complete clearance of the infection (Raimondo et al., 2006). Thus depending on the complex of HLA and KIRs, either stimulation or inhibition of NK activity will be initiated (Antonelli et al., 2006). Similar to the influence of HLA—KIR genes and their alleles on NK in case of infectious diseases; conjugation of HLA and KIR different genotypes have been associated with host susceptibilities to many autoimmune diseases (Jain et al., 2013).

The types of KIRs and their number vary ultimately between haplotypes, there are near 30 distinguished KIR haplotype with discrete gene content (Rajalingam et al., 2008). KIR haplotypes are classified basically into two groups; group A haplotypes which include certain gene content encompassing: KIR3DL3−2DL3−2DP1−2DL1−3DP1−2DL4−3DL1−2DS4−3DL2 (Carrington & Norman, 2003; Hollenbach, Nocedal, Ladner, Single, & Trachtenberg, 2012; Solgi et al., 2011), while group B haplotypes include variable content of genes and alleles, some of them are not even on the A haplotype. Consequently, B haplotypes generally encode the most activating KIR compared to A haplotype that encodes a single activating receptor which is KIR2DS4 (Hsu, Liu, et al., 2002; Jiang et al., 2012).

Many researches revealed the impact of some KIR—HLA complexes on the NK cells activities (Carrington, Martin, & van Bergen, 2008; Jamil & Khakoo, 2011), whereas, certain complexes having the strongest inhibitory effect on NK cells and subsequently (Rölle, Meyer, Calderazzo, Jäger, & Momburg, 2018), other complexes that have less inhibitory effect lead to higher activation of NK cells and higher protection against viral infections (Rölle et al., 2018) or greater sensitivity in the case of autoimmune diseases (Körner et al., 2017).

Furthermore, some studies revealed that certain KIRs, KIR 2DLs require certain levels of $Zn^{2+}$ to achieve their inhibitory effect. Thus it is possible to refer the strength and weakness of inhibitory effect of KIR2DLs to the presence or absence of $Zn^{2+}$ and its concentrations (Epling-Burnette, Wei, & Djeu, 2010). HLA molecule is a peptide binding molecule that is encoded by certain genes located in chromosome 6 (Rajagopalan & Long, 2005). There are two classes of HLA, HLA—I and HLA—II (Epling-Burnette et al., 2010). HLA class I is composed of alpha heavy chain which binds to β chain ($\beta_2$m) that is encoded by a gene located on chromosome 15.

The classical HLA—I molecules include A, B, and C series; those are expressed in all nucleated cells (Bromley et al., 2001; Cassidy, 2014). These chains can bind to long fragments (8−10 amino acids) of $CD8^+$ T lymphocytes to form HLA—Peptide complex (Ding et al., 1998), whilst the nonclassical HLA—I molecules involve E, F, and G series, those are mainly responsible for binding with NK cells and express their function as ligands of these cells and they are showing less polymorphism in comparison to the classical HLA class I chains (Huan, Zhuo, Xiao, & Ren, 2019).

HLA class II molecule is composed from two heavy chains; Alpha (α) and Beta (β) chains, both of them include extracellular domains (Trolle et al., 2016), the peptide binding cleft is composed of their Alpha 1 (α1) (Remesh et al., 2017) and Beta 1 (β1) domains (Vigneron, Ferrari, Stroobant, Abi Habib, & Van den Eynde, 2017), these (α) and (β) chain loci encoding Dr, DQ and DP chains series of HLA class II molecule in HLA complex (Chan et al., 2018). Dr α chain is considered monomorphic, while Dr β, DQα, DQβ, DPα and DPβ chains show a high level of polymorphism (Li, Batliwala, & Bouvier, 2019) and in contrary to HLA class I, HLA class II molecules are expressed in Antigen Presenting Cells (APC) (Cheloha et al., 2019), as dendritic cells, through binding to longer peptide fragments ranged from 8−15 amino acids originating from endocytes exogenous protein and carry them to the cellular membrane (DeWitt et al., 2018). Beside, instead of $CD8^+$T cells that bind to HLA class—I; $CD4^+$T cells bind to HLA class—II (Antunes et al., 2017).

The high level of polymorphism in HLA class I loci can be observed mainly in exon 2 and 3, while in loci of class I (Grimholt et al., 2003), polymorphism can be detected in exon 2 only. These exons correspond to the Peptide Binding Region (PBR) of HLA molecules, and by analyzing of HLA molecules and their amino acids, the allelic variation is referred mainly to the substitution in the residues contributing to PBR, exactly in certain pockets of PBR, where the side chains of the peptide can bind to the molecule (dos Santos Francisco et al., 2015). Therefore different molecules of HLA bind with different sets of peptides and as a consequences, the diversity of HLA alleles is to a broad extent becomes functional (Sarri et al., 2018). This high diversity in PBRs and HLA molecules is required to bind a corresponding high variety of the pathogen—derived peptides ending with their presenting to the receptors of T—cells (Sarri et al., 2018).

## 4.4 Gene frequency analysis

To study HLA genetic diversity within population needs to consider population from medical point of view as the study is a comparison between patient and healthy population (Mulindwa et al., 2020; Prugnolle et al., 2005; Singh, Kaul, Kaul, & Khan, 2007). Here we will discuss some statistical methods used for disease associated studies.

The identification of HLA genotype is difficult to be defined even with complex (Angel-Pablo et al., 2020; Solberg et al., 2008) DNA techniques due to hidden or blank alleles. If we identify a unique allele then other are homozygous alleles with unknown nature, for this reason we should always look for HLA phenotype not the genotype (Boquett, Bisso-Machado, Zagonel-Oliveira, Schüler-Faccini, & Fagundes, 2020; Denis et al., 2005). To measure the allele frequency in ABO blood group Bernstein in 1924 derived an expression known as Bernstein formula for estimation of

frequency in the presence of recessive allele (Alferink et al., 2019; Farh et al., 2015; Sun et al., 2016). To study high probable frequency a more efficient method is developed which is based on maximum likelihood and expectation maximization (Moravvej et al., 2018; Yousefi & Behroozifar, 2010). The basic principle behind this technique is the iteration process, which stops when the difference between in iterations is less than the threshold (Fattahi et al., 2019; Jarvis, Bernstein, & Jain, 2004).

### 4.4.1 Estimation using linkage disequilibrium

To estimate individual haplotype level due to nonrandom connotation of alleles at two different loci, is used (Habel et al., 2020; Hill & Robertson, 1968; Loh et al., 2013; Vilhjálmsson et al., 2015). The equation used for linkage disequilibrium coefficient is:

$$D = Pij - Pi \cdot Pj$$

$D$ = Standardized coefficient

It depends on allele frequency and does not consider 0 and 1 as low and high values (Bentley, Callier, & Rotimi, 2020; Berisa & Pickrell, 2016; Qanbari et al., 2010). For this reason this standardized coefficient can neither be comparable amongst different or identical haplotypes.

The null hypothesis $D = 0$ for standardized coefficient can be used for statistical significance. This disequilibrium linkage is important as during the immunogenetic study sometime a very frequently studied haplotypes could be of no significance in particular with corresponding alleles like DRA1 and DSA1 or DTA1 loci (Ovsyannikova, Haralambieva, Crooke, Poland, & Kennedy, 2020; Waples & Do, 2010).

To measure $D$ significance, chi-square test may be used with Yate's correction; however it is necessary to keep a clear idea in for concluding the result, but an alternative method to measure nonrandom relation between alleles of 2 loci is by applying Fisher's test on $2^2$contingency table (Hohenlohe, Bassham, Currey, & Cresko, 2012; Sundaresh et al., 2018). Linkage disequilibrium is a very powerful tool to be applied on low-frequency classes. It can estimate the coexistence of two allelic antigens in the sample that is higher than the expected (Druet & Georges, 2010; Tang et al., 2020). During the test a significant result does not indicate the existence of two alleles on the same chromosome (Aranzana, Abbassi, Howad, & Arús, 2010; López-Cobo et al., 2018).

## 4.5 To Test disease associations

The association between HLA and disease can be measured using the same approach as used to study association between HLA and allele (de Almeida et al., 2018). The method which is best applicable in this situation is to apply

$2^2$contingency table between population carrying alleles in comparison to people without alleles (Berntsen et al., 2015; Silva, 2019). To verify the association between a locus and a disease, many alleles are tested together followed by Bonferroni's correction to be performed for multiple tests (Ahmed et al., 2012; Card, Thomas, Graves, Barrick, & Lenski, 2020).

A frequent task is to check whether two (or more) particular populations differ genetically from each other. As mentioned in the introduction, these populations may be of different origin or may represent patients and controls for a given disease (Dendrou, Petersen, Rossjohn, & Fugger, 2018). While being so common, this task is yet not so easily carried out with available HLA data. Indeed, one should compare phenotypic or genotypic distributions between populations. Comparing populations using allelic frequencies can only be done when Hardy-Weinberg equilibrium holds (Puig, Ginebra, & Graffelman, 2017; Zhu et al., 2015). In both cases, goodness-of-fit tests can be applied by taking into account the sizes of the samples to work on absolute frequencies. The usual problem related to low expected counts still persists (Chen et al., 2015; Hao & Storey, 2019).

## 4.6 Conclusion

The studies until now that used GMs, KIR and HLA as tools to study diversity in human population and to gather knowledge can also be used to study disease appearance and spread around the world. The study of mt DNA and Y chromosome markers are found to be very useful to reconstruct gender specific phylogenies of human genome. SNPs and DNA sequencing techniques and many other genome based applications are helping to reveal genetic variation and polymorphism in human population around the world. Thus immunogenetic is an important tool in anthropological studies with some limitations which is used to reconstruct peopling history of human population and the effect of these factors on human genetic diversity.

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Chapter 5

# Immunogenetic surveillance to histocompatibility

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## 5.1 Introduction

Scientifically speaking, the human Major Histocompatibility Complex (MHC) was identified half a century ago (Dausset, 1981) and first recognized along with its function in implantation via histocompatibility antigens, which gave rise to the term "human leukocyteantigens" (HLA) (Snell, 1968; Dausset, Le Brun, & Sasportes, 1972). A few years of intensive study have represented the impressive genomic atmosphere of the MHC as well as how genetic variation inside this area influences sensitivity to autoimmune, infectious, as well as other diseases. The MHC exhibits high amounts of gene intensity as well as polymorphism. The genetic variation has proven to be significant in terms over its composition, coinheritance, and practical implications. Overall, impressive discoveries were made, and even this genomic region will have emerged as a model for human genomics (Fernando et al., 2008; Lie & Thorsby, 2005; Ryder, Svejgaard, & Dausset, 1981; Trowsdale, 2005). Each genes of the MHC embed proteins that play an important role in immune response.

The MHC is a different biological structure that is approximately 4 Mb in length and is positioned within short-arm of chromosome number 6. HLA loci, which become code primarily for antigens present on the nucleated cell surfaces and has vital part in transplantation connectivity (Krausa & Browning, 1996). The HLA particles regulate an immune reaction by recognizing "self" and "non-self." They are members of an Immunoglobulin Supergene Family, that also contains immune-globulins, as well as CD4 and

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00010-5**
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CD8. The antigen is presented to T-lymphocytes by HLA molecules, which then initiates a particular immune reaction (Tiercy, 2002).

The HLA process was discovered in the early 1950s, since leukoagglutinating antibodies was discovered in the sera of polytransfused patient populations and primiparous females (Dausset, 1958). Overview of reactive structures of such sera revealed where each serum identified only cells from specific individuals, without an autologous responses reported. In 1958 Jean Dausset identified the first HLA antigen, HLA-A2, which has now been identified as HLA-A2 (Dausset, 1958). Within following years, many total HLA antigens raised, as well as the sharing of sera across various laboratories enhanced the discovery of new compounds (Payne, Trip, Weigle, Bodmer, & Bodmer, 1964; Van Rood & Van Leeuwen, 1963).

The first International Histocompatibility Workshop (IHWS) were held in 1964 to facilitate cooperation, the distribution of well-characterized sera, analytical comparisons, and the distribution of observational results (Terasaki & McClelland, 1964). Significant information collected from this conference formed the design of the study and resulted to the inclusion of histocompatibility evaluations in clinical transplantation, particularly after initial investigations revealed that the HLA structure adversely effected kidney and skin compatibility (Patel & Terasaki, 1969). The discovery of new loci in the HLA system resulted in the formation of a Taxonomy Review panel to standardize a identities of recognized HLA antigens and to collaborate the mentioning of new designs. Advancements in science, the concept of Class I and II HLA antigens, and, most recently, the implementation and standardization of analytical tools that revealed the HLA system's strong polymorphism, have all contributed to the development of data about the HLA system (Charron, 2003). The DNA-based methodology seems to have huge benefits over the serological technique. Since these, proofreading materials focused particularly on sequencing of nucleotides. This is much more versatile since current substances can be developed as latest alleles are revealed, and studies are being performed at various points of the process based on a proofreading recommendation. It is also more stable even though it does not require specialized type of cell or effectiveness, which is less influenced by the patient's condition (Cano et al., 2007; Erlich, Opelz, & Hansen, 2001). Here as conclusion, the current study describes particular MHC-related conditions, the MHC's genomic environment, but many of the obstacles, most of which represent an outstanding challenge in understanding human genetic variation that holds the MHC in at frontline of human population genetics.

## 5.2 Major Histocompatibility Complex genomics and human disease

New kinds of genomic studies of the species MHC are already remarkably helpful in identifying many disease connections across several generations.

This really is understandable given that the MHC location is just about the majority gene-dense and polymorphic peaks in the DNA of individual (Trowsdale & Knight, 2013). It really encodes proteins, which are relevant for immune response, along with many that regulate antigen production and discussion. SNP genotyping and HLA imputation just allow for the analysis of potential data set, which is aided by among rising sequencing (Pröll, Fischer, Michelitsch, Danzer, & Niklas, 2017).

All such techniques have the potential to provide more accurate data of MHC variable contributors to disease. Consequently, interpreting MHC-disease correlations in view of variable forms has proven difficult. Importantly, the MHC is linked to circumstances apart from infections and autoimmunity, including some cancers and neuropathies (Kwok, Mentzer, & Knight, 2020; van Hateren, Bailey, & Elliott, 2017; Wieczorek et al., 2017). The MHC has been investigated for over 60 years, but also its historical backdrop is well reviewed (Klein, 1986). Serological typing has shown links among the MHC and numerous significant immune phenotypes even earlier the clones of group I and group II genes. Until 1980, once DNA cloning and MHC class I structure were discovered, and molecular existence of the different phenotypes mapping in a MHC was unclear (Janeway, Travers, Walport, & Shlomchik, 2001). The idea of T cells recognizing peptides in grooves got my attention. It introduced a series of results that clarified a previously complex and perplexing region from mixed lymphocyte responses to suppressor T cells. A limited number of class I and class II receptors are involved in a variety of MHC phenotypes (Trowsdaleand Knight, 2013). The MHC area of the genome is linked to more illnesses (primarily autoimmune and infectious) than some other region of a gene (Powis et al., 1992).

Some correlations were first discovered using HLA typing for the class I as well as class II (McDevitt, 2002). Because of the large amount of linkage disequilibrium, whether class I and class II is important to diagnose illness correlation in certain cases, while quantifying causative genes was more complicated. For example, mutation in the class I related gene HFE cause hemochromatosis, a recessive iron—mediated dysfunction. Such a disorder was initially linked to HLA-A*03, prompting a few institutes to begin sequencing patients with HLA-A. Detailed cosmid analysis later revealed the HFE genes associated to class I and map to an area on the MHC, which is several mega basestelomeric. Diseases are often generally linked to multiple alleles of class I and II loci, through some MHC loci having less effect in certain circumstances (Horton et al., 2008). Humans must be in a prime place to elucidate the context underlying MHC-associated disease with this knowledge and current molecular methods derived via genomics and immunology. The challenge is that good mapping correlations in this area and establishing structural coding or regulatory features is already a significant challenge just at genetic locus.

Moreover, since MHC class II molecules and I be conceptually concerned in several distinct immune cells activities, identifying the process at which disease-associated allelic molecules function has taken years (Trowsdale & Knight, 2013). The popular diseases related deviation in MHC be due to lower impact of certain alleles, or natural variation. There are a few humans that lack functioning class I and class II molecules but are immune-compromised, as in pure lymphocyte syndrome types I and II (Steimle, Otten, Zufferey, & Mach, 1993). There seems to be a lack of awareness about what influences MHC polymorphism; which specific alterations among the sea of variability are biologically relevant, purely in term of resolving particular amino acid alterations related to diseases; and whether the diseases method is in molecular scale. This is humbling to consider that, later so many years, just the second of such things has progressed; but even then, the advancement has appeared to be improvement rather than additional perspective. In type 1 diabetes, the main publications on amino acid role 57 in HLA-DQB reported 25 years ago (Todd, Bell, & McDevitt, 1987). Distinct variations in groove receptors were found to be the key genetic leads to autoimmune disorders. Simultaneously period, the process for storing peptides onto MHC molecules was discovered (Karttunen, Trowsdale, & Lehner, 1999), with features like TAP encoded inside the MHC. Despite their significance, certain studies have not resulted in a better explanation of how peptides in MHC grooves cause organ-specific disorder but drive the previously unknown polymorphism (McMichael & Jones, 2010).

## 5.3 The Major Histocompatibility Complex locus and genetic susceptibility to autoimmune and infectious diseases

The MHC locus, also claims that human leukocyte antigen (HLA) locus, is located also on short arm of chromosome 6 and spans approximately 4 Mbp (6p21.3) (Fig. 5.1). Antigen presentation, inflammatory modulation, the complement mechanism, and adaptive and innate immunity defenses all include compounds from this area, suggesting the MHC's importance in immune-mediated, allergic, and autoimmune conditions (Shiina, Hosomichi, Inoko, & Kulski, 2009). Polymorphisms in the MHC locus were shown to affect a variety of therapeutic factors as well as an organism's vulnerability to variable, autoimmune, and autoimmune diseases across the last fifty years. In relation to immune-mediated inflammatory illnesses, the MHC played a critical part in certain neurological conditions, suggesting that autoimmune elements can play a key role in such illnesses (Hamza et al., 2010; Purcell et al., 2009; Song et al., 2016).

MHC single nucleotide polymorphisms (SNPs), alleles, and amino acids challenging. Consequently, the latest generation of detailed genotyping tools, like the custom-built IlluminaInfinium SNP chip (Immunochip), and MHC comparative frames has benefited in the fine mapping of the locus,

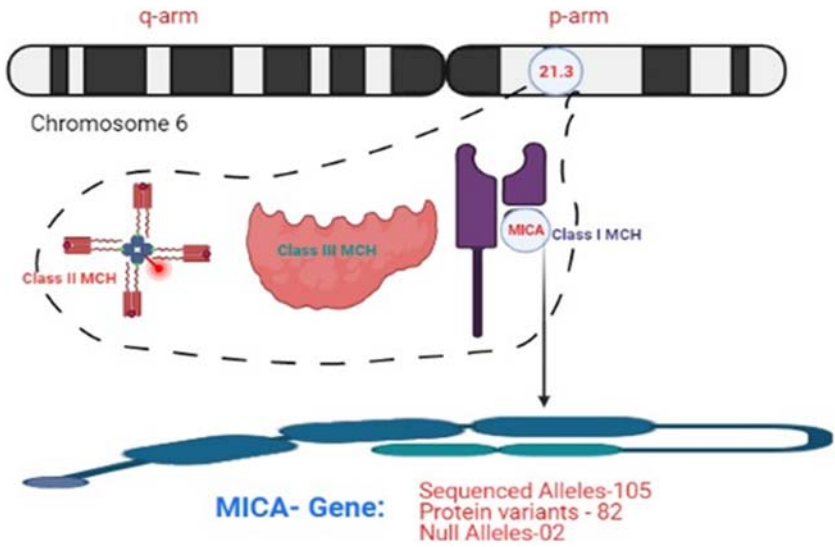


**FIGURE 5.1** Location of the MICA gene on the short arm of chromosome 6, centromeric to HLA-B locus. *Adapted and modified from Baranwal, A. K., & Mehra, N. K. (2017). Major histocompatibility complex class I chain-related A (MICA) molecules: Relevance in solid organ transplantation.* Frontiers in Immunology, 8*, 182.*

enhancing our understanding of the disease interactions and our ability to differentiate functional variants (Cortes & Brown, 2011).

## 5.4 Role of Major Histocompatibility Complex variants in human diseases

Affiliations in between MHC and autoimmune conditions reviewed in the 1970s are amongst the earliest explained genetic linkage (Mulder, 1974), and they continue to be the most powerful health issues for autoimmune disorders. MHC tabulation and fine mapping was studied in European and Asian communities after the introduction of broad genotyping platforms and imputation pathways for the most specific autoimmune disorders, including RA (Kim et al., 2016; Okada, Han, et al., 2014), CeD (Gutierrez-Achury et al., 2015), psoriasis (Okada, Kim, et al., 2014), ankylosing spondylitis (AS) (Cortes et al., 2015), systemic lupus erythematosus (SLE) (Kim et al., 2014), T1D (Howson, Walker, Clayton, Todd, & Diabetes Genetics Consortium, 2009), multiple sclerosis (Ms) (Hu et al., 2015), Graves' disease (Okada et al., 2015), inflammatory bowel disease (IBD) (Goyette et al., 2015), and dermatomyositis (DM) (Zhang et al., 2016) (Table 5.1).

The strongest correlation with the HLA-DRB1 of class II genotype were reported through MHC fine-mapping survey in people of European ancestry

**TABLE 5.1 Association studies showing possibilities of using human leukocyte antigen (HLA) genotyping to predict predisposition to diseases (Kuznetsov, Voronina, Govorun, & Arapidi, 2020).**

| Disease | Associated Alleles of the HLA I and II Genes | Correlation with disease | References |
|---|---|---|---|
| Parkinson's disease | HLA-B*07:02, HLA-C*07:02, HLA-DRB5*01, HLA-DRB1*15:01, HLA-DQA1*01:02, HLA-DQB1*06:02 | Positively correlated | Sulzer et al. (2017) |
| | HLA-C*03:04, HLA-DRB1*04:04, HLA-DQA1*03:01 | Negatively correlated | |
| Birdshot chorioretinopathy | HLA-A*29:02 (>95% of cases carry the HLA-A*29 allele) | – | Kuiper et al. (2014) |
| Systemic sclerosis | HLA-DRB1*15:02, HLA-DRB1*16:02 | Major Systemic sclerosis risk allele subtypes | He et al. (2014) |
| | HLA-DRB1*01:01, HLA-DRB1*04:06 | Strong systemic sclerosis -protective | |
| Psoriasis | HLA-B*08, HLA-C*06:02, HLA-B*27, HLA-B*38, HLA-B*39 | Positively correlated | FitzGerald, Haroon, Giles, and Winchester (2015), Wiśniewski, Matusiak, Szczerkowska-Dobosz, Nowak, and Kuśnierczyk (2018) |
| SARS | HLA-B*46:01 | Positively correlated | Lin et al. (2003) |
| Allergic rhinitis | HLA-B*27 | Positively correlated | Waage et al. (2018) |
| Lung cancer | HLA-B*08:01, HLA-DQB1*06 | Positively correlated with lung cancer risk for Europeans | Ferreiro-Iglesias et al. (2018) |
| | HLA-DQB1*0401, HLA-DRB1*0701 | Positively correlated with lung cancer risk for Asians | |

(*Continued*)

**TABLE 5.1** (Continued)

| Disease | Associated Alleles of the HLA I and II Genes | Correlation with disease | References |
|---|---|---|---|
| Ankylosing spondylitis | HLA-B*27 | Positively correlated | Brewerton et al. (1973), Cauli et al. (2002) |
| Behçet's disease | HLA-B*51 | Positively correlated | Ohno et al. (1982), Wallace (2014) |
| Tuberculosis | HLA-DQA1 | Positively correlated | Sveinbjornsson et al. (2016) |
| Crohn's disease | HLA-C*01 | Significant associations with Crohn's Disease | Jung et al. (2016) |
| Type-1 diabetes | HLA-B*39:06 | Positively correlated | Howson, et al. (2009), Noble et al. (2010), van Lummel et al. (2019) |
| | HLA-B*38 | Protective for Type-1 diabetes | |
| | Heterozygous HLA-DQ2/8 (DQA1*05:01-DQB1*02:01/ DQA1*03:01-DQB1*03:02) | Highest risk in Type-1 diabetes Heterozygous | |
| | HLA-DQ6/8 (DQA1*02:01-DQB1*06:02/ DQA1*03:01-DQB1*03:02) | Protective against Type-1 diabetes | |
| Immunoglobulin A deficiency | HLA-DQB1*02, HLA-DRB1*03 and HLA-DRB1*07 | Strong immunoglobulin A deficiency risk factors | Ferreira et al. (2012) |
| | HLA-DRB1*15 | Protection from Immunoglobulin A deficiency | |
| Autoimmune polyglandular syndrome type 2 | HLA-DRB1*15 | Protection from Immunoglobulin A deficiency | Weinstock et al. (2011) |

with RA (Raychaudhuri et al., 2012). Earlier, a series of amino acid sequences at positions 70−74 in the HLA-DRB1 gene, were designated "shared epitope" locus, and are implicated in the pathogenesis of RA (Gregersen, Silver, & Winchester, 1987). The two amino acids at location 11, positioned in a peptide-binding groove of the HLA-Dr heterodimer shows more solid relationship. It mentioned that amino acid had a vital function in attaching the RA-triggering antigen. Some autoimmune disorders were subjected to equivalent fine-mapping research (Table 5.2). In overall, fine mapping documented the key linked locus of specific MHC locus in certain autoimmune ailments revealed through serotype evaluation. These techniques have already revealed a number of precise variants or amino acids, and autonomous derivatives in various HLA classes. In CeD, for example, the high degree of correlation was to the documented DQ-Dr locus, and five certain individual sensors in classes I and II have also been recognized. CeD is now the lone autoimmune condition with a recognized and also sound antigen, gluten.

Gluten is a protein found in wheat, barley, and rye grains. These are absorbed in the intestine and deamidated by tissue transglutaminase enzymes; helping it to fit nicely into the binding sites of a specific CeD-risk DQ heterodimer (encoded by the DQ2.2, DQ2.5, and DQ8 haplotypes). MHC fine mapping reported such an affiliation, indicating functions for 4 amino acids in the DQ genes with the significant determinant affiliations to CeD risk (Gutierrez-Achury et al., 2015). Accordingly, the key relationships for T1D, Ms, and SLE were reported inside the MHC class II locus (the relations for such three diseases will be to a specific HLA-DQ-Dr haplotype), with distinct but poorer correlations only with class I and/or III regions. In diabetes, fine mapping in an Asian population confirmed MHC interactions pushed by subtypes in the MHC class II area, with HLA-DP1*17 is now the most meaningful (Zhang et al., 2016).

In addition, the main and toughest affiliations in psoriasis and AS are with MHC class I molecules, even as IBD and Graves' disorder often had positive correlations to a class I locus. Despite the fact that class III mutants are still only slightly linked to inflammatory disorders, many correlations throughout the MHC class III domain have been discovered for Ms, like the connection to rs2516489 that corresponds to a broad haplotype in between MICB and LST1 genes. SLE sensitivity has often been linked to the interaction signal to rs419788-T in the class III region gene SKIV2L, which represents a unique locus discovered by fine-mapping in UK parent child trios (Fernando et al., 2007). A main report of European SLE patients and controls, especially upstream of NOTCH4, recognized an autonomous organization message to class III (rs8192591) (Morris et al., 2012). Conversely, more research is required to understand why these genetic changes relate to SLE susceptibility.

MHC fine-mapping analyses, in recognizing individual variants, allow for the observation of epistatic and non-additive influence in the locus. Such

**TABLE 5.2** Major histocompatibility complex (MHC) associations to autoimmune diseases, as described by fine-mapping studies (Matzaraki, Kumar, Wijmenga, & Zhernakova, 2017).

| Disease | Classic associated locus | Fine-mapping: top locus | HLA class for top association | Fine-mapping: independent associations at $P < 5 \times 10^{-8}$ | HLA class for Independent association | Sample size | References |
|---|---|---|---|---|---|---|---|
| Rheumatoid arthritis | Shared epitope (HLA-DRB1 AA70–74) | HLA-DRB1: AA 11, 13, 71, 74 | II | HLA-B AA 9 | I | 5018 individuals with seropositive RA, 14,974 unaffected controls | Raychaudhuri et al. (2012) |
| | | | | HLA-DPB1 AA 9 | II | | |
| | | HLA-DRB1: AA 11, 57, 74 | II | – | – | 2782 seropositive RA cases, 4315 controls | Okada et al. (2014) |
| | | HLA-DRB1: AA 11, 71, 74 | II | HLA-DPB1 AA 84 | II | 6244 cases, 23,731 controls | Okada et al. (2016) |

(*Continued*)

**TABLE 5.2** (Continued)

| Disease | Classic associated locus | Fine-mapping: top locus | HLA class for top association | Fine-mapping: independent associations at $P < 5 \times 10^{-8}$ | HLA class for Independent association | Sample size | References |
|---|---|---|---|---|---|---|---|
| | | | | HLA-DOA (rs378352) | II | | |
| | | | | HLA-B 40:02 | I | | |
| Celiac disease | HLA DQ locus (DQ2, DQ8) | HLA-DQA1: AA 25 and 47;HLA-DQB1: AA 57 and 74 | II | AA9 in HLA-DPβ1 | II | 9247 patients, 13,589 controls | Gutierrez-Achury et al. (2015) |
| | | | | rs2301226 (near HLA-DPB1 gene) | II | | |
| | | | | HLA-B*08:01 | I | | |
| | | | | HLA-B*39:06 | I | | |
| | | | | s1611710 (HLA-F) | I | | |
| Psoriasis | HLA-Cw6 (HLA-C 0602) | HLA-C*06:02 | I | HLA-C*12:03 | I | 9247 patients, 13,589 control | Okada et al. (2014) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | HLA-B amino acid 67 | I | | |
| | | | | HLA-B amino acid 9 | I | | |
| | | | | HLA-A amino acid position 95 | I | | |
| | | | | HLA-DQα1 amino acid position 53 | II | | |
| Ankylosing spondylitis | HLA-B27 | HLA-B*27:02 and B*27:05 | I | HLA-A*02:01 | I | 9069 AS cases, 13,578 controls | Cortes et al. (2015) |
| | | | | HLA-DPB1 (rs1126513) | II | | |
| | | | | HLA-DRB1*01:03 | II | | |
| | | | | other HLA-B alleles (B*07:02) (protective), B*13:02, B*40:01, B*40:02, B*47:01, B*51:01 andB*57:01 (protective) | I | | |

(*Continued*)

**TABLE 5.2** (Continued)

| Disease | Classic associated locus | Fine-mapping: top locus | HLA class for top association | Fine-mapping: independent associations at $P < 5 \times 10^{-8}$ | HLA class for Independent association | Sample size | References |
|---|---|---|---|---|---|---|---|
| Systemic lupus erythematosus, | HLA-DRB1 (*03:01 and *15:01); TNF-308 G/A; C4Anull allele | HLA-DRB1 AA 11, 12, 26HLA-DRB1*15:01-HLA-DQB1 *06:02 | IIII | HLA-DQB1*02 | II | 849 SLE cases, 4493 controls | Kim, et al. (2014) |
| | | DRB1*0301 | II | rs419788 in intron 6 of the SKIV2L | III | 314 trios | Fernando et al. (2007) |
| | | rs1150753 in intronic region of TNXB | III | HLA-DRB1*03:01 | II | 3701 cases, 12,110 controls | Morris et al. (2012) |
| | | | | HLA-DRB1*08:01 | II | | |
| | | | | HLA-DQA1*01:02 | II | | |
| | | | | rs8192591 (upstream of NOTCH4) | III | | |
| | | | | rs2246618 near MICB | I | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | HLA-DRB1 AA 13, 11; HLADRB1*15:01-HLA-DQB1*06:02 | II | | | 4478 cases, 12,656 controls | Sun et al. (2016) |
| Type 1 diabetes | DRB1, DQB1, in particular heterozygosity for HLADR3/HLA-Dr4 | HLA-DQβ1 position 57; HLADRβ1 positions 13; HLA-Drβ1 positions 71 | II | HLA-B*39:06 | I | 8095 cases, 10,737 controls | Hu et al. (2015) |
| | | | | HLA-B*18:01 | I | | |
| | | | | HLA-B*50:01 | I | | |
| | | | | HLA-DPB1*04:02 | II | | |
| | | | | HLA-DPB1*01:01 | II | | |
| | | | | HLA-A AA62 | I | | |
| | | | | HLA-A*03 | I | | |
| | | | | HLA-A*24:02 | I | | |
| | | DRB1, DQB1 | II | HLA-B*39 | I | 2300 families | Howson et al. (2009) |
| | | | | HLA-B*13 | I | | |
| | | | | HLA-B*50 | I | | |

(*Continued*)

**TABLE 5.2** (Continued)

| Disease | Classic associated locus | Fine-mapping: top locus | HLA class for top association | Fine-mapping: independent associations at $P < 5 \times 10^{-8}$ | HLA class for Independent association | Sample size | References |
|---|---|---|---|---|---|---|---|
| | | | | HLA-B*18 | I | | |
| | | | | HLA-B*38 (protective) | I | | |
| | | | | HLA-A*24 | I | | |
| Multiple sclerosis | HLA-DRB1*15:01 | HLA-DRB1*15:01 | II | DRB1 alleles: *03:01, *13:03, *04:04, *04:01, and *14:01 | II | 5091 cases, 9595 controls | Patsopoulos et al. (2013) |
| | | | | HLA-A*02:01 | I | | |
| | | | | AA 65 of HLA-DPβ1 | II | | |
| | | | | rs2516489 in MICB-LST1 locus | III | | |
| | | | | HLA-B*37 | I | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | HLA-DRB1*15:01 | II | HLA-DRB1*03:01, *13:03, *08:01 | II | 17,465 cases, 30,385 controls | Moutsianas et al. (2015) |
| | | | | HLA-DQB1*0302 | II | | |
| | | | | HLA-A*02:01 (protective) | I | | |
| | | | | HLA-B*44:02, *38:01, *55:01 (protective) | I | | |
| Crohn's disease | HLA class II and HLA-C | HLA-DRB1*01:03 | II | HLA-C*06:02 | I | 18,405 cases, 34,241 controls | Goyette et al. (2015) |
| Ulcerative colitis | HLA class II | rs6927022(HLA-DQA1); HLADRB1*01:03 | II | HLA-C*12:02 | I | 14,308 cases, 34,241 controls | |
| Graves' disease | DRB1*03:01-DQA1*05:01-DQB1*02:01 (Dr3-DQ2 haplotype), HLA-C | HLA-DPB1 AA35, AA55, DPB1 *05:01 | II | HLA-DPβ1 AA9 | II | 1956 cases, 7047 controls | Okada et al. (2015) |

(*Continued*)

**TABLE 5.2** (Continued)

| Disease | Classic associated locus | Fine-mapping: top locus | HLA class for top association | Fine-mapping: independent associations at $P < 5 \times 10^{-8}$ | HLA class for Independent association | Sample size | References |
|---|---|---|---|---|---|---|---|
| | | | | HLA-A AA9 | I | | |
| | | | | HLA-B AA45, AA67 | I | | |
| Dermatomyositis | HLA-DPB1 | HLA-DPB1*17 | II | HLA-DBP1 (rs7750458-A) | II | 127 cases, 1566 controls | Zhang et al. (2016) |

things happened whenever the influence of one allele on disorder personification is dependent on the genotype of an allele in the locus (nonadditive effect) or the genotype of the "modifier" gene in that other locus (additive effect) (epistasis). In CeD, non-reactive MHC influences are discovered, and understanding gluten would be the responsible antigen aided research into the antigen-specific design of the DQ-heterodimer. The involvement of a few HLA-DQ haplotypes, such as the DQ2.5, DQ2.2, and DQ8 haplotypes, mediates CeD risk. These haplotypes develop a particular region that conveniently delivers gluten to T cells.

Such haplotypes has been encapsulated in cis (both DQA1 and DQB1) on the same chromosome, but on separate chromosomes in trans. Few DQ allelic strains generate CeD sensitivity only when they are associated with other haplotypes, resulting in a CeD-predisposing trans-combination. HLA-DQA1*0505-DQB1*0301 (DQ7), for example, confers CeD risk only when paired with DQ2.2 or DQ2.5, resulting in the development of vulnerable haplotypes in trans. DQ7/DQ2.2 heterozygozygosity, in specific, provides an advanced chance for CeD than homozygosity for both alleles, revealing a non-additive outcome including both alleles. Apart from CeD almost all autoimmune disorders lack precise haplotypes and their associated functions; furthermore, studying non-additive outcomes may reveal new information about disease-causing antigens.

Lenz et al. noticed nonadditive outcomes for autoimmune disorders such as CeD, RA, T1D, and psoriasis, confirmed through correlations among several HLA alleles (Lenz et al., 2015). For example, particular combinations within HLA-DRB1*03:01-DQB1*02:01/ DRB1*04:01-DQB1*03:02 genotypes or many correlations of the specific HLA-DRB1, HLADQA1, and HLA-DQB1 haplotypes have been documented that maximize T1D disease risk (Koeleman et al., 2004; Hu et al., 2015). Epistatic relation was found for HLA-B60 and HLA-B27 correlations in AS, demonstrating that persons with HLA-B27 + /HLA-B60 + genotype have higher threat of infection (van Gaalen et al., 2013). Furthermore, even though their relation to the insufficient general intelligence in Ms was mild, a recent report in Ms received reports for 2 studies have identified class II alleles: HLA-DQA1*01:01-HLA-DRB1*15:01 and HLA-DQB1*03:01-HLA-DQB1*03:02 (Moutsianas et al., 2015). Relations in both MHC class I and unique killer immunoglobulin receptor (KIR) genes are highly imperative in autoimmune disorders including psoriatic arthritis, scleroderma, sarcoidosis, and T1D predisposition (Nelson et al., 2004). KIR genes are encapsulated by a leukocyte binding site on chromosome 19q13 and are represented on natural killer cells and T cell subpopulations (Moretta & Moretta, 2004). Eventually, for AS, psoriasis, and Behçet's disease, epistatic correlations among MHC class ERAP1 and I are already represented (Kirino et al., 2013).

The development of innovative MHC variants as well as the recognition of attraction impacts inside the MHC are expanding our overview of a

biology associated immune—mediated inflammatory illnesses. The primary amino acid sites in the DQ or Dr Heterodimer have also been identified leads to fine mapping of a specific correlated locus around HLA-DQ-Dr haplotypes. The formation and significance of alternative antigens for immune or inflammatory diseases could be characterized by identifying particular amino acids via attaching assays and molecular dynamics. Because such regions are identified in peptide-binding grooves, they can play an important role in antigenic peptide transmission to T cells, during initial thymic production or after distant immune responses. Overview of nonadditive responses in MHC linked loci sometimes allows for identification of antigen-specific binding sites and specific amino acid sequencing. The evaluation of the defensive, DERAA sequence in RA-protective HLA-DRB1:13 allele, as well as resemblance to living organism and bacterial peptide, resulted in the creation of (citrullinated) vinculin but little pathogen variants as innovative RA antigens. Many immune disorders have been linked to the discovery of autonomous signals in MHC I and III, assuming the unique channel factors are responsible. CeD's affiliation with class I molecules forms a basis for innate-like intraepithelial leukocytes, limited to class I expression are also essential in epithelial integrity and microbe identification (Sheridan & Lefrançois, 2010). Class I correlations with RA, T1D, as some immune disorders imply the CD8 + cytotoxic cells, and CD4 + helper T cells, are participating in genetic susceptibility.

The discovery of MHC and non-MHC loci's epistatic impact could cast doubt on infection processes. According to the findings, ERAP1 controls the cut of similar epitopes so that the HLA-B27 molecule (Cortes et al., 2015) will introduce them. Mentioning that such epitopes will be inactivated by ERAP1 in order for CD4 + and CD8 + cells to remain employed it would be a crucial step in recognizing unique autoimmune disease triggers. The latest discovery of genetic components among MHC alleles and autoimmune disorders is exceptional, and has the chance of leading to an analysis of disease-causing antigens. This will be a significant step forward in the development of new drugs and infection control. As such, we don't know why most correlated alleles or haplotypes operate, and more quantitative research is required to fully know their role in disorder.

## 5.5 Genetic restraint of the immune reaction

The body's protected systems develop a broad set of tools for detecting and eliminating infectious agents. The HLA-A, -B, and -C, whom purpose is to represent just at binding site either native and external antigens show a crucial character in this microbial protection and host protected system. This stimulates the adaptive immunity, which includes antigen-specific CD8 + T cells (Zinkernagel & Doherty, 1997) and NK cells, which have an innate capability to disrupt tumor cells (Parham, 2005). As a result, HLA class I

compounds serve as signaling molecules for retained and innate immune responses to bacterial infections, especially viruses. Specific HLA class I compounds can impose restrictions on the types of peptides that can be attracted (Bjorkman et al., 1987), giving a basic structure for what is popularly referred to as the immune response's 'genetic restriction' (Zinkernagel & Doherty, 1997). With such essential functions in view, intense research into HLA class I gene correlations with contagious ailments has been initiated (Debebe et al., 2020).

### 5.5.1 Progression of polymorphism of human leukocyteantigens class I genetic factor

HLA class I encoded by genes found on a short arm of chromosome 6 (MHC) is the utmost genotype portion of the human species (The MHC sequencing consortium, 1999). The prolonged MHC gene spans approximately 7.6 Mb and contains more than 80 different immune system loci (Horton et al., 2004). Various HLA loci, genes taking part in peptide binding and HLA receptor transmission, immune molecules, coreceptors, and regulatory receptors, supplement and heat-shock proteins, but also inflammation and signal transduction are among them. The sequencing of several immune system genes in a tiny region of the genome is probably to have emerged to allow for organized expression of closely linked and coordinating substances (Parham, 1999). HLA class I genes are highly pleiotropic, encoding tens of alleles, the significant proportion where such cause structurally distinct variants to be expressed. HLA-B is the mammalian genome's most polymorphic locus. The observation of over 2000 molecularly described HLA-B alleles reveals that this locus is evolving rapidly. This variation, caused by a variety of biological progressions like gene replication and recombination, interallelic transfer, and the preservation of desirable point mutations, is prone to be affected by strong bacterial positive selection (Prugnolle et al., 2005). As a result, the strong polymorphism of HLA are being viewed as a unique footprint of mutation on the entire genes, representing the mechanism of natural adaptation by microbes operating on an adaptive system, which leads to individual persistence, divergence, and extension.

### 5.5.2 Human leukocyteantigens class I supertypes and supermotifs

HLA I genes have significant effects on a biological imperative of their materials related to excessive polymorphism. The majority of polymorphisms affect the basic amino acids that shape a sequence of antigen-binding pockets (A−F) in the class I cleft, thus affecting the kind of microbial peptides delivered to antigen-specific T cells. The protein sequences of antigenic peptides attached to various class I molecules defined distinct classes of HLA class I alleles or supertypes that have an affinity for small antigenic peptides or

supermotifs with such a common assembly (Sidney, Peters, Frahm, Brander, & Sette, 2008). An example of biological evolution could have chosen the present HLA submotifs, since there is an overlap of favored motifs in genes located within and during classes, and across loci (Sette et al., 2003). Besides, knowledge of HLA class I linking expectations is helping to guide the current vaccine selections. An example of biological evolution could have chosen the present HLA submotifs, since there is an overlap of favored motifs in genes located within and during classes, and across loci (Sette et al., 2003). HLA class I polymorphism causes significant variations in allele percentages within both African, Asian, and Caucasoid ethnic groups at the community level (Cao et al., 2004). In African communities differences in HLA class I allele concentrations has the largest extent, which is presumably due to the greater incidence of regional pathogen variability and systematic constraint on such communities (Prugnolle et al., 2005). Additional distinguishing characteristic of HLA class I genes is association imbalance, resulting in balanced haplotypic alleles passing down through generations in a Mendalian manner. Therefore complexity and prevalence of HLA haplotypes differs significantly across ethnic communities (Alter, Gragert, Fingerson, Maiers, & Louzoun, 2017).

### 5.5.3 Human leukocyteantigens typing and nomenclature

The component of individual transplant immunology prerequisite to directly influence appropriate tissue forms to minimize the chance of organ rejection gave rise to the methods of typing HLA compounds and its allelic alternatives. HLA sorting was once done using mixed lymphocyte culture (MLC) proliferative assays or serological phenotyping approaches using sera of multiparous women produced antibodies to their biological partners' HLA antigens. Although the introduction of polymerase chain reaction (PCR)-related procedures, a range of molecular detection systems based on PCR-amplification of DNA in coordination with sequence-specific oligonucleotide probes and primers or direct sequencing emerged (Bentley et al., 2009; Gabriel et al., 2009). The subsequent rise in molecularly described alleles necessitated the development of a logical nomenclature scheme.

The new WHO-Guideline foreign structure uses a simple 4-figure system, with the title of the class I locus preceded by an equal sign and at least 4 figures. The first two numbers identify the large HLA form, which is approximately equal to the serologically specified antigen or serotype (allotype), while the 3rd, 4th, and often 5th numbers are now used to recognize specific amino-acid variations divided by a colon (Marsh et al., 2010). Some more numbers were added to certain HLA class I alleles to indicate non-coding, silent, or identical nucleotide base shifts, and intronic, 50-, and 30-prime polymorphism, if present. Alleles that are barely articulated or "null," and

some are poor sources or secreted, are designated by the prefixes N, L, or S, accordingly (Marsh et al., 2010).

### 5.5.4 Human leukocyteantigens relations with infection

The hereditary interactions of infectious diseases are commonly screened through two main approaches. There are genomic approaches that presume that the genetics of patients is important. A comprehensive research including HLA or blind interaction research in which a significant number of patients ( $>$ 500,000), in a correlation study with susceptibility, genes (SEG) are examined with a single nucleotide polymorphism (Tibayrenc, 2007). The length and race of the study participants, the prevalence and configuration of the observed HLA alleles and haplotypes, etc. can all affect the analysis of HLA interactions with disease (Cardon & Bell, 2001). Significant surveys of at last many hundred scientifically excellently participants, as well as geographically and ethnically balanced unaffected monitors, are needed to identify HLA class I interactions with statistical significance strength. To be genuinely significant, an HLA correlation with illness are being repeated in 2 or even more reports in these and separate cultural minorities. Implementations of such reproducibility criteria are perhaps better demonstrated by research on HIV/AIDS, in which a microbial disease was already studied in considerable detail for the first time, showing a significant function for HLA class I genes and alleles in identifying sensitivity and resilience to apparent medical sickness (Blackwell, Jamieson, & Burgner, 2009).

### 5.5.5 Immunogenetic surveillance and vaccine design

The production of successful HIV therapies has received a lot of attention. The virus's ability to mutate, maximize, and evade immune detection, a shortage of adequate animal studies, and uncertainty about the design of HIV-1 vaccines and effectiveness studies have all hampered this effort (Burton et al., 2004). Three big phase 3 placebo-controlled HIV-1 shot field trials were conducted to date. The first two studies, which were performed in a number of high-risk communities for HIV-1 clade B and CRF01 AE, struggled to show potency (Buchbinder et al., 2008; McElrath et al., 2008). Fortunately, immunogenetic evaluations of individuals in some of those trials confirmed that injected participants with HLA class I alleles usually linked to better viraemia control (HLA-B 57, B 58, B 27) had lower concentrations of viral RNA and better vaccine-induced immune cells to HIV-1 than placebo receivers (Fellay et al., 2007; Fitzgerald et al., 2011). The third trial, which used a main boost regimen (RV144) to elicit both CTL and diffusing antibody responses to a clade B and CRF01 AE forms circulated in mainland Southeast Asia, recently reported moderate immunity in ethnic Thais (Rerks-Ngarm et al., 2009), showing proof of concept that HIV vaccine protection is

feasible (Dolin, 2009). It's also harder to specify and comprehend the exact criteria of vaccine-induced defense towards HIV-1 infection (Kim, Rerks-Ngarm, Excler, & Michael, 2010). HLA-B constrained CTL reactions were observed in ethnic Thais in immunogenetic research of the RV144 vaccine-induced CTL responses (Paris et al., 2004), that is compatible with older reports of HLA-B limited T cells dominating CTL defenses to HIV-1 (Kiepiela et al., 2004) and effectively attacking several RNA viruses (Kiepiela et al., 2004). (Hertz et al., 2011). In ethnic Thais, however, HLA class II gene profiles showed allele and haplotype interactions with RV144 vaccine-induced neutralizing immune response (Paris et al., 2004). Considering the practical involvement of HLA class II groups in regulating antibody responses, as well as the remarkable growth in designing vaccines that elicit protective antibody reactions to HIV-1, more research into HLA class II gene interactions with HIV/AIDS, which has so far been mostly incomplete, should be encouraged. A variety of algorithms have been designed to screen microbial genomic and proteomic sequence to predict the location of conserved immunogenic epitopes and supermotifs that are HLA allele and supertype-specific (Flower, 2003; Lund et al., 2004; Sylvester-Hvid et al., 2004). Publicly accessible international databases are constantly collating and updating HIV-1 proteomic data. They revealed highly conserved regions that can act as hot spots of HLA-restricted immune recognition in diverse populations (Stephens, 2005). As a result, both HIV-1 diversity and HLA heterogeneity have been kept in mind in the formulation of vaccinations, that is a significant catalyst for more studies into immunogenetic interactions of HIV-1.

## 5.6 Major Histocompatibility Complex class I chain-related molecule (MICA) antibodies in transplantation

The MHC class I-related sequence genes A and B (MICA and MICB) are a recent group of proteins located inside the human HLA class I genes that have since been identified in 1994 by two separate study groups (Bahram, Bresnahan, Geraghty, & Spies, 1994) (Fig. 5.2). Although the latter participant labeled them Perth beta block transcript 11, Bahram and coworkers coined the word MIC for them, which was later accepted by the WHO nomenclature committee for HLA framework causes. Such proteins, unlike traditional HLA molecules, were never active in antigen presentation to T cells. Rather, they work as receptors for the natural killer (NK) group 2, member D (NKG2D) stimulating C-type lectin-like receptors that is represented on NK cells, T cells, and CD8 + T cells. MICA's association with NKG2D causes immune response cytotoxic T-lymphocyte-mediated cytotoxicity, NK cell defenses, and cytokine output to be enabled (Suárez-Álvarez et al., 2009a). Furthermore, in the form of counterpart, polymorphic MICA antigens can induce antibodies which can destroy target cells (Zou et al., 2002).

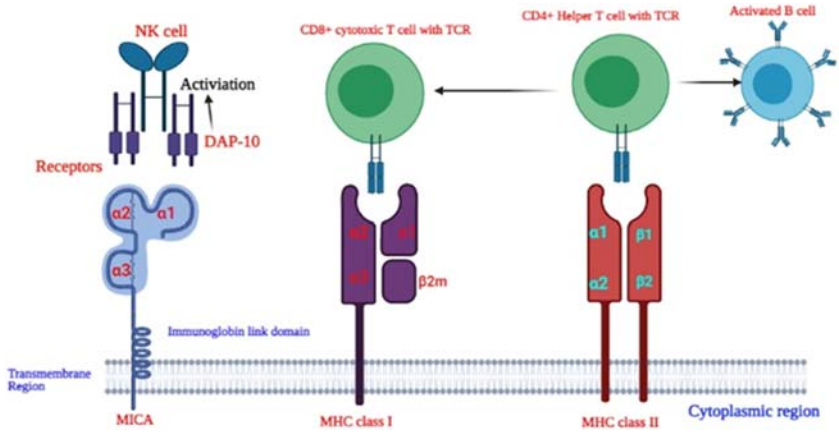


**FIGURE 5.2** Structural similarities between Major Histocompatibility Complex (MHC) class I and II molecules with MICA. The latter is equivalent to the heavy chain of MHC class I molecule without the β2 microglobulin. While the MHC I and II present peptides to CD8 and CD4 cells, respectively, the MICA recognizes NKG2D receptors on the surface of natural killer (NK) cells. *Adapted and modified from Baranwal, A. K., & Mehra, N. K. (2017). Major histocompatibility complex class I chain-related A (MICA) molecules: Relevance in solid organ transplantation.* Frontiers in Immunology, 8*, 182.*

As a result, MICA is one of a variety in that it connects the natural and adaptive immune responses in organ transplants. Apart from the overarching effect of anti-HLA antibodies on general graft viability, the immune reaction to non-HLA antigens has been a hot topic in latest studies. A multi-centric researchers studied renal allograft participants and reported under that same aegis of the Collaborative Transplant Study (CTS) generated much-needed impetus for recognizing the contribution of non-HLA antibodies (Zhang & Reed, 2016). Following that, numerous studies have pointed out that non-HLA antibodies play a significant role, not just in kidney transplants, as well as in several involving various organs including the heart and lungs (Hiemann et al., 2012; Valenzuela & Reed, 2013; Baranwal et al., 2020; Zhang & Reed, 2016). MICA, the only well with all non-HLA antigenic sites, is already linked to allograft rejection in hyper acute, intermittent, and/or chronic states (Baranwal & Mehra, 2017; Sumitran-Holgersson, 2008). The MICA gene is found inside the MHC class I domain on chromosome 6, centromeric to a HLA-B locus, which is strongly polymorphic, of 109 alleles. Endothelial cells, monocytes, keratinocytes, dendritic cells, fibroblasts, and epithelial cells, mostly in the gastrointestinal tract, produce MICA compounds biologically derived, but not immunocompetent cells (Zwirner et al., 1999). Their expression, on the other hand, may be stimulated in active CD4 + and CD8 + T cells (Molinero et al., 2006). MICA antigens can also be used to kill grafts in strong organ transplantation.

Antibodies found in patients' sera were able to directly react with various recombinant MICA molecules, according to a study involving 73 transplant recipients (Zwirner, Marcos, Mirbaha, Zou, & Stastny, 2000). Later, Zou et al. (2002) confirmed MICA as a candidate for complement-dependent cytotoxicity using both mouse MICA monoclonal antibodies and human alloantibodies. As a result, Sumitran-Holgersson, Wilczek, Holgersson, and Soderstrom (2002) confirmed a connection between MICA antibodies and renal allograft loss. Pregnancy, blood transfusions, and prior transplantation are all recognized risk factors for the production of MICA antibodies. MICA antibodies are thought to be present in between 7% and 27% of people around the world, according to studies (Sanchez-Zapardiel et al., 2013). About 30% of those receiving renal allografts and about 30% of those undergoing heart transplantation (Suárez-Álvarez et al., 2006a). Furthermore, their existence has been confirmed in pre-transplant sera, as well as after transplantation but before graft rejection, and in patients with kidney allograft rejection (Mizutani et al., 2005; Zou, Stastny, Süsal, Döhler, & Opelz, 2007). There is strong evidence that pre-transplant MICA antibodies are linked to poor allograft outcomes in renal transplant recipients who were HLA-matched to their donors but did not express HLA-DSA (Zou et al., 2007). Similarly, MICA antibody-positive recipients (425 renal allograft recipients) had greater overall graft survival than MICA-negative recipients. MICA antibodies have been shown to have no major effect on allograft rejection in other studies (Solgi, Furst, Mytilineos, Pourmand, & Amirzargar, 2012; Yu et al., 2011). Despite abundant evidence that MICA antibodies are linked to poor graft outcomes, a clear consensus on this relationship has yet to be reached. Indeed, compared to non-donor specific antibodies, very few studies have focused on the effect of MICA donor specific antibodies (DSA) on graft outcome (NDSA) (Table 5.3).

## 5.7 Immune response to MICA

Zwirner et al. (2000) confirmed the existence of anti-MICA antibodies in the sera of solid organ transplant recipients, which was the first evidence that MICA could function as a new polymorphic alloantigen. Similar antibodies were later discovered in mice immunized with recombinant MICA, and they were identified as a target for complement-dependent cytotoxicity (Zou et al., 2002). Zhang and Stastny (2006) went on to show that immunizing mice with recombinant MICA*001 containing all three extracellular domains could elicit responses in both T and B cells. By cell-mediated cytotoxicity, activated CD8 + T cells were able to destroy target cells pulsed with MICA. In addition, MICA-stimulated CD4 + T cells were Th2-biased, secreting high levels of IL-4 but low levels of INF-$\gamma$. This led to the conclusion that MICA-induced T-cell responses are limited to classical MHC molecules, which is consistent with MICA peptides being recognized indirectly by host

**TABLE 5.3 Correlation between HLA antibodies and graft outcomes (Valenzuela & Reed, 2013).**

| Organ | Frequency | Correlation with outcome | References |
|---|---|---|---|
| Heart | 75% of AMR + Px had HLA-DSA (14% of total patients); 11% of total had MICA-DSA out of total 14 patients | HLA or MICA-DSA associated with CAV | Nath et al. (2010) |
| | 35% developed de novo post anti-HLA out of 71 total patients | Correlated with HLA-A mismatch; CAV correlated with HLA II Ab | Tambur et al. (2000) |
| | 60% of AMR + Px had HLA-DSA, the remainder had MICA Ab out of total 168 patients | HLA ± MICA Ab correlates with chronic rejection | Zhang et al. (2011) |
| | 16% had preexisting anti-HLA, 48% had post-Tx anti-HLA out of total 950 patients | Patients with persistent HLA Ab had significantly reduced survival (post ± pre) | Ho et al. (2011) |
| | 32% had anti-HLA preTx; 63% had anti-HLA postTx out of total 219 patients | preTx anti-HLA correlated with rejection in the first year | Tambur et al. (2000) |
| Intestine | 12% had positive PRA, 7% had HLA-DSA out of total 88 patients | Correlated with reduced survival | Farmer et al. (2010) |
| | 21% had positive CM | Correlated with early poor function | Wu et al. (2004) |
| Liver | 70% had pre anti-HLA I out of total 80 patients | Lower outcome 1 year, higher rejection rate | Bishara et al. (2002) |
| Lung | 9% had positive PRA (post Tx) out of total 200 patients | PRA positivity increased post-Tx Complications | Lau et al. (2000) |
| Renal | 5% had HLA-DSA, 11% had non-DSA HLA Ab (post Tx) out of total 1229 patients | Both reduced survival | Hourmant et al. (2005) |
| | 30% of Px had anti-HLA, 31% of those had HLA-DSA (post Tx) out of total 1014 patients | DSA reduced survival | Lachmann et al. (2009) |
| | 15% of non-sensitized Px developed de novo HLA-DSA | HLA-DSA reduced 10 year survival | Wiebe et al. (2012) |

(*Continued*)

**TABLE 5.3 (Continued)**

| Organ | Frequency | Correlation with outcome | References |
|---|---|---|---|
| | (post Tx) out of total 315 patients | | |
| | 11% had anti-HLA, 4% had HLA-DSA (post Tx) out of total 251 patients | Correlated with reduced function | Cardarelli et al. (2005) |
| | 17% had C1q fixing HLA DSA (pre Tx) out of total 64 patients | C4d fixing ability of DSA was not predictive of early rejection | Hönger et al. (2010, 2011) |
| | 22% were FCXM +, 66% of those had HLA I-DSA, 34% had HLA II-DSA (pre Tx) out of total 308 patients | Persistent DSA reduced survival | Kimball, Baker, Wagner, and King (2011) |
| | 57% had HLADSA (pre Tx) out of total 28 sensitized Px patients | HLA-DSA increased acute rejection incidence, especially HLA II DSA | Song et al. (2012) |
| | 11% had MICA-DSA (preTx) out of total 1910 patients | MICA Ab correlated with rejection, especially when HLA matching was good | Zou et al. (2007) |
| | 16% had MICA Ab; 13% had preTx PRA; 16% had HLA Ab postTx out of total 185 patients | HLA ± MICA Ab increased rejection episodes and decreased survival | Panigrahi et al. (2007a, 2007b, 2007c) |

MHC antigens. Stastny et al. (2006) proposed that "in addition, to the adaptive immune response of T and B cells against an alloantigen, MICA also has the ability to activate innate immunity mechanisms. Co-stimulating T and B cells by engaging NK cells can have the effect of potentiating their responses. Another possibility is that MICA is immunogenic in and of itself, capable of inducing a response from a wide range of cells through a variety of mechanisms. This may be due to cross-reactivity with unknown microbes, an expansion of the repertoire of reacting immune cells, other genetic factors that influence the size of the specific immune response, or structural features of MICA molecules that make them immunogenic." These results back up the theory that MICA antigens play a role in human allograft rejection by

triggering both humoral and cellular mechanisms. Furthermore, interleukin-mediated upregulation of NKG2D, NK cell activation (in inflammatory conditions), NK cell-induced dendritic cell maturation, and subsequent activation of alloreactive T cells, as well as NKG2D-mediated decrease in regulatory T cells, may all contribute to graft rejection and graft loss in transplantation (Suárez-Álvarez, López-Vázquez, Balta, Ortega, & López-Larrea, 2009b). It was suggested that either cellular stress-induced MICA expression, such as on renal tubules, could enhance NKG2D-mediated cytotoxic T cell co-stimulation or direct activation of alloreactive CD8 + T cells through a TCR-independent mechanism (Verneris, Karami, Baker, Jayaswal, & Negrin, 2004; Suárez-Álvarez et al., 2009b). Furthermore, Zou and Stastny (2009) suggested that anti-MICA antibodies could be linked to graft rejection. Anti-MICA antibodies, MIC recognition on allografts, and NKG2D-mediated cytotoxicity are all possible MIC-mediated allograft rejection pathways. Following the first demonstration of MICA antigen expression on endothelial cells, researchers looked into the possibility of these molecules being used to kill grafts in solid organ transplantation (Zwirner et al., 1999). Antibodies in the patient's serum can also respond differently with different recombinant MICA molecules (Zwirner et al., 2000). Using both mouse MICA monoclonal antibodies and human alloantibodies, the expression of MICA on renal and pancreatic allograft biopsies proved it to be a candidate for complement-dependent cytotoxicity (Hankey et al., 2002). Sumitran-Holgersson et al. (2002) found a strong association between MICA antibodies and graft failure in 139 renal allograft recipients. As a result, a significant milestone in demonstrating that MICA expression in graft tissues can lead to antibody-mediated lysis and that MICA antibodies can play a role in triggering antibody-mediated rejection (AMR) was reached. Patients who had both antibodies rejected their grafts more often than those who did not have either of these antibodies, according to a retrospective analysis of "serial ten-year follow up of HLA and MICA antibody development prior to kidney graft failure" (Hankey et al., 2002). Antibodies to MICA found during the pretransplant period can play a role in the process of AMR (Mizutani et al., 2006). There has been a rise in studies looking into the influence of MICA antibodies on graft outcome in solid organ transplants, especially kidney and heart transplants. Table 5.4 summarizes findings on the impact of MICA antibodies on graft outcome in various solid organ transplants. Role of Soluble MICA (sMICA) in solid organ transplantation. A soluble isoform of MICA (sMICA) derived from the proteolytic shedding of membrane bound molecule occurs in the serum in addition, to the membrane bound form. MICA, a ligand for NKG2D receptors, forms a complex with ERp5, a disulfide isomerase/chaperone, which causes MICA to undergo a conformational transition, allowing ADAM proteases to cleave it. The interaction of NKG2D with sMICA causes the receptor−ligand complex to be endocytosed and degraded, suppressing NKG2D-mediated host innate immunity. The majority

**TABLE 5.4** Presence of MICA antibodies and their effect on allograft outcome in solid organ transplantation (Baranwal & Mehra, 2017).

| Organ | Time after Tx | Year | Number of patients | Transplant | Outcome | References |
|---|---|---|---|---|---|---|
| Heart | Pre | 2002 | 139 | Deceased donor | ↑ antibody-mediated rejection | Sumitran-Holgersson et al. (2002) |
| | | 2007 | 1910 | Deceased donor | ↑ antibody-mediated rejection, ↓ graft survival | Zou et al. (2007) |
| | | 2010 | 425 | Not specified | no adverse effect | Lemy et al. (2010) |
| | | 2012 | 40 | Live donor | no adverse effect | Solgi et al. (2012) |
| | | 2013 | 727 | Deceased donor and live donor | ↑ antibody-mediated rejection | Sánchez-Zapardiel et al. (2013) |
| | Post | 2005 | 145 | Deceased donor and live donor | ↓ graft survival | Mizutani et al. (2005) |
| | | 2007 | 185 | Live donor | ↑ antibody-mediated rejection | Panigrahi et al. (2007a) |
| | | 2007 | 1921 | Deceased donor and live donor | ↓ graft survival | Terasaki et al. (2007) |
| | | 2009 | 284 | Deceased donor | ↑ antibody-mediated rejection | Suárez-Álvarez et al. (2009a) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 2011 | 442 | Deceased donor and live donor | ↓ chronic rejection | Cox et al. (2011) |
| | | 2012 | 779 | Deceased donor and live donor | no adverse effect | Lemy et al. (2012) |
| | | 2012 | 147 | Deceased donor and live donor | no adverse effect | Seyhun et al. (2012) |
| Kidney | Pre and Post | 2007 | 44 | Deceased donor | ↑ antibody-mediated rejection | Suárez-Álvarez et al. (2007) |
| | Pre | 2009 | 491 | Deceased donor | ↔ antibody-mediated rejection /cardiac allograft vasculopathy ↑ graft survival | Smith et al. (2009) |
| | Pre | 2010 | 63 | Deceased donor | no adverse effect | Pavlova et al. (2010) |
| | Post | 2010 | 95 | Deceased donor | ↑ antibody-mediated rejection, ↑cardiac allograft vasculopathy | Nath et al. (2010) |
| | Post | 2011 | 168 | Deceased donor | ↑ antibody-mediated rejection | Zhang et al. (2011) |
| | | 2015 | 05 | Animal experiments (rat-to-mouse cardiac transplantation model) | ↓acute rejection | Yu et al. (2015) |
| Liver | | 2008 | 84 | NS | no adverse effect | Uzunel et al. (2008) |
| | | 2013 | 123 | NS | no adverse effect | Ciszek et al. (2013) |

of research on soluble MICA release in the serum has focused on its effect on tumor development, with very little information on the biology behind it. A few studies have highlighted the complex relationship between the science behind sMICA's function in cancer and transplant rejection. Suárez-Álvarez et al. (2006b), for example, found an inverse relationship between sMICA levels and AR when assessing the effect of MICA on heart graft acceptance. The research followed 31 heart transplant recipients for a year, and eight of them developed AR, while the other 23 did not. Further investigation revealed that 17 of the 23 patients who did not have AR had detectable levels of sMICA, compared to two patients in the rejected group. The authors discovered a propensity for MICA antibodies to occur in the absence of sMICA in the AR community of patients based on a combined study of MICA antibodies and sMICA. sMICA levels were observed in patients without MICA antibodies and in the absence of MICA antibodies. They also looked at sMICA levels in pre-transplant serum samples, as well as at 15 days, 3 months, and a year after the transplant in 34 heart transplant recipients (Suárez-Álvarez et al., 2006a). sMICA was virtually undetectable in pre-transplant sera, but it was found in 21 patients 15 days after transplantation. Surprisingly, 20 of the 21 patients did not develop AR, while 9 of the 13 patients with no detectable sMICA in their serum did develop AR. These findings are consistent with a previous study that found that the existence of sMICA promotes graft acceptance. A recent animal experiment (rat-to-mouse cardiac transplantation) also revealed a negative relationship between sMICA and AR. Anti-MICA antibodies in xenografts with AR continued to evolve in the absence of sMICA, according to the researchers (Yu, Xu, Wang, Cai, & Xu, 2015). There was no substantial difference in the presence and quantity of soluble MICA between the three classes in a study involving 30 patients with coronary artery disease, transplant recipients with stable grafts, and 15 healthy controls (Assadiasl et al., 2015). Using sandwich ELISA to determine the effect of sMICA on the outcome of liver transplantation, 37.6% of recipients had significantly higher pretransplant sMICA than the healthy population. Although recipients with lower posttransplant sMICA levels had a lower incidence rate of biliary cast syndrome (BCS) than those with high posttransplant sMICA levels, indicating that complex variations in these levels are linked to the development of BCS (Zou et al., 2009). Clearly, there is a paucity of published research examining a potential connection between circulating sMICA levels and graft outcome in solid organ transplantation. To determine the applicability of sMICA as a potential prognostic biomarker in solid organ transplantation, studies with larger cohorts and diverse ethnic groups are required.

## 5.8 Conclusion and future perspectives

Recent advancements for comprehending genetic discrepancy in the MHC in association with autoimmune and communicable infections have been

addressed. For infectious diseases, traditional GWAS is difficult to apply; however, other approaches could improve the power of genetic studies. Overall, launching collaborative initiatives to enhance patient cohort figures, constructing larger tests with even more adequate access, homogeneously clinically established patient phenotypes, and employing inference with population specific genetic markers will serve as a stepping-stone for studying the genetic makeup of viral infections. Furthermore, several studies have shown the additional advantage of fine-mapping the MHC to identify hereditary menace influences for autoimmune illness. Though MHC-related imputation approaches based on genotype data, MHC discrepancy accompanying with multifaceted sicknesses has been established.

Despite the difficulty of identification, genetic discrepancy in the MHC is important due to the two theories (pathogen-driven evolutionary assortment of defensive genes or pathogens as causes of autoimmunity). Firstly, it throws light on the expansion of autoimmunity, and secondly, it contributes to a better understanding of the human immune system's complexity. Furthermore, despite strong evidence that MICA antibodies have a negative effect on graft ending, a decisive conclusion on this association has not been extended until date. Based on current evidence, flowing heights of sMICA might be a useful factor in the evaluation of patients who have undergone renal transplantation. Now, there is a shortage of available research examining a potential connection among sMICA development and titers and graft result in renal transplantation. However, larger cohorts and varied ethnic groups in future studies will help to confirm the current findings. This awareness would allow improved prophylactic and therapeutic strategies to be developed in the future, resulting in more controlled patient−immune responses during treatment.

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Chapter 6

# Gestational immunogenetics: an overview

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## 6.1 Introduction

Pregnancy is an unusual physiological condition, where a mother carries a fetus with distinct genes and is like that of a grafted foreign organ. It is a state whereby cells from two individuals who share only half of their genomes coexist while blastocyst implantation and fetal development occur. The genetic dissimilarities between the fetus and mother have presented a challenge for immunologists, which was first presented by Peter Medawar's significant contemplation on this subject (Medawar, 1953). Scientists have debated on the immune system of the pregnant mother which does not eliminate the genetically immuneincompatible fetus in her womb. At the time of delivery, the maternal immune system is evoked to make antibodies against the components coded by paternally inherited alleles that significantly differ from the maternal genes. Such antibodies are alloantibodies and are raised against intraspecies (allogeneic) molecules against their target antigens called alloantigen (Thorsby, 2009).

Three main events occur during gestation which prevents the recognition and rejection of fetal tissues by maternal immune system. These mechanisms are (1) Placental barrier: which acts as an anatomical separation between fetus and mother; (2) Changes in placental Human Leukocyte Antigen molecules; (3) Silencing of maternal cell mediated immunity (Tersigni et al., 2020).

With the discovery of major histocompatibility complex (MHC) and self/nonself immune recognition hypothesis proposed lymphocytes receptors for nonself antigens and allogeneic cells that lead to rejection of the allograft, and this transplantation immunology model was also applicable to gestational immunology. Acute graft rejection occurs when donor MHC molecules are identified as nonself by recipient T lymphocytes. Stimulation occurs by classical HLA class I and II differences, which are highly polymorphic. Many theories initially postulated the absence of this phenomenon

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00012-9**
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during pregnancy or a constant maternal immune suppression. Nevertheless, this mechanism cannot explain the later findings of antigen-specific T cell in fetus and antibody-facilitated reactions in mother (Van Kampen et al., 2002).

Recent studies have demonstrated that during implantation, the presence of adaptive immune environment prevents the antigen specific immunity (Boudreau et al., 2016). This tolerance is mediated by regulatory T cells (Treg), a subclass of T cells. Treg cells act as powerful intermediators between natural killer (NK) cells, dendritic cells (DCs), macrophages, and other components of adaptive and innate immunity, to suppress of inflammatory responses (Burt, 2013). An overview of interaction of immune cells at fetomaternal interface is shown in Fig. 6.1.

## 6.2 Placenta as an anatomical barrier

Gestational immunity largely focuses on the interactional events between HLA molecules and ligands that happens at fetomaternal interface, the placenta. The formation of placenta and its function can affect survival of fetus, development, growth, and influence the immune response of mother. Following fertilization, the embryo is formed from the blastocyst which is the inner layer of cells and the outer layer or trophectoderm forms the placenta. The placenta forms once implantation occurs, as extravillous trophoblast (EVT) cells located at the edge of anchoring villi attach and invade the

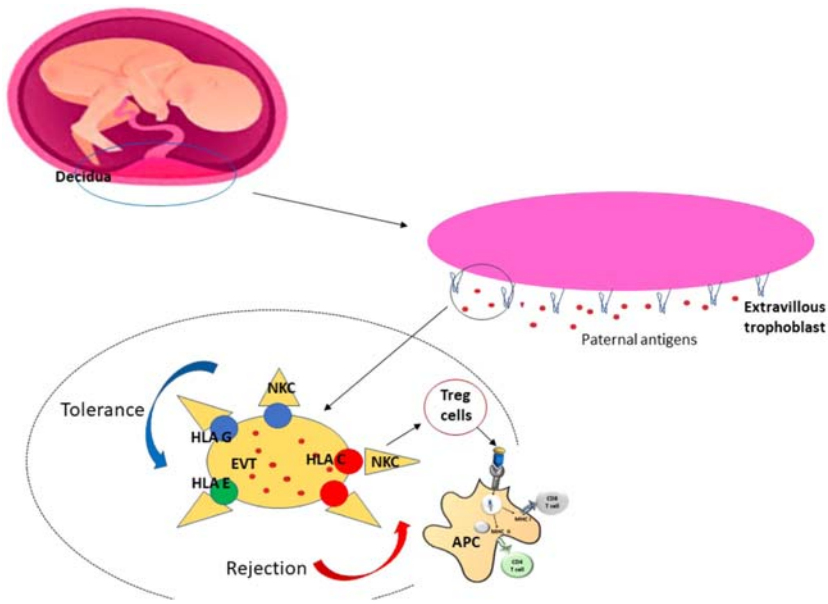


**FIGURE 6.1** Immunological events at maternal fetal interface during early pregnancy.

maternal decidua that develops from the uterine mucosa, by a phenomenon called decidualization. Decidualization requires alterations in the functional morphology of stroma cells in endometrium, remodeling of the maternal spiral arteries, and immune cell infiltration and action. Approximately 45% of the decidual cells are made up of cells of immune response (Vince, Starkey, Jackson, Sargent, & Redman, 1990). Among these, 66% are NK cells, macrophages form 25%, one tenth are T cells, B cells, and DCs are very smaller in number (Abelius et al., 2015; Mor, Aldo, & Alvero, 2017; Muzzio et al., 2014). The immune cells at decidua and myometrium can come into direct contact with the EVTs. The inhibition of signal pathway or the rejection of immune cells at the position of implantation can have serious consequences that result in the loss of pregnancy (Abrahams, Straszewski-Chavez, Guller, & Mor, 2004).

The placenta acts as a barrier during fetal growth, prevents interaction with the somatic cells of the fetus, limits the contact of the maternal immune cells to the trophoblast, promotes an antiinflammatory environment, enables tolerance, and facilitates evasion of maternal immune surveillance (Moffett-King, 2002). However, the placenta does not provide a complete immunological barrier. The villous trophoblast cells fuse into syncytiotrophoblasts (ST) and encounters the blood from mother present in the intervillous site. Syncytial cells enter the circulation as soluble fetal antigens, that can lead to the development of anti-HLA alloantibodies (Moffett, Chazara, & Colucci, 2017). Besides, a form of genetic sharing effectively occurs because trophoblast cells can be released directly into the mother's circulation by a phenomenon called fetal microchimerism (Bianchi, Zickwolf, Weil, Sylvester, & DeMaria, 1996; O'Donoghue et al., 2004; Yeung & Dendrou, 2019). Similarly, maternal microchimerism can also occur when maternal immune cells and platelets are present in umbilical cord blood, a phenomenon seen in almost 40% of healthy pregnancies (Maloney et al., 1999).

## 6.3 Placental human leucocyte antigen molecules

During pregnancy, a controlled expression of HLA class I and II occurs in EVT and chorionic, and is significant for a successful pregnancy outcome (Moffett et al., 2017). The villous ST cannot express any HLA I or II molecules, thus making T cells unable to identify and attach to the placenta (Apps et al., 2009). This hiding mechanism is highly effective in protecting the placenta from the rejection by maternal immune cells. Only HLA class I is expressed by the EVT, hence it cannot act as an antigen presenting cell and cannot lead to recognition by maternal T cells (Burton & Jauniaux, 2015; Parham, Norman, Abi-Rached, Hilton, & Guethlein, 2012). Although trophoblasts have IFN gamma receptors, they cannot initiate the mechanism of transduction as they are unable to express HLA class II antigens (Murphy & Tomasi, 1998).

The paternal derived HLA antigens are prevented from maternal T cell allo-immune response by the lack of HLA class II and HLA-A molecules on EVT and by expression of HLA-B antigens. The destruction of EVT by decidual NK cells and T cells is further prevented by the expression of HLA-C, HLA-E and HLA-G molecules, which are the components of class I antigens (Datema, Van Meir, Kanhai, & Van Den Elsen, 2003; Tersigni et al., 2020).

The HLA-C1 on EVT is specifically recognized by dNK cells, which recognize the expression of Killer cell Ig like receptors (KIRs). These cells utilize inhibitory KIR2DL2/3 receptors for HLA C1 and activating KIR2DS1 receptor expression for HLA-C2 (Carrillo-Bustamante, Kesmir, & de Boer, 2016; Papúchová, Meissner, Li, Strominger, & Tilburgs, 2019; Sharkey et al., 2008).

The pregnancy related complications are protected by presence of haplotype KIR B, while the risk is increased in its absence (Hiby et al., 2010). The KIR B haplotype has activating KIR2DS1 receptor which interacts with its specific ligand on NK cells to secrete cell mediated immune factors that lead to invasion of placental trophoblast (Bari, Bell, Leung, Vong, & Chan, 2009). On the other side, the complications in pregnancy increase if the fetus has addition copy of HLA-C2 born to homozygous KIR A mothers, such as the mother is C1/C2 and fetus is C2/C2 and when the second allele is of paternal origin (Hiby et al., 2014). The homozygous KIR A mothers with KIR AA haplotype have two copies of inhibitory KIR for HLA-C2 or KIR2DL1 receptors. The pregnancy related complications occur when the maternal uterine NK cells having KIR2DL1 receptors interact with HLA-C2 of fetal trophoblasts, inducing the inhibitory pathway and lead to the strong rejection of placenta (Singh, Rajak, Kadam, & Rajadhyaksha, 2019).

There is another inhibitory mechanism adopted by decidual NK cells in addition to the KIR-HLA C interaction. The cytotoxicity of NK cells at fetal-maternal interface is induced by the interaction of NKG2D receptor with ligands UL-16 proteins (ULBPs) and MHC class I chain related proteins A/B. The interaction activates the perforin mediated cytotoxic pathway that eliminates the defective or infected cells. The expression of these ligands in humans is highly regulated and released by STEVs in pregnancy in a soluble form (Sharkey et al., 2008). These soluble ligands also downregulate the receptor NKG2D which in turn downregulates the cytotoxic pathway (Mincheva-Nilsson et al., 2006; Tersigni et al., 2020).

During pregnancy, HLA-G contributes significantly to the survival and growth of the fetus by maintaining maternal tolerance. Since the beginning of first trimester, the HLA-G antigens are expressed by embryonic trophoblast cells, and interaction of HLA-G protein with decidual immune cells at fetomaternal interface leads to fetal tolerance by inhibiting cytotoxic action of CD8 + T cells and NK cells. This further leads to suppression of CD4 + T cells, B cell activity, and Treg cells stimulation (Hedlund et al., 2009).

The immunosuppressive mechanism of HLA-G is mediated by the interaction of its α1 domain with inhibitory receptors of immune cells. The ILT 4 receptor expressed by macrophages, DCs, and monocytes interact with monomeric HLA-G molecules, whereas ILT 2 displayed by CD8 + T cells, CD4 + T cells, monocytes, macrophages, DCs interact with heterodimers of HLA-G (Mincheva-Nilsson et al., 2006). The interaction of HLA-G negative cells like NK cells with trophoblast cells expressing HLA-G induces the negative immune cells to acquire HLA-G and making them positive HLA-G cells by a phenomenon called Trogocytosis.

Thus trogocytosis is a mechanism by which surface molecules are transferred by cell-cell contact from one cell to another. Immune suppressive environment is created by these negative immune cells once HLA-G is acquired (Tilburgs, Roelen, van der Mast, van Schip, & Kleijburg, 2006). Trogocytosis of HLA-G by decidual NK cells helps in the maintenance of immune function and tolerance by NK cells during pregnancy (Van der Zwan, Bi, et al., 2018).

## 6.4 Immune responses at the fetomaternal interface

The fetomaternal interface is formed from maternal decidua and fetal placenta. The decidua is formed only during pregnancy and is synthesized from the uterine endometrium and is shed at the end of the pregnancy or parturition. During the process of decidua formation, both fetal and maternal remodel spiral arteries so that maternal blood is continuously supplied to the placenta, which enables the exchange of gases, nutrients, and waste. Post implantation, the lining endothelial cells in spiral arteries are sloughed off, forming a fibrinoid layer implanted with invasive fetal placental trophoblasts (Tuckey, 2005). The trophoblasts begin to invade endometrial tissue and uterine spiral artery after implantation of the blastocyst in the endometrium. The activation markers are expressed at the surface of maternal lymphocytes including CD4 + T cells, CD8 + T cells, and CD16 − CD56 NK cells, implying that maternal lymphocytes recognize trophoblasts (Manaster & Mandelboim, 2010). At the fetal-maternal interface formed by the maternal immune regulation and trophoblast-derived molecules, a tolerogenic environment is created. Maternal leukocytes (NK cells and macrophages) play a crucial role in this renovating procedure. These processes by fetal trophoblasts and maternal leukocytes result in the dilation of the spiral arteries, resulting in the decrease of force and maximizing the volume of the maternal blood to placenta (Liu et al., 2017).

The placenta is formed by the third week of gestation by both floating and anchoring villi. The key cellular barrier between the fetus and maternal blood is formed by a single layer of syncytial SYN lines present on the outermost surface of the placenta villous tree. Under the SYN layer exist the undifferentiated mononucleated cytotrophoblasts (CTBs). CTBs are precursor

trophoblasts that are capable to fuse to replenish the SYN layer or differentiate into mononucleated EVTs, located at the tips of the anchoring villi. The SYN layer functions as a main endocrine part of the placenta producing human chorionic gonadotropin (hCG) and progesterone hormones. It also facilitates nutrient exchange, in addition to exchange of gases, and waste across maternal-fetal interface (Malassine, Frendo, & Evain-Brion, 2003).

Maternal leukocytes constitute 40% of decidua, dNK cells comprise the majority (~70%) of immune cells, followed by decidual macrophages (20%–25%), and T cells (3%–10%). Maternal leukocytes remain present in decidua during pregnancy, while dNK cells and decidual macrophages are present at the initial stages of pregnancy (Williams, Searle, Robson, Innes, & Bulmer, 2009). These maternal leukocytes are recruited by chemokine gradients produced by decidual stromal cells and trophoblasts (Carlino et al., 2008).

### 6.4.1 Decidual natural kill cells

Decidual natural kill cells (dNK) form the group of maternal immune cells that accumulate at fetomaternal interface and contribute significantly to decidualization and implantation. Unlike other immune cells, they secrete significant amounts of growth and angiogenic factors plus cytokines (Hanna et al., 2006). They increase the availability of maternal blood, remodel spiral arteries of decidua and endorse the invasion of trophoblasts at fetal-maternal interface (Kalkunte et al., 2009; Saito et al., 1993; Wang, Umesaki, Nakamira, Tanaka, & Nakatani, 2000). The dNK cells are highly granulated and recognized as CD56brightCD16$^{-}$ (Ander, Diamond, & Coyne, 2019). They have an increased ability to secrete various chemokines and have regulatory effects on different types of cells in the vicinity (Yang, Wang, Li, & Li, 2019). Various tissue-resident markers such as CD9, CD69, and CD49a, and adhesion molecules, such as CD9, CD62L, and $\alpha$-1 integrin, which might contribute to their accumulation are expressed by dNK cells. Although dNK cells have huge amounts of lysozymes, such as perforin and granzyme, but they have very low cytotoxicity (Koopman et al., 2003; Montaldo et al., 2015; Vacca, Moretta, Moretta, & Mingari, 2011). Furthermore, dNK cells highly express KIRs and NKG2A, which can attach to different types of HLAs on trophoblasts (as discussed earlier), indicating a very significant role of dNK cells in the regulation of the biological behavior of trophoblasts (Yang et al., 2019).

The dNK cells are divided into the following three groups: dNK1,2 and 3 cells, that express tissue specific markers like CD49a and CD9. Decidual NK1 cells express CD39, CYP26A1, and B4GALNT1; dNK2 cells express ITGB2 and ANXA; and dNK3 cells express CD103, CD160 and KLRB1. Decidual NK1 cells highly express the activating receptors like KIR2DS1/4 and the inhibitory receptors like KIR2DL1/2/3, which can bind to HLA-Cs on trophoblasts. LILRB1, which has a high affinity for HLA-Gs on EVTs, is

expressed only in dNK1 cells, and both dNK1 and dNK2 cells express the HLA-E receptors NKG2C, NKG2E, and NKG2A, suggesting that dNK1 cells might play unique roles in recognizing and interacting with EVTs, that specifically express HLA-G. A subpopulation of dNK cells has been recently found in multigravida women, which are involved in enhanced secretion of IFN-γ and VEGFα (Yang et al., 2019; Yeung & Dendrou, 2019).

The dNK cells express KIRs, NKG2A/CD94 receptors, and ILT2 receptors, which bind to HLA-C, HLA-E, and HLA-G in trophoblast, respectively (Liu et al., 2017). The adhering of HLA-E and NKG2A can inhibit NK cell activation and prevent trophoblasts from being killed. Also, the attachment of HLA-G and ILT2 can stimulate the secretion of inflammatory and angiogenic chemokines like IL6, IL8, IL 1 and TNF α, by dNK cells. These cells are engaged in the formation and preservation of decidual immune. They also play roles in cytotoxicity and virus clearance in pathogenic infection (Yeung & Dendrou, 2019). The direct contact between trophoblast and $CD49a^{+}$ Eomes + NK via HLA-G and ILT2 stimulus the secretion of growth promoting factor of this NK cell subset, which further promote the fetal growth. While it was also revealed that increased CD107 and NKp46 expression, release of perforin was related to apoptosis of trophoblast and miscarriage in fetal/neonatal alloimmune thrombocytopenia, showing that antibody-dependent NK cell-mediated cytotoxicity of invasive trophoblasts as a pathological mechanism of miscarriage and hemorrhage in neonates. HLA-Gs expressed on trophoblasts also promote the invasion and angiogenesis by dNK cells, and attenuate regulatory function to dNK cells of trophoblast by downregulating HLA-G expression which can be a possible mechanism of recurrent miscarriage (Vacca et al., 2011). The dNK cells secrete many chemokines and cytokines like IL8, TNF α, IFNϒ, TGFβ, VEGF-C, VEGF-An and PGF [40]. Receptors such as CXCR1 (receptor for IL-8), CXCR3 (receptor for CXCL-10), TNFR1, VEGFR-1 (receptor for VEGF-A), and VEGFR-3 (receptor for VEGF-C) are also expressed in EVT. Both IL-8 and CXCL-10 after binding with the receptors are involved in the migration of primary trophoblasts that block the expression of VEGFR and thus inhibiting the invasion of trophoblasts (Zhang, Dunk, & Lye, 2013).

The dNK cells participate in the formation of an inflammatory environment in early pregnancy. Localizations of dNK cells and neutrophils have been reported to overlap forming an interactive relationship between dNK cells and neutrophils. Cytokines secreted by dNK cells, for example, granulocyte-macrophage colony stimulating factor and IFN-γ can stimulate the activation of neutrophils, contribute to the survival of neutrophils, and promote the expressions of CD64 and CD11b in neutrophils. NK cells in the decidua inhibit the expansion of Th17 cells and synthesis of IL-17 by secreting IFN-γ. Women with recurrent spontaneous abortions have decreased $CD56brightCD27^{+}$ NK cells, that lead to inhibition of Th17 cell proliferation and IL-17 secretion, hence affecting immune tolerance. Decidual NK cells

also promote TGF-β secretion in monocytes through IFN-γ, thus inducing the differentiation of Treg cells and promoting maternal−fetal immune tolerance (Costantini & Cassatella, 2011).

### 6.4.2 Macrophages

Macrophages which are mononuclear phagocytic cells are highly plastic and alter their phenotype in response to external stimuli. These cells have been suggested to play role in implantation, placentation, fetal development, and parturition. Decidual macrophages play an important role in the suppression of maternal immune responses to fetal antigens and induce tolerance by secreting immunomodulatory factors, such as TGFβ, IL10, PGE2, and IDO. They also maintain homeostasis and efficiently makeover tissue and are also be involved in regulating blastocyst implantation and facilitating trophoblast invasion. They utilize cytokines, components of matrix, growth factors, and various proteases for angiogenesis and tissue reconstruction. They also initiate apoptosis and remove any unwanted cells and debris (Ning, Liu, & Lash, 2016; Renaud & Graham, 2008).

During the peri-implantation period classically activated macrophages (M1) and the invasion of EVT cells to uterine stroma they transit to mixed M1 and to activated macrophages (M2). These mixed macrophages remain in the decidua till sufficient placental blood supply is established. The decidual macrophages then shift specifically towards M2 type playing a significant role in parturition and preventing fetal rejection. The upregulated M2 genes include surface markers like tertraspanin CD9, mannose receptor CD209 and CD206 and have been associated with tissue regeneration, immune regulation, cell proliferation and metabolism during early pregnancy. The interleukins secreted by these cells particularly IL10 are actively involved in immunosuppression (Gustafsson et al., 2008; Laskarin et al., 2005).

In first-trimester decidua two subclasses of CD14 + decidual macrophages are present that express CD11c (67% is CD11clo and 33% id CD11chi) and both of them proinflammatory and antiinflammatory cytokines. Both CD11chi and CD11clo have similar phagocytotic capacities. Based on mRNA array studies, increase CD206 and CD209 are expressed by CD11clo decidual macrophages, which are involved in the regulation of smooth muscle cells, formation of extracellular matrix, and tissue growth. High levels of CD304, CD206, CD163, ICAM-3, and low levels of CD11c are expressed by CD209 + macrophages. (Houser, 2012).

Normal decidua also has high levels of CD16 + and CD14 + macrophages. These cells emerge at the implantation site and direct the spiral arteries remodeling. Chorionic villi also contain Hofbauer cells, which are the mature macrophages that express receptors like CD163, LYVE-, DC-SIGN, and phosphatidylserine receptor Tim-3. Tim-3 has recently gained more attention and is both antiinflammatory and proinflammatory regulator and

maintains immune tolerance. Tim-3 expressing macrophages also have high phagocytotic activity in placenta.

Both DCs and uterine macrophages share a majority of surface markers and both cells also express MHC II antigens, as they have a common origin, that is, from myelo-monocytes. (Svensson et al., 2011).

### 6.4.3 Dendritic cells

DCs play a significant role in fetal immune tolerance and maternal decidualization and angiogenesis, despite their low concentration in decidua (Laskarin et al., 2007). They respond to hormone changes in pregnancy and interact with other immune cells such as NK cells, macrophages, and T cells (Blois, Klapp, & Barrientos, 2011; Tagliani & Erlebacher, 2011). There are four main subtypes of DCs: myeloid DCs or conventional DCs (cDCs), lymphoid DCs or plasmacytoid DCs (pDCs), the monocyte-derived DCs (mono DCs), and Langerhans DCs (LDCs). The two subclasses of DCs (pDCs and cDCs) are present in decidua throughout the pregnancy, with cDCs is slightly higher concentration than pDCs (Miyazaki et al., 2003). The increase secretions of the hCG during pregnancy maintains an intricate balance of cDC and pDC (Sauss, Ehrentraut, Zenclussen, & Schumacher, 2018).

During pregnancy, the naïve T cells are differentiated by DCs into subclasses, these are Treg cells, helper cells type 1 (Th1), type 2 (Th2) and type 17 (Th17). Th1 and Th2 immune balance is regulated by CDCs and pDCs respectively. The T cells are proliferated and differentiated to Treg cells by decidual DCs through TGFβ. These induced Treg cells exhibit immunosuppression via Th2-type cytokines, which promotes HLA-G expression in trophoblasts and reduces the cytotoxicity in dNK cells (Tagliani & Erlebacher, 2011). The DCs may also activate Treg cells by secreting IL10, which induces an adaptive form of Treg cells (Wei, et al., 2021).

The adaptive immune response via NK mediated maturation and proliferation of DC, and innate immunity through DC mediated NK cell stimulation has been well defined. The NK cells and DCs are in proximity in fetomaternal interface, which cannot be found in the nonpregnant uterus, indicating pregnancy related interaction of NK cells and DCs. Implantation sites lacking DCs show decreased levels of IL5, IL12 transcription which causes decreased expression of IFNϒ in NK cells (Dietl, Honig, Kammerer, & Rieger, 2006).

DCs share the same monocytes origin with macrophages and thus have similar phenotypic and functional characteristics. DCs and macrophages present the main antigen presenting cells at fetomaternal interface. In the first trimester, these cells are induced to produce cytokines via NFκβ/MAPK pathway, which leads to their accumulation in decidua. Other inducers like macrophage growth factor colony stimulating factor (M-CSF) also affect the activities of these cells via CCR2 pathway. In the second trimester. Decidual

macrophages differentiate into DC like cells that show immunesuppressive behavior and to DC like cells in third trimester that have immunestimulatory actions, indicating their role both in immune suppression as well as in tolerance (Li, Wu, Yang, & Huang, 2011; Wang et al., 2016). Thus DCs influence the polarization of macrophages during pregnancy.

### 6.4.4 T cells

Form 15%−25% of the total leukocyte at the decidua. About 30%−46% are CD4 + T cells and 46 to 74% are CD8 + T cells, with most of the cells are antigen presenting CD45RO + or CD45RA-, only 2% and 5% of first trimester CD4 + T cells are TH17 and TH2, while as TH1 form 10 to 30% of these cells. Treg cells which are CD25hiFOX3 + form 5% of CD4 + T cells. In the third trimester, CD8 + T cells form 5% of decidual T cells (Bulmer, Morrison, Longfellow, Ritson, & Pace, 1991; Tilburgs, Claas, & Scherjon, 2010).

The specific arrangement of T cells at maternal-fetal interface, regulate chemokine synthesis by trophoblasts and decidual cells during the development of placenta (Ali, Majid, Ali, & Taing, 2020). The series reaction that controls T cell behavior and the synthesis of decidual associated factors are very poorly understood, although several models have been suggested. These models explain the inhibitory PD-L1 molecule, death ligand FasL and tryptophan metabolizing enzyme idoleamine 2,3-dioxygenase, that are expressed at the interface of placenta and inactivate or waste away infiltrating effector T cells. Galectin-1, which is a carbohydrate binding protein has been shown to promote a favorable uterine environment that supports pregnancy by regulating the functions of decidual T cells (Baban et al., 2004; Patrice & Adrian, 2014).

### 6.4.5 Regulatory T cells or Treg cells

Regulatory T cells or Treg cells help in maintaining immune tolerance in allogeneic pregnancy. They contribute to the establishment of active immune tolerance toward the fetus by preventing inflammatory immune responses to self-antigens. FoxP3hi Treg cells are secreted during the first trimester that has a phenotype of CD45RO/HLA-Dr/CTLA4 + . The term Treg cells express GITR and have high levels of CD69, CD25, and CTLA-4. Both effector and central memory T cells synthesized during pregnancy remain active after pregnancy as well. However, the formation of memory Treg cells after pregnancy has not been established and remains to be elucidated. They increase in number and concentration systematically with each pregnancy. Treg cells are essential for maintaining tissue regeneration and establishing immune tolerance. The concentration of Treg cells increases from the first trimester and reaches maximum concentration in the second trimester. Treg cells have been shown to have high suppression towards fetal cord blood indicating the

presence of fetal specific Treg cells at placental interface. The high suppressive function is shown by CD4 + CD45RA-FoxP3high subclass of Treg cells and low suppression is exhibited by CD4 + CD45RA + FoxP3low subclass of Treg cells (Tsuda, Nakashima, Shima, & Saito, 2019).

Treg cells inhibit in vitro the function and proliferation of DCs via few chemokines like IL10, TGFβ and CTLA-4, the mechanism however is not clear. The importance of Treg cells in gestational immuno-tolerance is implicated by the absence of CD25 + Treg cells that lead to pregnancy-related complications (Jørgensen, Persson, & Hviid, 2019; Tsuda et al., 2019). In addition to the classical Treg cells CD4 + CD25 + /hiFoxP3 + , other types of Treg cells are also associated with pregnancy. The HLA-$G^+$ Treg cells exhibit memory and an activated phenotype (CD25 + CD45RO + ) but these cells lack the expression of FoxP3 (Hsu, Santner-Nanan, Joung, Peek, & Nanan, 2014; Van der Zwan, van der Meer-Prins, et al., 2018; Wang et al., 2015). The peripheral blood in pregnant women has elevated levels of CD4 + HLA-G + T cells. One study reported that the placenta was enriched with CD4 + HLA-G + T cells compared to the peripheral compartment, and cases of ectopic pregnancies have reduced CD4 + HLA-G + T cells levels in the blood as wells in the decidua (Darrasse-Jèze & Podsypanina, 2013; Wang et al., 2018). Treg cells are thus important regulators at the mother-fetal interphase and induce immune tolerance in pregnancy. They, however, cannot work alone and require various cells and immune modulatory molecules to regulate their action.

## 6.5 Conclusion

Pregnancy is a challenging physiological state for the immune system. An equilibrium is required between anti-and proinflammatory conditions that remodel the cells of the uterus, fetal growth, and birth at term. During gestation, fetal tolerance must be achieved without compromising the mother's immune system, which involves various immune and nonimmune components. Cell-mediated maternal-fetal tolerance performs an important role to protect allogeneic pregnancy. Immune cells like decidual NK, Macrophages Treg cells, and DCs initiate and regulate an immune response at fetal-maternal interface and are essential for the maintenance of immunotolerance and homeostasis. The dysfunction of these cells would lead to various pregnancy-related complications. A delicate HLA-KIR interaction between decidual NK cells and fetal EVT is essential for acquiring fetal tolerance and tissue remodeling during gestation. An intricate yet specific communication of dNK cells with trophoblasts, vascular endothelial cells, and immune cells of decidua, like Th17 cells, regulatory T cells, CD14 + monocytes, and DC, results in successful implantation, remodeling of placental vascular, and helps in maintaining placental immune tolerance. Decidual immune cells acquire a specific phenotype due to unique microenvironment and have a

significant function to maintain a viable and healthy pregnancy. They also influence angiogenesis, tissue homeostasis, invasion of trophoblast, remodeling of the spiral artery, and placental apoptosis. Their abnormal activity leads to various pregnancy-related disorders like miscarriage, fetal growth arrest, and preeclampsia. The understanding of how immune cells function at maternal-fetal interface could elucidate the mechanism by which these cells participate in normal and defective pregnancy. However, there are still many discoveries to be made and many mechanisms to be explained to understand antigen presentation by immune cells that will efficiently pave the way to explore the role of cellular-based immunotherapy in pregnancy-associated diseases.

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Chapter 7

# Gene polymorphisms and their role in autoimmunity

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## 7.1 Introduction

Autoimmune disorders are complex inflammatory disorders (Gregersen & Olsson, 2009). These disorders are generally characterized by failure of body to recognize its own organs or tissue and induction of aberrant immune response towards itself which leads to pathological changes and clinical manifestations (Davidson & Diamond, 2014). Although the exact etiology of autoimmune disorders remains elusive, several genetic, pathogenic and environmental factors have been recognized that play a role in establishment of autoimmune diseases (Zhang & Lu, 2018). A number of polymorphisms have been in reported in association with several autoimmune disorders (Ciccacci et al., 2019). Familial and twin studies report increased risk of several systemic autoimmune diseases among the relatives of patients in systemic autoimmune disease setting and monozygotic twins show more concordance than dizygotic twins (Raychaudhuri, 2010). This reflects the role of genetic inheritance in autoimmune diseases however, absence of complete disease concordance in monozygotic twins vouches for the role of additional factors (Aslani, Rezaei, Jamshidi, & Mahmoudi, 2018). Some genetic variants are shown to increase the susceptibility while others may provide protection against the disease (Ramos, Shedlock, & Langefeld, 2015). Genetic variability in the components of both innate and adaptive are implicated in autoimmune diseases (Knight, 2013). With no definitive cure of autoimmune diseases available that could cause total remission till now the

Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00009-9
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goals of treatment are to keep the symptoms controlled, prevent complete organ failures and manage the therapies side effect (Chandrashekara, 2012). The advances in field human genetics has led to identification of number of single nucleotide polymorphisms (SNPs) and the insights in molecular mechanism with which these SNPs affect autoimmune disease has helped to develop target specific therapies that has changed the paradigm of the approaches made. SNPs locate in genes or a regulatory region near gene and alter the expression of genes leading to translation of a modified protein leading to development or increase in susceptibility of a particular diseases (Al-Koofee & Mubarak, 2019). The SNPs identified underline the need for proper genetic evaluation and can act as biomarker for presence and characterization of specific autoimmune disease which can lead to early interventions and improved outcomes (Stalin et al., 2020).

## 7.2 Autoimmunity and autoimmune genes

The principle of immune system discriminating between self and foreign molecules is fundamental to the survival of the species. The self-responsive T lymphocytes are destroyed in the primary lymphoid organs by clonal deletion via apoptosis, clonal diversion or are anergized (Xing & Hogquist, 2012). The self-reactive T cells escape central negative selection are anergized in the periphery and responsiveness of self-recognizing immune cells is downregulated or may be suppressed by interaction with other types of cells as regulatory-T cells or Natural Killer cells (Macián, Im, García-Cózar, & Rao, 2004). Likewise self-reactive B lymphocytes are also deleted and anergised when B cells encounter membrane-associated self-antigens. The self-antigens crosslink with surface Ig receptors with high avidity, the cells become downregulated and die via apoptosis (Ghosh, 2020). If these B cells encounter a soluble monomeric antigen which is incapable of cross-linking with surface Ig receptors, they are rendered anergic by downregulation of surface IgM or by the formation of antiautoantibodies (antiidiotypes) that can recognize the antigen receptor on autoreactive B cells lymphocytes (Tobón, Izquierdo, & Cañas, 2013). This tolerance mechanism when disturbed leads to a wide spectrum of auto immune diseases. Autoimmune disorders are marked by inappropriate inflammatory response against self-molecules, resulting in organ or tissue damage (Ganapathy, Vedam, Rajeev, & Arunachalam, 2017). Autoimmune diseases can be organ specific in which self-antigens are localized to a particular tissue like Addisons disease, insulin dependent diabetes milletus (IDDM), Graves disease to name a few or it may be systemic with autoantigens spread through various tissues in body like that in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Janeway, Travers, Walport, & Shlomchik, 2001a). Autoimmune disorders are complex multifactorial conditions influenced by several genetic and environmental factors. The application of genome-wide association studies

(GWAS) to autoimmune diseases has ledto identification of large number of genetic variants that are implicated in regulating T cell and B cell tolerance and several additional genes that predispose to autoimmunity (Kochi, 2016). Most of the studies conducted have shown significant association of HLA alleles in establishment of autoimmune diseases, however, a number of non-HLA variants as AIRE, FOXP3, TLR that affect a range of immunological pathways are now known to have important role in progression and phenotypic expression of diseases (Serrano, Paula, & Páez, 2006). The autoimmune disease causing genetic variants are being extensively studied with the intention of understand the pathogenesis and underlying mechanism of autoimmune disorders and lead to recognition of potential therapeutic targets. We in this chapter have tried to discuss few earlier reported genetic variations and their role in autoimmune disorders.

### 7.2.1 The autoimmune regulator (AIRE)

AIRE protein is a transcriptional regulator primarily expressed in high levels in thymus specifically in medullary thymic epithelial cells (mTECs) of thymus and al thymic dendritic cells and macrophages (Roberto, 2018). Low levels of AIRE is expressed in other immunologic tissues as lymph nodes, pancreas, testis, spleen and fetal liver (Eldershaw, Sansom, & Narendran, 2011). AIRE plays an important role in establishment of self-tolerance by orchestrating the promiscuous gene expression of tissue-specific antigens (TsAgs) in mTECs. Naive thymocytes that recognize these TsAgs with high affinity undergo apoptosis in a process of negative selection and helps to establish self-tolerance (Abramason & Husebye, 2016). AIRE gene in humans is located on chromosome no 21q 22.3 and encodes a 545 aminoacids AIRE protein (Constantine & Lionakis, 2019). AIRE protein shares 71% homology with mice AIRE (Eldershaw et al., 2011). A number of studies on murine models have helped to although not identically produce important information on mechanisms and role of AIRE in human beings. AIRE localizes in nucleus and has features identical to other transcription factors (Pitkänen & Peterson, 2003). It bears three domains identical with the members of SP 100 family of transcriptional coactivators; at N-terminus, is a homogeneously staining region to which ability to dimerize is attributed, a nuclear localizing signal domain, the middle of the amino-acid chain, the SAND domain that is important for DNA binding and at C terminal are two zinc finger PHD (Plant homeodomains) −PHD1 and PHD2 which are important for binding DNA and interact with other proteins, PHD 1 binds unmethylated histone 3on DNA and AIRE also has 4 LxxL nuclear receptor binding motifs which are characteristic of proteins that act as enhancers or repressors in transcription (Fig. 7.1) (Perniola & Musco, 2014). However, AIRE does not act as a "classical" transcriptional factors, it associates with other protein and work in coordination to regulate the gene expression. The first protein to associate with AIRE identified was CREB-

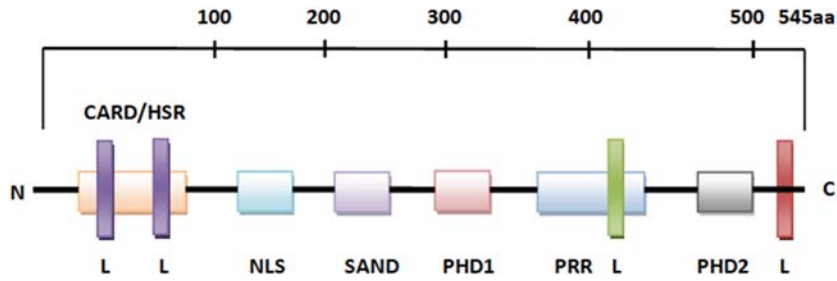


**FIGURE 7.1** Schematic representation of AIRE gene and its functional domain. CARD/HSR domain attributes to dimerization of AIRE, NIS (Nuclear localizing domain) is important for the import of AIRE, SAND domain is important for DNA binding. PHD1 and PHD2 are zinc finger binding domain that provide anchorage to other proteins. PHD1 binds unmethylated H3. 4LXXL are nuclear receptor binding motifs. *AIRE*, autoimmune regulator.

binding protein (CBP) (Peterson, Org, & Rebane, 2008). CBP acetylates AIRE and activates several other transcription factors (Incani et al., 2014). Other proteins found to associate with are DNA PK (DNA-dependent protein kinase) a serine-threonine kinase, P-TEFb which is also a transcription factor, Topoisomerase -II alpha which produces double breaks in DNA strands and recruits proteins DNA PK and PARP1 (poly [ADP-ribose] synthase 1), to form the multimolecular complex with AIRE and P-TEFb and play arole in expression ts −ag encoding genes and regulates the expression of other target gene (Fig. 7.2) (Abramson, Giraud, Benoist, & Mathis, 2010; Roberto, 2018). Dysfunction of this process allows self-reactive T cells escape into the periphery resulting in autoimmune destruction of certain tissues. It has been suggested that AIRE may promote self-tolerance further by other mechanisms as promoting development of regulatory T cells (Malchow et al., 2016). Loss of function mutations in AIRE gene causes autoimmune polyglandular syndrome type 1 (APS-1) also known as polyendocrinopathycandidiasis-ectodermal dystrophy (APECED). APS-1 is characterized by chronic mucocutaneos candidiasis and autoimmune manifestations effecting especially the endocrine glands like autoimmune hyperparathyroidism and Addison's disease (Golyan et al., 2019). Several other autoimmune disorders like type 1 Diabetes milletus, vitiligo, chronic active hepatitis have been reported in several patients (Husebye, Anderson, & Kämpe, 2018). About 129 different AIRE gene mutations have been identified in studies performed across several countries (Yan et al., 2020). Manifestation of autoimmune endocrinopathies highlights the role of AIRE in establishing self-tolerance and adds to the therapeutic intervention perspective of autoimmune disorders.

### 7.2.2 FOX-P3

Loss of function mutations in FOX-P3 cause Immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) (Park et al., 2020).

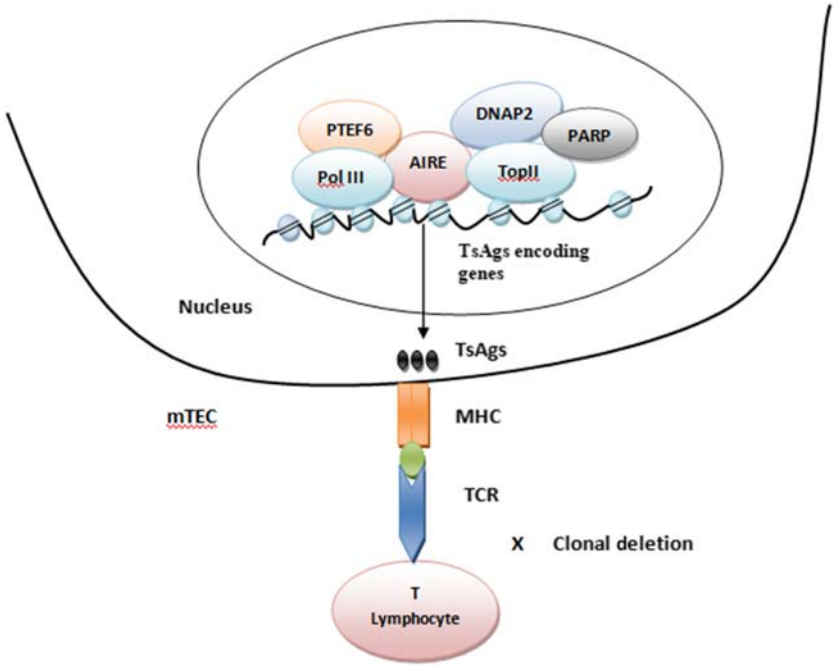


**FIGURE 7.2** Role of AIRE in establishment of self-tolerance. Acetylated AIRE binds through PHD1 domain to unmethylated histone 3. TopIIx induces breaks in DNA and attracts DNA PKA and PARP which causes chromatin relaxation and further recruits other mediators like PTEF6, releases the stalled Pol I and initiation of transcription. TsAgs binds the naïve T lymphocytes and mark it for clonal deletion. *AIRE*, autoimmune regulator.

IPEX is an autoimmune disorder characterized by early onset of severe enteritis, eczema, thyroiditis or and TID (Huang et al., 2020). Other autoimmune symptoms such as hepatitis, arthritis, alopecia and autoimmune cytopenia can develop in IPEX syndrome patients (Federica, Laura, & Rosa, 2012). FOX-P3 is a transcription factor and is essential for development and maintenance of CD4 + CD25 + T regulatory cells (Azizi et al., 2016). In humans FOX-P3 gene maps on chromosome Xq11.23 and consists of 11 exons encoding a 431 aminoacid FOX-P3 protein (Marques et al., 2015). FOX-P3 has a forkhead (FKH) DNA-binding domain at C-terminal, important for Dna binding and nuclear localization; proline rich (PRR) N-terminal repressor domain, it represses NFAT-mediated transcriptional activity; leucine zipper (LZ) and zinc finger (ZF) central domains important for protein −protein interactions (Mackey-Cushman et al., 2011). Majority of the mutations in IPEX patients are reported in FKH-binding domain (De Benedetti et al., 2006). Immunopathogenesis of IPEX syndrome is marked with loss of functional CD4 + CD25 + T regulatory cells (Tregs). Upon receiving TCR

signal FOX-P3 is constitutively expressed inCD4 + Cd25 + T cell subset (Ohkura et al., 2012). The expression of FOX-P3 is mediated by several transcription modulators.

FOX-P3 expressed then regulates the transcriptional programs of Treg cell, acts either enhancer or represser depending upon on the other proteins it interacts with. For instance FOX-P3 binds adjacent to NFAT binding site with corepressors Eos/Ct BP1 (C-terminal binging protein 1) in the promoter region of cytokine IL-2 and interferon-$\gamma$ and prevents its expression while it is with other transcriptional factors as RUNX1 upregulates Treg cell associated molecules as CD25, GITR, FR8, and CTLA-4 (Bacchetta, Barzaghi, & Roncarolo, 2018). Several mutations in FOX-P3 genes have been reported that have role in dysregulation of functions which affects tReg cell development and leads to immune dysregulation. The exact molecular mechanism by which it imparts its effects remains unknown.

## 7.3 Toll like receptors polymorphism and effects on autoimmunity

Toll-like receptors (TLRs) are pattern recognition receptors (PRR) which play a significant role in initiation of innate immune response (Hess, Felicelli, Grage, & Tapping, 2017). They are usually expressed on antigen presenting cells (APC) like macrophages, dendritic cells and monocytes. Till now 10 TLRS have been indentified in humans that bind specific pathogen associated molecular patterns (PAMPs) like flagellins, LPS and dinucleotides (Dolasia, Bisht, Pradhan, Udgata, & Mukhopadhyay, 2018). TLRs are single span transmembrane receptors consisting of leucine rich motifs in extracellular regions that form ligand binding horseshoe shaped domain, transmembrane domain and cytoplasmic signaling domain which is homologous to cytokine interleukin-1 receptor and is known as TIR (Toll/Interleukin-1) domain (Botos, Segal, & Davies, 2011). Ligand binding leads to dimerization of TLRs which then initiates downward signaling to express proinflammatory cytokines, chemokines, various antiviral and antipathogen proteins and initiate adaptive immune response.

TLRs 1, 2, 4, 5, 6 interact with extracellular ligands and are localized on the plasma membrane whereas TLRs 3, 7, 8, and 9 are situated in the membranes of lysosomes and endosomes and these recognize the internal microbial ligands endocytosed by these organelles (Pelka, Shibata, Miyake, & Latz, 2016). Besides recognizing the microbial components TLRs can also bind to the damage associated molecular patterns (DAMPS) which are the damage signals like intracellular components, heat shock proteins, chromatin proteins and nucleic acid fragments released by dying cells or injured tissues (Álvarez & Vasquez, 2017). The signaling pathways activated by TLRs depend on the adapters it recruits. Most of the TLRs recruit MyD88 except TLR3 that binds other adapter protein TRIF (TIR-domain-containing

adapter-inducing IFN-β factor) (Troutman, Bazan, & Pasare, 2012). TLR4 binds MyD88 when situated on plasma membrane, signals its endocytosis and TRIF when it is internalized to the endosomes (Gangloff, 2012). Depending upon the adapters associated TLR signaling cascade activates expression of certain transcription factors like NFkappa β, activator protein-1 (AP-1), interferon regulatory factors which lead to synthesis of pro-inflammatory cytokines and interferons (El-Zayat, Sibaii, & Mannaa, 2019). Though TLRs are crucial in establishing immune response it has been seen their over expression or malfunction due to mutations is associated with pathogenesis of several autoimmune diseases. Recent studies have identified number of polymorphisms in several types of TLRs that may have a role in susceptibility and intractability of several autoimmune disorder howbeitinformation of the molecular mechanisms by which they affect immune responses is limited. Minoretti et al. (2005) conducted a study in Italian population found patients with variation in TLR4 coding gene at 299Gly/399Ile had higher risk of developing bowel and Crohn's diseases (Also Asp299Gly polymorphism of the TLR4 gene has been associated with an attenuated receptor signaling and suppressed inflammatory response against the development of late-onset Alzheimer's disease (Zhou, Chen, Xu, Zhang, & Lin, 2020). TLR9 which is essential for recognition of CpG DNA is reported to be associated with many autoimmune diseases, such as SLE, autoimmune thyroid diseases, type 1 diabetes, vitiligo and RA (Liao et al., 2010). In a study conducted by S. Pabst et al. found missense mutations Asp299Gly and Thr399Ile in TLR4 gene were much more prevalent in the patient with chronic sarcoidosis group than in healthy control subjects or individuals with acute sarcoidosis (Pabst et al., 2006). The suggested that along with factors there is a significant association between the chronic course of sarcoidosis and TLR4 mutations. A study conducted by Liao et al. (2010) has shown T allele of TLR (rs352140) C/T polymorphism located in exon 2 to be associated with higher TLR9 expression and susceptibility to ophthalmopathy in Chinese patients with Graves Disease (Liao et al., 2010). In another study conducted in Egyptian population the association between the TLR9 polymorphisms and RA has been recorded. In the promoter region of TLR9 it was found that the $-1486C > T$ gene polymorphism was associated with the development of RA in TT genotypes (Hegazy, Auf, Neseem, & Al-Harrass, 2019). The exact role of TLR9 polymorphism in the pathogenesis of RA is not clear. However, it has been reported that TLR9 induces a T helper type 1 isotypic switching in B lymphocytes, which may be involved in the pathogenesis of RA (Jaen et al., 2009). TLR polymorphisms can lead to innate immune dysfunction which then affect adaptive immune responses, the exact mechanism involved in unknown. An improved understanding of the molecular mechanisms by which TLR signaling gets affected would facilitate the development of new therapeutic strategies for treatment of autoimmune diseases.

## 7.4 Vitamin D receptor polymorphism and their role in autoimmunity

Vitamin D the "sunshine vitamin" is synthesized when sunlight strikes our skin. When precursor of vitamin D3 7 dehydrocholesterol gets irradiated it converts to cholecalciferol which chemically inert (Chen et al., 2020). Activation of cholecalciferol occurs after it undergoes two hydroxylations steps mainly in liver and kidney. In liver cholicaciferol is converted to 25 hydrocycholicalciferol (calcefidiol) by hyroxylase enzymes like CYP2R1, CYP27A1. Calcefidiol is then hydroxylated to its metabolically active form calcitriol or1, 25-dihydroxyvitamin D3 [1, 25(OH) D] by hydroxylase CYP27B1 (Zhu & DeLuca, 2012). 1, 25(OH) D binds vitamin d3 receptors (VDR) on cells located throughout the body (Carlberg, 2019). Upon activation, besides its role in calcium-phosphorus metabolism it elicits a wide variety of biological responses, that influence cellular growth, proliferation, apoptosis, and several immunological effects (Kutner & Brown, 2018). VDR is a transcription factor and binds to vitamin D responsive elements (VDREs) in the DNA, mostly to Dr3-type which is a response element of repeated arrangements of two hexameric binding sites with three spacing nucleotides (Meza-Meza, Ruiz-Ballesteros, & de la Cruz-Mosso, 2020). When ligand is not present VDR mostly binds to non-Dr3-type VDREs and co repressor proteins (Dankers, Colin, van Hamburg, & Lubberts, 2017). 1, 25(OH)2D3 binding to VDR induces a conformational change leading to dimerization with the retinoid X receptor (RXR) and the 1, 25D-VDR-RXR heterodimer then formed binds to the Dr3-type elements in the promoter regions of vitamin D responsive genes and induces expression of these genes (Campbell, Xu, El-Tanani, Crowe, & Bingham, 2010). VDR is also expressed in neutrophils, APC such as macrophages and dendritic cells and activated B or T lymphocytes (Penna et al., 2007). Upon ligand binding, VDR initiates the signaling cascade in the cells participating in innate and adaptive responses and thereby modulates several immunological responses. It inhibits proliferation of inflammatory cells and upregulates production of antiinflammatory cytokines (White, 2012). In addition, macrophages and dendritic cells are shown to express CYP27B1 hydroxylase and are thus able to take 25 D and can locally form 1, 25 D (White, 2008). Expression of 1, 25 D on stimulation with signaling pathogens leads to production of Cathelicidin antimicrobial peptides, defensin β2 in cells mediating innate immunity as macrophages, monocytes and neutrophils which initiates response against the pathogens (Lin, 2016). In dendritic cells expression of 1, 25 D leads to generation of tolerogenic DCs, inhibiting their differentiation marked by low expression of costimulatory molecules CD80, CD40, and CD86, inhibited expression of HLA-Dr and increased expression of antiinflammatory cytokines like IL-10. 1, 25 D also promotes the production of Tregs cell which play important role in maintaining immune tolerance

(Barragan, Good, & Kolls, 2015). Thus 1, 25 D is a suppressor of antigen presentation to and activation and differentiation of Th1 cells. 1, 25 D signaling directly acts on T lymphocytes suppressing expression of IL-12, interferon-γ and IL-16 and IL-23 (Daniel, Sartory, Zahn, Radeke, & Stein, 2008) which are important for the development of Th1 and Th17, respectively. Furthermore suppression of IL-12 increases the development of Th2 cell phenotype (Van Etten & Mathieu, 2005). Development of Th2 cells leads to increased expression of IL-4, IL-5, IL-13 which inhibit development of Th1 cells (Bikle, 2017) (Fig. 7.3).

A large number of studies conducted suggest that different polymorphic variants of VDR, through their effect on receptor structure or expression, could influence the risk of autoimmune disorders (Dupuis, Pagano, Pierdominici, & Ortona, 2021). To date, four SNP of VDR gene have been most extensively studied in AIT patients: FokI in exon 2, BsmI and ApaIin intron between exon 8−9, and TaqIin exon 9 (described by altering restriction endonuclease sites). Growing evidences have shown significant associations in VDR polymorphism and susceptibility of autoimmune diseases like

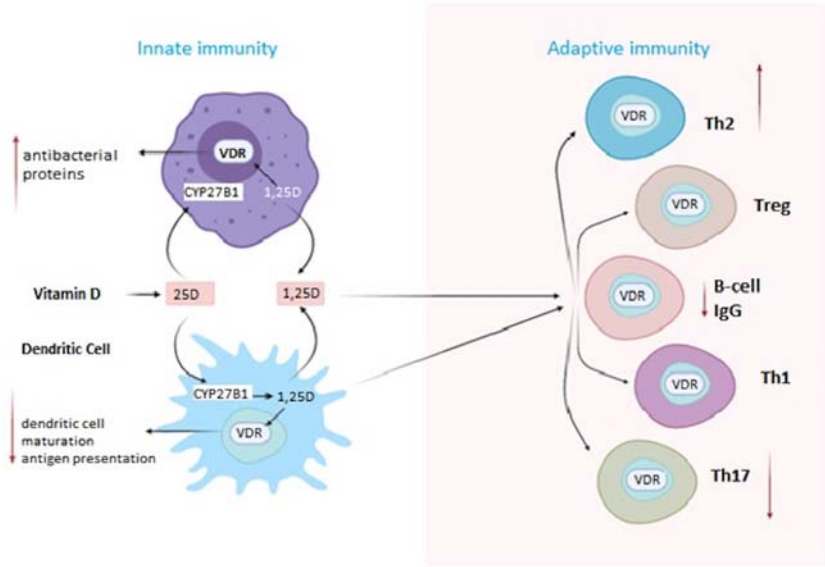


**FIGURE 7.3** Action between vitamin D and immune system; almost all proliferating immune cells express the VDR and some cells from the innate immune system also expressed by CYP27B1 as such they are able to take the precursor 25D and convert this locally to 1, 25D and drive endocrine responses. In case of macrophages, the vitamin D stimulates the expression of antibacterial protein, and enhances the autophagy to enhance the intracellular bacterial killing. In case of dendritic cells, local synthesis of 1, 25D inhibit cell maturation and suppress antigen presentation. This is a potential mechanism by which vitamin D can mediate the interface between adaptive and innate immunity.

SLE, type-1 diabetes milletus, RA and multiple sclerosis (Ms). However, it has been found that impact of VDR SNPs on predisposition to autoimmunity may also vary depending on studied population or ethnicity (Maciejewski et al., 2019).

In a metaanalysis studies conducted by Zhou et al. in 2014 including 13 studies to evaluate the association of VDR BsmI, Fok1, ApaI and TaqI gene polymorphisms to SLE susceptibility was found that BsmI B allele and bb genotype, Fok1 f allele and ff genotype, and ApaI aa genotype were linked with the risk of SLE in all the populations. However, in Asians, the BsmI B allele, BB and bb genotype, Fok1 f allele and ff genotype were found to be associated with the risk of SLE. In Africans ApaI An allele, AA genotype and aa genotype were also shown to contribute to the risk of SLE.

These VDR gene polymorphism were not found to be associated with the risk of SLE in Caucasians (Zhou, Jiang, Lin, & Su, 2015). Polymorphism of ApaI An is also found to be associated with susceptibility to Ms among different populations (Imani, Razi, Motallebnezhad, & Rezaei, 2019). Not much data is available on the biological consequences of these polymorphism. It has been reported that ApaI, BsmI, and TaqI polymorphisms do not affect the VDR protein structure. They may influence the differences in stability and/or translation efficiency of the RNA molecules. Only FoxI polymorphism in exon 2 is known to shorten VDR protein as it leads to alteration in start codon (Triantos et al., 2018). Although Bsm I and ApaI are intronic polymorphisms, they may affect gene expression through disruption of splice sites for mRNA transcription, by altering RNA stability, or, by changing intronic regulatory elements. TaqI is a polymorphism of exon 9 of VDR genes, it is a synonymous mutation and could affect the mRNA stability and decrease the levels of mRNA (Wang, Cheng, Ma, & Zhu, 2017). In a study it was shown the polymorphisms of VDR in patients with autoimmune liver diseases primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) could have association with their quality of life. PBC and AIH are characterized by extensive liver damage and chronic fatigue, mental health problems and is mostly found in women and is independent of age, region or ethinicity. The study showed Taq I CC and Apa I AA genotypes in PBC and AIH patients to be significantly associated with high susceptibility and disturbed emotional responses and mental disorders (Kempinska-Podhorodecka et al., 2020).

It is now known that vitamin D also plays a role in neurotransmission and neuroprotection, and VDR is found brain tissue and cells (Anjum, Jaffery, Fayyaz, Samoo, & Anjum, 2018). This may explain the influence of VDR polymorphism on mental well-being. However, further studies in this area would help in better understanding of the mechanisms responsible for the occurrence of symptoms associated with cognitive dysfunction and other mental disorders.

## 7.5 Major histocompatibility complex gene polymorphism and autoimmunity

The MHC/HLA gene family is located on chromosome no 6 in humans, clustered in groups of three main sub families MHC I, MHCII, and MHC III, with MHCIII lying between the two. MHC genes encode a set of glycoprotein molecules expressed on the surface of nucleated cells (Janeway, Travers, Walport, & Shlomchik, 2001b). Molecules encoded by class I MHC I genes are present on all nucleated cells and present antigens derived from endogenous sources to CD8 + cytotoxic T lymphocytes (CTLs) (Hewitt, 2003). Stimulated CTLs then promote proliferation of T cells and lysis in the target cells. MHC class II molecules are found on surface of APC as dendritic cells, macrophages, B cells and present peptides primarily of exogenous origin to the CD4 + T helper cells (Duffy, Drake, & Harton, 2017). Effector T helper cells then initiate a cascade of immunological events that results in activation of CD8 + cytotoxic cells and plasma B cells (Alberts, Johnson, & Lewis, 2002).

The MHC system contains the most polymorphic gene cluster in the entire human genome. A number of genetic studies has provided evidence of association of MHC gene complex in association with autoimmune disorders, for example, RA, SLE, type 1 diabetes mellitus (T1D), multiple sclerosis (Ms), celiac disease, psoriasis, Crohn's disease and Graves' disease to name a few (Matzaraki, Kumar, Wijmenga, & Zhernakova, 2017). The molecular mechanisms of association of MHC genetic variants with the autoimmune diseases are not fully resolved. It is hypothesized that polymorphisms in HLA class I and HLA class II molecules affect the aminoacids in the peptide-binding groove and thus altering their binding specificity which may lead to alterations in immune response (Cruz-Tapias, Castiblanco, & Anaya, 2013a). MHC or HLA complex is a polygenic system. There are three class I genes HLA-A, HLA-B, and HLA-C and three MHC class II regions DP, DQ, and Dr and containfunctional genes encoding a human MHC class I $\alpha$ chain and MHC class II $\alpha$ and $\beta$ chains respectively (Wieczorek et al., 2017). Till date a number of polymorphisms have been reported in these genes that may predispose individuals to certain autoimmune disorders. For example, genetic studies have revealed that in Type 1Diabetes (T1D) a chronic autoimmune disease, characterized by T cell-mediated destruction of pancreatic islet beta cells, resulting in irreversible insulin deficiency and abnormal glucose metabolism, major genetic contribution to T1D susceptibility arises from the MHC class II mainly HLA-DQ and HLA Dr (Noble & Erlich, 2012). The effects of individual alleles however, may be modified by the haplotypes on which they are carried. It has been established the individual carrying a specific heterozygous genotype, comprised of DRB1*03:01-DQA1*05:01-DQB1*02 and DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04 (Dr3/Dr4) have higher risk of developing the disease then the general population.

Whereas some haplotypes strongly contribute to protection from T1D. The most significant example is DRB1*15:01-DQA1*01:02-DQB1*06:02, which is the most common Dr-DQ haplotype in Caucasians (Noble & Valdes, 2011). Only a handful of studies have reported association of T1D with class I HLA.

Several studies have explored association of HLA complex polymorphism and Multiple Sclerosis, by far HLA II has shown greatest association in its susceptibility. The most common association has been found with the DRB1*1501 allele. It has been detected as highest risk factor in populations of several ethnicities like Caucasians, Han Chinese, North Africans, South Americans, Caribbeans. In few ethnic populations like Colombians, Middle Eastern, Japanese and South American patients besides HLA-DRB1*15:01 another allele HLA-DQB1* 06:02 has been found to have significant association with Ms susceptibility (De Silvestri et al., 2019).

A significant association of HLA genes has been reported in Celiac Disease (CD). CD is a complex autoimmune disorder of small intestines caused by an inappropriate immune response to ingested wheat gluten (Caio et al., 2019). HLA-DQA1*05:01-DQB1*02:01 or DQA1*03:01-DQB1*03:02 polymorphic alleles from HLA Class II are expressed in CD patients and code for HLA-DQ2 and DQ8 variants (Megiorni & Pizzuti, 2012). The principal disease triggering component of wheat gluten is α2-gliadin. Gliadin belongs to a family of closely related proline-rich and glutamine-rich proteins and because of its rich proline content is hard to digest (Helmerhorst, Zamakhchari, Schuppan, & Oppenheim, 2010). When genetically predisposed individuals who express HLA-DQ2 or DQ8 are exposed to certain gliadin epitopes, these epitopes are presented on the surface of APC (Cruz-Tapias, Castiblanco, & Anaya, 2013b). APCs' then stimulate proliferation of gliadin-specific CD4 T cells and abnormal immune response gets triggered and leads to intestinal inflammation. Recent studies provide little insights on the mechanisms by which MHC variants can influence the tendency to the expression of autoimmune disease. Further validation on MHC polymorphisms and their affects would provide rationale for designing specific immunotherapeutic interventions.

## 7.6 Genetic polymorphism and autoimmune disorders

### 7.6.1 Alzheimer's disease

Alzheimer's disease (AD) is by far the most common type of dementia and accounts for upto 70% of the cases (Alzheimer's disease facts & figures, 2020). It is a progressive disorder with symptoms like episodic memory loss, behavioral issues and cognitive deficits (Martina Zvěřová, 2019). AD is characterized by accumulation of senile plaques and neurofibrillary tangles (NFT) in neuron cells. The senile plaques are made up of insoluble

β-amyloid peptides (Aβ) which are the fragments of Amyloid precursor protein (APP) (Takahashi, Nagao, & Gouras, 2017). APP is fragmented by beta-site APP cleaving enzyme 1 (BACE1) to give a APP soluble fragment (sAPPβ) beta-site amyloid and a carboxy-terminal complex linked to cell membrane which is then acted upon by γ-secretase complex and Aβ peptide is formed (O'Brien & Wong, 2011). NFT are mainly composed of hyperphosphorylated tau protein, a protein involved in formation of microtubules. It is been found that Aβ promotes accumulation of chemically altered tau proteins in the cells (Iqbal, Liu, Gong, & Grundke-Iqbal, 2010). The accumulation of Aβ and hyperphosphorylated tau proteins interferes with the normal functioning of neuronal cell processes that ultimately leads to cell death, loss of neuronal synapses, and progressive neurotransmitter deficits.

It has been seen that HLA loci doesn't have strong association with susceptibility of AD, it may have modifier effect on the risk of development of disease (Mansouri et al., 2015). Several cytokines including interleukin 1α, interleukin 1β, IL-6, tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) have been reported to be associated with the risk of AD. In a metaanalysis study conducted by Mun et al. in 2016 − 889 C > T polymorphism in interleukin 1 α was found strongly associated with the increased risk of AD (Mun, Kim, Choi, & Jang, 2016). Also several other genes like presenilin-1, presenilin-2, APP, and apolipoprotein E have shown significant association with inherited risk for AD (Tanzi, 2012).

### 7.6.2 Multiple sclerosis

Multiple sclerosis (Ms) is a chronic inflammatory autoimmune-mediated disease that affects the central nervous system (CNS). Ms attacks the myelinated axons in the CNS, destroys the myelin sheath and axons to different extents creating lesions that can lead to severe physical or cognitive disability and neurological defects (Goldenberg, 2012). Ms can develop in any age however, majorly the age of onset is between 20 and 40 years and women being affected more affected than men (Harbo, Gold, & Tintoré, 2013). Ms is characterized by wide range of symptoms that vary among patients. Changes in vision (unilateral visual loss, diplopia), muscle weakness, numbness and tingling, neuropathic pain, sensory loss or distortions, changes in bowel and bladder function, mood disturbance, sexual dysfunstion, are few symptoms of Ms (Ghasemi, Razavi, & Nikzad, 2017). Progression of disease could eventually cause severe disability. Four clinical forms of Ms have been identified: relapsing remitting Ms (RRMS), secondary progressive Ms (SPMS), primary progressive Ms (PPMS), and progressive relapsing MD (PRMS) (Brownlee, Hardy, Fazekas, & Miller, 2017). RRMS is the most common form approximately affecting about 85% of the patients (Nicholas, Electricwala, Lee, & Johnson, 2019). It is marked by exacerbations of symptoms followed by periods of remission. About 60%−70% of patients with

RRMS develop SPMS. In this condition, symptoms continue to worsen with or without periods of remission and may cause increase in disability and new brain lesions over time (Dutta & Trapp, 2014). PPMS approximately affects 10% of Ms patients, this disease gets steadily worse, with few or no relapses (Ontaneda & Fox, 2015). PRMS is a rare form, affects less than 5% of patients. It is progressive from the beginning, with sporadic flare-ups of worsening symptoms along the way (Loma & Heyman, 2011; Weiner, 2008). The etiology of Ms is not fully understood, however, the studies suggest that genetic, environmental and infectious agents may lead to development of Ms.

Genetic studies have confirmed association of HLA class II region, mainly HLA DRB 15:01 in Ms susceptibility. Another variant found to have protective effect in Ms is HLA A02 and a number of other non HLA risk alleles for Ms have been indentified and validated (Waubant et al., 2019). SNP s in CD6, IL 2, and IL-2 recetor, Apo-1/Fas gene have been recognized as risk allele for Ms.

### 7.6.3 Irritable bowel syndrome

Irritable Bowel syndrome (IBS) is a very common disorder characterized by presence of abdominal pain with altered bowel habits. The three main subtypes of IBS are constipation IBS (C-IBS), diarrhea predominant IBS (D-IBS) and mixed IBS with features of both constipation and diarrhea (Chey, Kurlander, & Eswaran, 2015). A patient is said to have IBS if there is discomfort for at least 3 day per month during the past months, change in frequency and form of stools with symptomatic relief after defecation (Lacy, Weiser, & De Lee, 2009). The pathophysiology and underlying mechanism of IBS is not clearly understood. IBS is identified as a product of multiple genetic factors, inflammation activity, altered bowel motility and stress. In patients suffering from IBS there is increased colonic vulnerability to factors such as infection, chronic inflammation, food, gut micro-flora (Vahedi, Ansari, Mir-Nasseri, & Jafari, 2010). Although a number of genes have been identified the role of their variants needs to be further validated. Most of the studies have focused on serotonin (5-hydroxytryptamine or 5-HT) release, serotonin receptors (5-HTR), and the serotonin transporter (SERT) because of their recognized role in gut motility and mood. Serotonin (5-HT) is a neurotransmitter molecules stored in granules of enterochromaffin (EC) cells and when released in gastrointestinal tract effects appetite regulation, gut motility, stimulating production of mucous and visceral sensitivity (Gershon, 2013). Abnormal levels and activities of 5-HT have been reported in IBS (Ng, Soh, Loke, Lim, & Yeo, 2018). Studies conducted have shown polymorphisms in serotonin modulators having significant association with IBS.

The best-studied polymorphism is 5-HT transporter gene linked polymorphic regions (5-HTTLPR) in the promoter region of serotonin transporter

gene, which results in either a long (L) or short (S) transcript. The short 5-HTTLPR allele is likely to be associated with increased visceral sensitivity in IBS-C patients (Zhang et al., 2014). However, the studies conducted have shown heterogeneity and further confirmations are required. Several other candidate genes for IBS are being analyzed for their association. In TNFSF15 that encodes for Tumor necrosis factor (TNF)-like cytokine 1 A (TL1A), a tumor necrosis superfamily member proinflammatory cytokine, polymorphisms TNFSF15 rs4263839 (A > G) and TNFSF15rs6478108 (C > T) are found to increase the susceptibility of IBS (Zhu, Wang, Jia, & Duan, 2019). Not very much is known about role of genetic variations may have in onset and progression of IBS. A better understanding of the pathophysiological mechanisms of IBS to improve the prevention, intervention, and treatment of the disease is required.

### 7.6.4 Rheumatoid arthritis

RA is a chronic, inflammatory systemic autoimmune disease that primarily affects the synovial joints and can lead to progressive disability. RA is characterized by inflammation of synovial lining and joint damage (mostly symmetrical joints get involved), production of autoantibodies rheumatoid factor (RF) and anticitrullinated protein antibody, bone deformities and other systemic manifestations (Guo et al., 2018). The characteristics of RA are end results of activity of components of both the innate and adaptive immune systems that orchestrate production of number of effector molecules like IL-1, IL-6, and TNF -α involved in abnormal inflammatory processes (McInnes & Schett, 2017).

There are two major subtypes of RA based the presence or absence of anticitrullinated protein antibodies (ACPAs) named as ACPA positive and ACPA negative respectively. In a susceptible individual, there is a loss of tolerance of self-proteins that contain a citrulline residue (de Brito Rocha, Baldo, & Andrade, 2019). These proteins are generated through post translational modification of arginine residues by the enzyme peptidylarginine deiminase to citrulline. A number of proteins like fibrin, vimentin, fibronectin, α-enolase, type II collagen, and histones undergo this modification (Puszczewicz & Iwaszkiewicz, 2011). ACPAs are found in 70%–90% of RA patients and may be considered as most distinctive marker for this disease (Aggarwal et al., 2009; Song & Kang, 2010). Like other autoimmune disorders prevalence of RA is multifactorial triggered by various genetic and environmental factors.

The main risk factor for predisposition of RA is found in genes of HLA II complex, strongest association with the class II HLA-DRB1 gene has been reported. HLA-DRB1 alleles encode unfavorable variants of the shared epitope (SE), a five aminoacid sequence motif in positions 70–74 of HLA-Drβ which is important for the correct T cell antigen presentation process (van

Drongelen & Holoshitz, 2017). Also the polymorphisms leading to substitutions of aminoacid residues at positions 11, 13, of HLA-DRB1 and position 9 in HLA-B and HLA-DPB1, have a great association with RA development (Mikhaylenko et al., 2020).

A number of non HLA variants have shown association with RA susceptibility in individuals. Within the TNF gene, SNP has been identified with guanine (G) to adenine (A) transition at position −308 in promoter region. This uncommon An allele could facilitate deregulation of the cytokine network, affecting the pathology of RA Other polymorphisms within this gene, such as −1031 T/C, −863 C/A, −857 C/T or +1304 G/A, have shown increased susceptibility to RA by increasing TNF-α production. Occurrence of rare alleles at IL-1α (+4845) or IL-1β (+3953) have also shown an increased susceptibility to RA and increased severity of joint destruction (Rego-Pérez, Fernández-Moreno, Carreira-García, & Blanco, 2009).

## 7.7 Immunogenetics and immune therapy

The ultimate goal of immunotherapy of autoimmune diseases is to either prevent or downregulate inflammatory responses. An improved understanding of the molecular mechanisms by which these mutations alter signaling in autoimmune disorders is expected to facilitate the development of new therapeutic strategies. It has been observed the polymorphism of target molecule affects the treatment efficacy and response which is challenge for developing a general treatment for a particular disease. In addition, to various molecules involved in immune mechanisms, various autoantigens may be of importance and corresponding strategies of immunotherapy may be designed to prevent the occurrence of, or to interfere with, established autoimmune disease. In this regard, effective immunotherapy and induction of tolerance to the autoantigen resulting in the elimination or energy of self-reactive T cells may point to a potential to reduce the proinflammatory response or directly block the activation pathway and alleviate symptoms of the disease.

The current approach of treatment of autoimmune disorders is to give either symptomatic or immunosuppressive therapy. Drugs like nonsteroidal antiinflammatory drugs (NSAIDS), glucocorticoids, antimalarials in combination with other modulators as methotrexate, sulfasalizine to name a few, are being widely used for the treatment autoimmune disorders (Chatenoud, 2020). However, they are mostly nonspecific, do not lead to complete remission and prolonged use of these drugs has its own adverse side effects compromising the immune system which makes the patient susceptible to other diseases and infections which sometimes can be life threatening. The recent advances made in approach to the autoimmune disorders have led to development of more specific and effective treatments with less toxicity. The introduction and development of biologic agents targeting T cells, B cells, signal transduction molecules, cytokines and TLRs on APCs has

revolutionized the treatment of many autoimmune disorders. Many of these biologic agents have been approved for use in many autoimmune disease settings and have shown promising results. TNF inhibitors (TNF i) are one of the first biologics to be used and now four TNF inhibiting Monoclonal antibodies (M Abs)—infliximab, adalimumab, certolizumab, golimumab and an altered peptide ligand etanercept have been approved for use in rheumatic disorders (Atiqi, Hooijberg, Loeff, Rispens, & Wolbink, 2020). Use of the TNF inhibitors (TNF i) in combination with MTX in RA has shown improvement where conventional disease-modifying antirheumatic drugs fail to achieve the target (Johnson, Sanchez, & Schoenbrunner, 2019). In patients in early stage of disease it has shown increased clinical remission and reduced bone erosion and cartilage destruction (Jin, Chang, & Wei, 2010). Since many cytokines exert their affect through JAK STAT pathway, JAK inhibitors are now being recognized as the target to inhibit the effect of the cytokines. Three JAK inhibitors Tofacitinib and ruxolitinib have been approved for clinical use in humans by FDA and EMA. Tofacinitib was the first JAK inhibitor approved for RA patients failing to conventional treatments and with poor prognostic factors (Schwartz et al., 2017). Its use in combination with MTX has also been approved for patients with active Psoriasis arthritis adults (Paik & Deeks, 2019). Several JAk inhibitors are currently undergoing clinical trials for use SLE, IBD, psoriasis and other autoinflammatory diseases. These MAbs have made it possible to consider a more specific therapy target. Success with such biologic agents has paved way for identification of novel biologic agents targeting other pathways.

B cell depletion therapies that were used initially in treatment of lymphomas have successfully made their way in the expanding armamentarium of autoimmune disorder therapies. Rituximab an anti-CD20 chimeric MAb which selectively depletes CD 20 + B cells has shown efficacy in clinical trials in patients with ANCA vasculitis (Jones et al., 2010). Several 2nd generation humanized anti CD20 as belimumab, ocrelizumb, veltuzumab and ofatumumab have been developed to reduce immunogenicity and shown to be more potent at least in vitro. Ocrelizumab has now been approved for use in treatment of Ms, both relapsing and primary progressive multiple sclerosis (PPMS) (Du, Mills, & Mao-Draayer, 2017), belimumab has shown positive effects in patients of SLE (Jones et al., 2019). Several biologic agents are being tested, their use is still is in early stages and they still have side effects and are not very cost effective.

Application of Gene therapy is another promising approach towards treating autoimmune disorders. Although it is making progress in modern medicine it is still in infancy in this field, but could lead to new possibilities in future. Identification of biomarkers and understanding of molecular mechanisms of disease would provide a great impetus to application of gene therapy in treatment of autoimmune diseases (Table 7.1).

**TABLE 7.1** Gene (TLR, VDR, HLA) polymorphisms in several autoimmune disorders.

| Genes | Polymorphism reported/genotypes | Disease setting | References |
|---|---|---|---|
| TLR-4TLR-9 | 299Gly/399IleC/T exon 2-1486 C/T | Bowel and Crohn s disease, SarciodosisGrave s diseaseRheumatoid arthritis | Minoretti et al. (2006), Pabst et al. (2006), Liao et al. (2010) |
| VDR | Taq I (CC)Apa I (AA)BsmI (BB; bb), FokI (ff)Apa I (A) | AIH, PBCSLESLE, MS | Zhou et al. (2015), Imani et al. (2019) |
| MHC | DRB1*1501DQB106:02DQA1*05:01/DQB1*02:01 DQA1*03:01/DQB1*03:02DRB1*03:01-DQA1*05:01-DQB1*02DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04 | MsCDT1D | Megiorni and Pizzuti (2012), Noble and Valdes (2011), Noble and Erlich (2012) |

*AIH*, autoimmune hepatitis; *CD*, celiac disease; *MS*, multiple sclerosis; *PBC*, primary biliary cholangitis; *SLE*, systemic lupus erythematosus; *T1D*, type 1 diabetes mellitus.

## 7.8 Conclusion

With common prevalence of autoimmune disorders and obscure information of their etiology, improvement in life quality in patients suffering from an autoimmune disorder is a challenge. Continuous efforts made from several decades to study the role of gene polymorphisms have proven beneficial. A large number of genetic polymorphisms have been reported in autoimmune disorders and shown to influence susceptibility, clinical manifestations as well as the therapeutic response. It is seen that similar variations have different manifestations between different subgroup of patients. Further investigations and trials would help to understand the role of these polymorphisms better, identify reliable biomarkers, and may also help in developing more appropriate specific immune therapies.

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Chapter 8

# Role of immunogenetics polymorphisms in infectious diseases

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## 8.1 Introduction

The rising infectious diseases represent a serious global economical and public health issue (Binder, Levitt, Sacks, & Hughes, 1999; Lederberg, Hamburg, & Smolinski, 2003; Morens, Folkers, & Fauci, 2004). It has been reported that in the late 1800s, better living conditions like proper hygiene, better water quality, etc., specifically in higher-income countries led to the decrease in the infectious disease burden and by the middle of the 20th century, the availability of more potent and secure vaccines/antibiotics resulted in the reduction of the occurrence of the infectious diseases in such countries (Holmes et al., 2017). Although the infectious diseases and the death rates linked with them have decreased to a large extent, they still represent a remarkable global threat. Currently, the world is fighting old (e.g., plague) as well as new pathogens for example, human immunodeficiency virus (HIV). Some infectious diseases like Malaria and tuberculosis (TB) are endemic to many areas, causing considerable rather steady havocs (Bloom & Cadarette, 2019). Until the end of the 20th century, the world's greatest burden of early deaths and various disorders were found to be associated with infectious diseases. Over the last few centuries, the global pandemics of various infectious diseases like cholera, smallpox, etc., have repeatedly terrorized the survival of the whole human population. In the case of human beings, in the recent past, the occurrence of emerging infectious diseases has increased drastically and is also likely to rise shortly as well (Dikid, Jain, Sharma, Kumar, & Narain, 2013). The process of infection is undoubtedly the most important issue of microbiology and immunology domains (Casanova, 2015). Infectious diseases still represent one of the leading global public health

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00006-3**
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issues responsible for causing millions of deaths yearly, inspite of the adoption of different safety, prevention, control, and treatment measures (Cohen, 2000; Qadri, Qureshi, Mir, & Shah, 2021; Qadri, Shah, & Mir, 2021). Various kinds of bacterial, parasitic, and viral diseases are responsible for causing thousands of deaths yearly in the case of developed countries mainly affecting the older generation and the individuals with a weak immune system in comparison to the millions of deaths mainly involving children in case of developing countries (Murray & Lopez, 1997).

Infectious diseases are being continuously imposing threats on global health and economies, an area that is required to be constantly explored, researched, and updated, respectively (Cupertino, Resende, Mayer, Carvalho, & Siqueira-Batista, 2020; Sheikh, Bhat, Mehraj, Mir, Hamadani, & Mir, 2021). Infectious diseases are considered as the global major terminators of children/young adult human populations (Selgelid, 2005). The developing countries face a great disadvantage in terms of infectious diseases as reported by various health statistic measures (Murphy, 2006). Infectious diseases are bidirectionally associated with poverty, which represents yet another important factor besides the two main associated factors that is morbidity and mortality (Gallup & Sachs, 2001). The proper control and management of the global issue of increasing human infectious diseases are strongly based on the identification and development of possible molecular/genetic strategies/approaches to enhance the immune system potential for sound human health (Mir, 2015; Mir, Bhat, Sheikh, Rather, Mehraj, & Mir, 2020; Nicholson, 2016).

Human genome analysis jointly with novel approaches in promoting and exploring the molecular-immunological features have proven to exhibit a significant influence on the development of new perspectives/avenues in the field of molecular medicine. Thorough knowledge and comprehension of the genetic foundation of complicated, multifactorial infections are quite essential for the identification of the predisposing factors, assessment of the impact of various genetic associations, identifying individual reaction to certain drugs/medicines, and finding/exploring novel strategies for the proper design and development of drugs (Mehra, Kaur, & Jaini, 2004). The human genome consists of enormous and diverse immune response genes pointing to the evolutionary significance of various immunological responses to a broad range of infections/infectious organisms (Apanius, Penn, Slev, Ruff, & Potts, 1997; Archbold et al., 2009). The growing acclaim of the human infectious disease, immunogenetic studies offers the accessibility of a wide range of novel polymorphic candidate genes and the potential to undergo and explore a genome-wide strategy of mapping and eventually the process of identification of novel resistance and/or susceptibility genes/mechanisms (Hill, 1998). Susceptibility to different human infections/diseases generally emerges via the complicated interplay and association of various environmental and host genetic elements. Making significant contributions in the process of the system of genetically complex human disease susceptibility,

diverse genetic loci have been identified to study their impact on different human diseases/infections.

The human leukocyte antigen (HLA) loci represent the potential factors for the phenomenon of infectious disease susceptibility. Multiple studies have reported positive interactions between classical HLA loci and many infectious disease conditions like HIV/acquired immune deficiency syndrome (HIV/AIDS), malaria, hepatitis, TB, etc (Blackwell, Jamieson, & Burgner, 2009). The field of immunological sciences faces a major challenging issue that is the proper knowledge/understanding of the complexities/effects/mechanisms and the processes of the host/pathogen interactions. As far as the immune activation (innate immune response activation) in response to an intracellular bacterial pathogen is concerned, the nature killer (NK) cells and the macrophages are actively involved in the process. The process involves the direct activation of the NK cells by the intracellular bacterial pathogen or the stimulation of the macrophages for the production of cytokines activating the NK cells, resulting in a wide and robust response against the microbial pathogens significant for the control of the process of microbial pathogen dispersion. However, the innate immune response activation procedure restricts the growth of the bacterial pathogen to some extent but to succeed in the process of the complete eradication of the disease/infection, the system of acquired immunity is triggered via the process of cell action (Cardoso, Marangon, Sell, Visentainer, & De Souza, 2014). The proper knowledge and understanding of the HLA/MHC system are of immense significance in the area of medical sciences. The system represents a significant instrument for comprehending the pathogenesis of different infectious diseases; the alleles/HLA haplotypes inherited by a person could speculate different infection-associated risk and defensive elements generated by several agents. Moreover, the connection of HLA/MHC complex with different types of infectious diseases is growing constantly (Cardoso et al., 2014).

In the year 2010, WHO settled a fresh *HLA* naming procedure, however, the task was first initiated in the year 1989 when a massive amount of *HLA* allele sequences were initially examined and named (Nazahah & Koh, 2015). The MHC/HLA system of genes coding antigen-presenting cells/molecules, forming the basis of the process of immune reaction/response initiation, has been ensnared in diverse disease conditions (infectious and autoimmune) through direct and/or indirect association/connection with disease etiology (Mehra et al., 2004). The activation of T cell needs particular identification of the antigen presented as peptides bound to molecules of the major histocompatibility complex (MHC) system. The identification of a specific MHC associated peptide takes place via extremely specific thymus selected T cell receptor (TCR). Following TCR triggering, costimulation and the availability of polarizing cytokines jointly regulate the T cell activation pattern and escort the final T cell differentiation. Simply, the CD4 + T cells identify antigens foraged extracellularly by the APCs which are presented in MHC

class II, while the CD8 + T cells identify endogenic antigens accorded/presented by MHC class I (Benvenuti, 2016; Moreau & Bousso, 2014). The interaction of diverse microbial infections (bacterial, fungal, viral, and parasitic) with the host's HLA system is broadly explored and the immunogenetic analysis of disease susceptibility has strongly supported the identification of strong HLA links with multiple infections/diseases (Alves, Souza, Meyer, Toralles, & Brites, 2006).

## 8.2 The major histocompatibility complex/human leukocyte antigen system: general structure and gene organization

Genetic polymorphism represents a significant characteristic of the system of human biology. The genetic changes/variations strongly influence the human immune system (Jin & Wang, 2003). The immune response process/system includes a series of sequential events wherein the complex antigen is subjected to absorption, procession, and presentation to the T helper lymphocytes, activating the suppressor subpopulations, B lymphocytes, and, cytotoxic T cells respectively. Such activities in the case of humans are modified by highly polymorphic genes, generally categorized under the MHC/HLA complex/system (Compston & Swingler, 1988). Found in all types of jawed vertebrates, the system of the MHC represents a huge complex of genes performing an essential part in different immune system processes (Klein, 1986). The MHC has got its name based on its part in graft rejection and tissue compatibility among the pair of donor and recipient and moreover, the MHC compatibility among persons has been found accountable for the process of proper graft transplantation. The system of MHC/HLA complex has been found marked by the property of large polymorphism, providing the immune system with the ability to identify the pathogenic organisms invading the system (Mosaad, 2015). From over the last five decades, the high degree of polymorphism displayed by the MHC/HLA loci has been recognized to impact various significant biological characteristics and a person's susceptibility to various diseases including infectious, complicated, and autoimmune diseases. The HLA system has also been recognized to perform a significant part in case of various neurological diseases besides the autoimmune and inflammatory diseases, exposing the autoimmune elements in such diseases/disorders (Hamza et al., 2010; Hill-Burns, Factor, Zabetian, Thomson, & Payami, 2011; Purcell et al., 2009; Shatz, 2009; Song et al., 2016). Generally, the immune system of humans is modulated by the molecules coded by certain genes, including the genes of the human histocompatibility complex, coding the HLA (Alves et al., 2006).

The HLA system is situated on the chromosome 6 (short arm) in the lateral segment of 6p21.3 band, around 1−2 cm in length and grouped into three classes (I, II, and III) (Fig. 8.1) (Allcock, 2012; Alves et al., 2006; Compston & Swingler, 1988). The two MHC regions (class I and II) are so

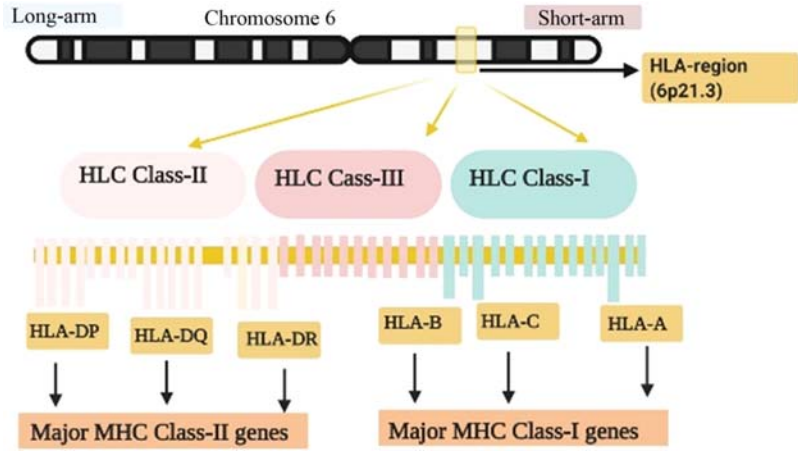


**FIGURE 8.1** Diagrammatic illustration of the MHC/HLA complex on the Human chromosome six.

named fundamentally due to the encoded HLA genes within them and the class III region having no such defining characteristic harbors a huge amount of genes of the variable role, inside/outside the immune system (Allcock, 2012). The class I region of MHC lies at the telomeric end, while as the class II region at the centromeric end of the short arm of the chromosome 6, respectively, and in between the two regions lies the class III region (central MHC) of about 1 Mb size (Allcock, 2012; Shiina, Hosomichi, Inoko, & Kulski, 2009). These three regions encode 3 classes of cell surface glycoprotein performing the most significant part in antigen presentation, self/non-self-identification, and directing the T cell-mediated immune responsiveness mechanism. The HLA antigens are usually mentioned as "Transplantation-antigens" because of their significance in graft exclusion and resistance procedures (Mehra et al., 2004).

The MHC/HLA complex is the major intensely studied human genome portion with a variety of distinctive characteristics. The complex is the major dense portion of the human genome in terms of genes, SNPs, complex haplotypes along with other essential characteristics. Greater gene density, short intergenic lengths, the greater number of SNPs, low recombination levels, etc. are some of the striking characteristics owned by the MHC/HLA complex, which are required to be analyzed (Allcock, 2012). Lying at the surface of nearly all the cells, the HLA molecules represents the polymorphic membrane glycoproteins. Inside the MHC/HLA complex, various genetic loci encode such proteins, and a single person expresses at the same time many polymorphic patterns from among a massive store of allelic population. Moreover, the entire class I and class II HLA molecule's structure is alike, having majorpolymorphic features/polymorphisms detected inside the

peptide-binding groove, a place for antigen recognition (Bjorkman et al., 1987). The MHC complex offers a background for the antigen identification by T lymphocytes (Singh, Agrawal, & Rastogi, 1997). Binding of the pathogen acquired peptide parts/fragments and their presentation on the surface of the cell for identification by the suitable T cells is the major part of the molecules/components of the HLA/MHC system (Janeway, Travers, Walport, & Shlomchik, 2001). The MHC class I and class II molecule's polymorphic binding site comprises of a ß-pleated sheet flanked by two α-helixes forming a groove accommodating a single microbial peptide ligand (Fig. 8.2) (Singh et al., 1997). The glycoproteins encoded by class I MHC genes are located on the cell surface of all the nucleated somatic cells, while the expression of glycoproteins encoded by class II MHC genes is mostly confined to the APCs like macrophages, dendritic cells, and B cells and that both the classes have extracellular areas forming the peptide-binding region. Moreover, the Class I MHC molecules direct the presentation of the peptide (antigen) fundamentally arose from endogenic sources to CD8 + cytotoxic T lymphocytes (CTLs), while the class II MHC molecules are particularly functional at directing the presentation of peptide fundamentally stemmed from exogenic sources to CD4 + helper T cells (Fig. 8.3A–B) (Saghazadeh & Rezaei, 2019). Moreover, due to its significant part in the process of autoimmune and infectious disease susceptibility, and diagnostic potential features as part of transplantation and rejection procedures, the HLA complex offers immense research value and potential (Allcock, 2012). The HLA

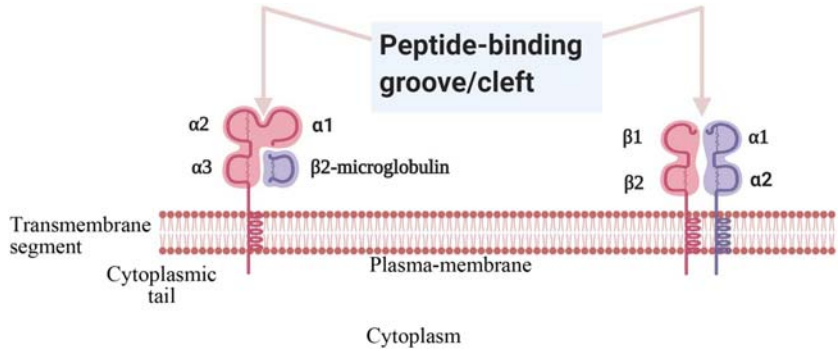


**FIGURE 8.2** Structural organization of MHC/HLA Class I and MHC/HLA Class II. HLA Class I is composed of a heavy chain having 3 globular domains (α1, α2, and α3) non-covalently bound to β-2m (β2microglobulin). On the other hand HLA Class II is composed of two heavy chains (α-chain and β-chain) each with two globular domains viz: α1 and α2 or β1 and β2. Moreover, HLA Class-1 (α1 and α2 domains) and HLA class II (α1 and β1 domains) , are involved in the formation of peptide-binding groove/cleft (Hickey et al., 2016).

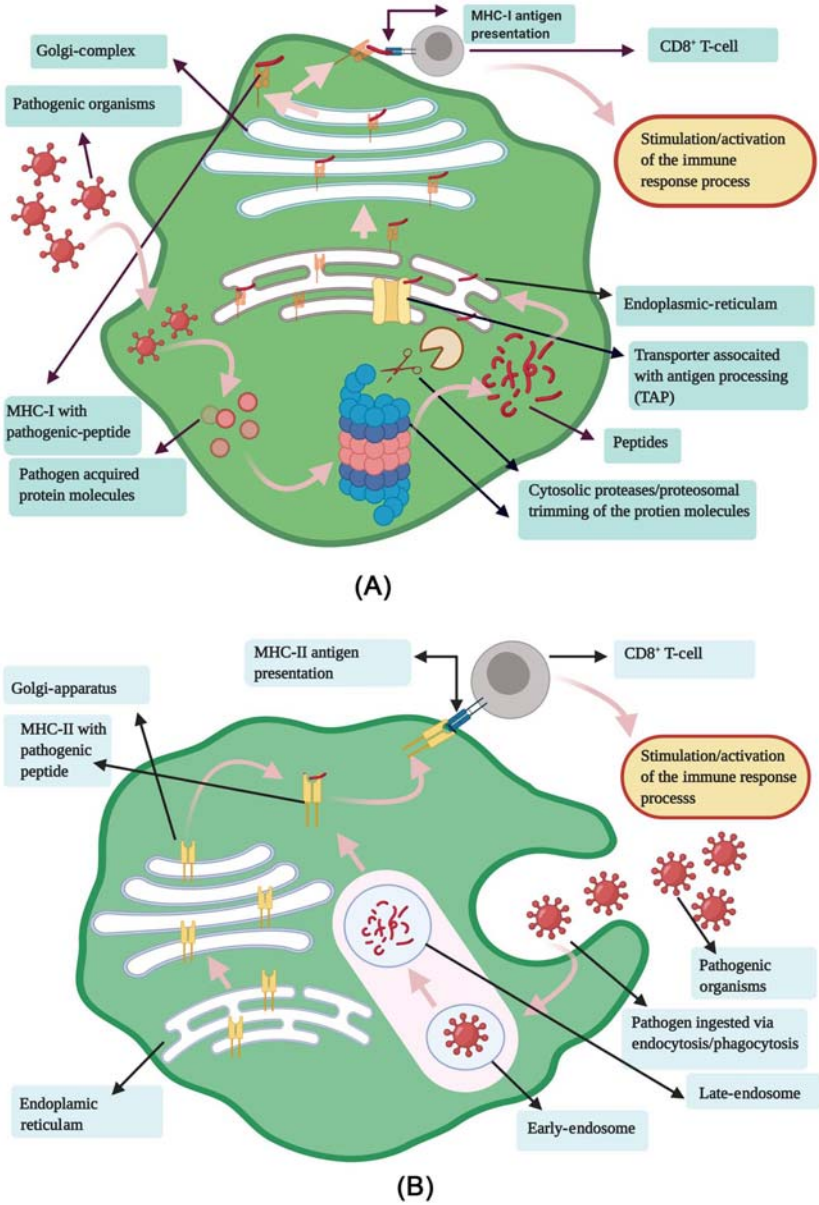


**FIGURE 8.3** (A & B): Schematic illustration of the processing and presentation of endogenous and exogenous antigen presentation through the major histocompatibility complex class I (MHC-I) and the major histocompatibility complex class II (MHC-II) for the activation/stimulation of the proper immune response processes.

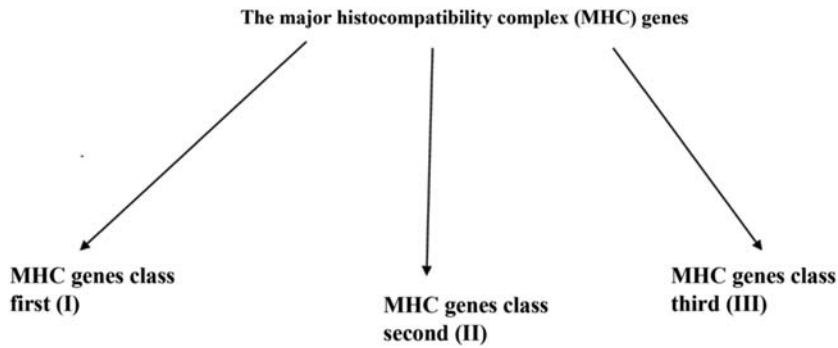


**FIGURE 8.4** A simple representation of the classification of MHC genes.

antigens are widely studied for their part in transplantation biology, population diversity, and human diseases (Hamed et al., 2018).

## 8.3 Classification of major histocompatibility complex genes

Spanning over a zone of around four million base pairs, the system of HLA/MHC complex includes above a hundred genes. Generally, MHC genes are grouped into regions encoding three classes of molecules, as represented in Fig. 8.4:

### 8.3.1 Major histocompatibility complex genes class I

The surface of all the nucleated cells express glycoproteins encoded by Class I MHC genes. The main role of class I MHC proteins is the presentation of endogenic peptide antigens from damaged intracellular proteins and peptides from invasive viruses to CD8 + cells. The HLA complex comprises of three major/minor MHC class I genes viz;

1. Major MHC class I: (i) HLA-A, (ii) HLA-B, and (iii) HLA-C.
2. Minor MHC class I: (i) HLA-E and (ii) HLA-F and (iii) HLA-G

A heterodimer is formed via the binding of HLA-G β2-microglobulin with the major/minor gene subunits, respectively.

### 8.3.2 Major histocompatibility complex genes class II

Class II MHC includes genes encoding glycoproteins which are principally expressed on APCs for example, macrophages, dendritic cells, and B cells, fundamentally process exogenic peptide antigens for presentation to CD8 + cells. The HLA complex comprises of three major and two minor MHC class II genes viz;

1. Major MHC class II: i) HLA-DP: a) α-chain encoded by HLA-DPA1 locus b) β-chain encoded by HLA-DPB1 locus.
   a. HLA-DQ: (i) α-chain encoded by HLA-DQA1 locus; (ii) β-chain encoded by HLA-DQB1 locus.
   b. HLA-Dr: (i) α-chain encoded by HLA-DRA locus; (ii) β-chains encoded by HLA-DRB1, HLA -DRB3, HLA-DRB4, and HLA-DRB5 loci.

Consistently expressed on the surface of APCs, the class II MHC genes integrate for the formation of heterodimeric (αβ) protein receptors.

2. Minor MHC class II: (i) DM and (ii) DO.

### 8.3.3 Major histocompatibility complex genes class III

Class III MHC includes genes encoding diverse proteins such as proteins with immune regulatory functions/properties, including the components of the complement system like tumor necrosis factor (TNF), heat shock proteins, etc. (Mosaad, 2015; Shiina, Inoko, & Kulski, 2004; Cruz-Tapias, Castiblanco, & Anaya, 2013; Mahdi, 2019; Trabace, 2000).

Moreover, the MHC class II region is comparatively uniform, comprising genes chiefly encoding class II HLA proteins (α and β subunits), and a few amounts of genes having a part in antigen processing for class I proteins (TAP and PSMB genes). In comparison, the class I region encodes HLA-A, HLA-B, and HLA-C (classical) and HLA-E, HLA-F, and HLA-G (non-classical) proteins, cluttered with the remains of diverse evolutionary evidence in the shape of pseudogenes. Additionally, class I MHC region encodes a remarkable amount of genes belonging to Tripartite-motif (TRIM) and butyrophilin (BTN) families, exhibiting various immune-associated functions, and also encoding a major quantity of genes exhibiting roles influencing the immune system directly/indirectly and not confined to the immune system alone. The different kinds of gene families present in the MHC-system are possibly due to the outcome of numerous duplication events and the individual gene pairs could be alike (like HLA-B and HLA-C), or very related to each other or very divergent (Allcock, 2012; Choy & Phipps, 2010; Posch, Cruz, Bradshaw, & Medhekar, 2003). The genes belonging to the HLA system are firmly associated and in a Mendelian trend from each parent, the whole MHC system is inherited as an HLA haplotype. HLA genes are firmly joined and the intact MHC is inherited as an HLA haplotype in a Mendelian manner from each parent. In a family, HLA haplotype segregation is allocated by family HLA research studies. In the case of 2 siblings, there are 25% chances of them being genotypically HLA similar, 50% chances of being HLA haploidentical, and 25% chances of sharing no HLA haplotypes at all. The potential random antigen combinations from various HLA loci on an HLA haplotype are infinite, but certain HLA haplotypes are present very often in few populations than expected by chance. The process is known as linkage disequilibrium (Choo, 2007).

## 8.4 Human leukocyte antigen system and the infectious diseases (function and association)

Infectious diseases are often linked with an impaired/weak immune system. Certain individuals escalate a powerful immune response on giving vaccines, while other individuals show poor responses to vaccines or no response at all. There are different factors which can determine the degree/extent of response, including the infection severity, factors associated with the host immune response intensity/severity, the state of T cells, the function of T cells, and finally the significant one that is the genetic element interacting with additional elements determining the consequences of the disease/infection. The study and research on infectious diseases are multifold, focusing on different genetic aspects of the disease/infection for example, study on genetic markers viz: the allelic forms of HLA complex molecules (Singh et al., 1997). In the case of humans, the HLA complex represents the major polymorphic genetic system, with multiple alleles, and therefore diverse feasible combinations (Alves et al., 2006; Fernandes, Maciel, Foss, & Donadi, 2003; Kazuo, 2000). HLA complex in humans is a group of gene multiplex, which encodes the MHC proteins referred to as antigens at the cell membrane surface of leukocytes in humans (Mahdi, 2019). The class I and II genes HLC encode molecules lying at the core of the acquired immune response against various infectious diseases (Martin & Carrington, 2005). The HLA complex genes are expressed co-dominantly and are highly polymorphic, having different alleles modulating the adaptive immune system which aids the body to differentiate its protein molecules from the foreign invading proteins such as bacterial, viral, and other pathogenic proteins (Trowsdale, 1993). The HLA complex polymorphism promotes the phenomenon of genetic diversity of the species and the variations in disease susceptibility within the genetically definite groups, thereby restricting the occurrence of mass-level epidemics (Van Rood, 1993). The HLA system associated molecules are involved in the process of antigen presentation and the T lymphocytes can recognize antigens only when connected to HLA molecules and because of their potential part in the activation of the immune response, the HLA antigens can eventually assist in the control and management of the resistance and susceptibility mechanisms of various diseases/infections (Alves et al., 2006; Fernandes et al., 2003; Kazuo, 2000; Singh et al., 1997).

As far as the genetic factors as the determinants of susceptibility to human infectious diseases are concerned, the HLA complex happens to be a significant system accountable for the diverse clinical forms of several human infectious diseases (Alves et al., 2006; Marcos, Souza, Ura, & Opromolla, 2000). For a successful, systematic, and well-organized activation of the immune response against a particular infectious agent, the HLA complex molecules should be able to connect the pathogen-derived

peptides, and that the concerned T cells should involve the clones to be activated by HLA-peptide linkage (Klein & Sato, 2000). If a person's HLA molecules do not function efficiently, the result could be an impaired immune response to the harmful infectious pathogenic organisms (Alves et al., 2006; Kazuo, 2000). The susceptibility to the infection/infectious disease is greatly influenced by the defects/impairment in certain stages of the system/complex (Klein & Sato, 2000). As a process of protection and safety, the HLA complex genes likely select and stimulate the T cells which proliferate and eradicate the invading pathogen, via the generation of inflammatory cytokines and/or by damaging the infected cells themselves (Mack et al., 1999).

The genes of the MHC/HLA system show coordination with different non-immunological genes such as the noncoding RNA genes, involving the expressed pseudogenes. These genes displaying haplotype-specific linkage disequilibrium patterns carry the strongest Cis eQTLs/meQTLs and TranseQTLs/meQTLs in the genome and are referred to as a hot spot for disease connections. The haplotype HLA-Dr/DQ is very inconstant in terms of structure and displays a greater amount of disease connections (Kennedy, Ozbek, & Dorak, 2017). Multiple genome-wide association studies (GWAS) have revealed the significant part/role of the MHC/HLA complex in disease association, such as autoimmune diseases (Trowsdale & Knight, 2013; Zhou et al., 2016). Over the last few decades, the different population studies/researches done have recognized various human diseases that are remarkably more frequent between persons carrying the specific HLA alleles involving autoimmune, inflammatory, malignant diseases/disorders (Holoshitz, 2013; Mosaad, 2015). The process of HLA disease association and its basic underlying procedure is a well-focused/studied hot topic in the field of medical science. Moreover, it has been studied and found that the process of social behavior is also influenced by HLA complex genes and the choice for HLA distinct partners may supply "better genes" for a person's progeny. Additionally, specific HLA genes have been found connected with the phenomenon of shorter/longer lifespan, the impact of which mainly depends on the genetic settings as well as the environmental conditions (Mosaad, 2015). The abundance and the occurrence of HLA alleles tend to vary between various populations. Multiple reports propose that the alleles conferring resistance to specific pathogenic organisms are prevalent in regions with endemic infectious diseases. Additionally, the genomic analysis in families has aided in the mapping and identification of the loci associated with multiple infectious diseases. Fortunately, with the aid of the system of genomic analysis of families, many infectious diseases are successfully mapped and also their allied loci have been successfully recognized accordingly (Cardoso et al., 2014). The process of HLA typing has been carried out in various medical areas, such as matching in transplantation, forensic medicine, disease connection and other related processes and procedures (Trabace, 2000).

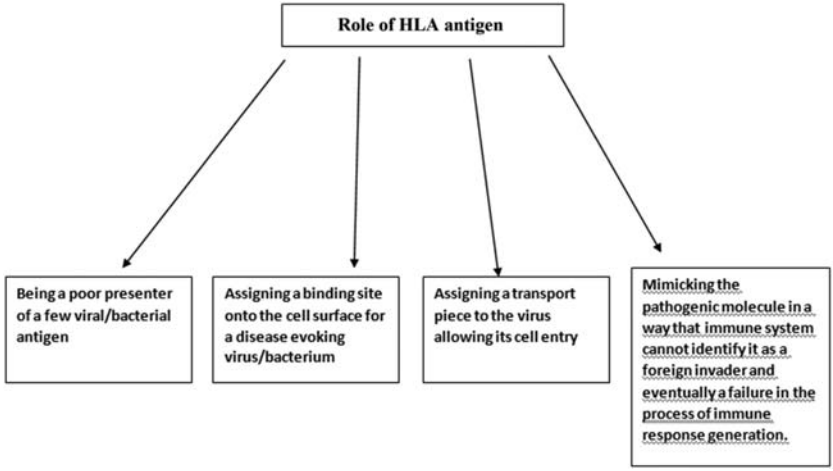


**FIGURE 8.5** Different models highlighting the important role of HLA antigen in different diseases.

Numerous genetic research studies have given valuable evidence in the favor of the association of HLA loci and various autoimmune and inflammatory diseases; gastrointestinal diseases; psychiatric disorders; neurological disorders; infectious diseases (Saghazadeh & Rezaei, 2019). Based on the observation that certain diseases are markedly very frequent in persons having a particular HLA allele/haplotype enabled studies on the phenomenon of HLA system and disease connections (Trabace, 2000). Possibly, there are 2 common descriptions of HLA and disease connections/associations. Firstly, there could be linkage disequilibrium among the alleles at a specific disease-linked locus and the associated HLA allele with that disease like in the case of HLA-A3 and idiopathic hemochromatosis. The second common description for such associations could be the HLA antigen itself performing an important part in the disease, which can be understood by one of the following models as represented below (Trabace, 2000) (Fig. 8.5). Probably, all these processes are included, in a diverse range of various diseases. It has been reported that the MHC/HLA system in association with additional non-linked genes impacts the environmental factors response thereby evoking disease (Trabace, 2000). Association between some infectious diseases and the HLA complex is represented in Table 8.1.

## 8.5 Human leukocyte antigen system and the human immunodeficiency virus

Since the 1918 influenza epidemic, the HIV/AIDS, represents the worst human pandemic. The disease has been found associated with millions of

**TABLE 8.1 Association between infectious diseases and human leukocyte antigen.**

| S. NO | Concerned disease | Associated HLA antigen/allele | References |
|---|---|---|---|
| 1. | Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS) | *HLA-B*35, HLA-Cw*04HLA-B*57HLA-B*1503, HLA-B*0801; HLA-B*w4* | Carrington et al. (1999), Catano et al. (2008), Boulet et al. (2008), López-Larrea et al. (2005), Yindom et al. (2010), Welzel et al. (2007), Shankarkumar and Sridharan (2011) |
| 2. | Tuberculosis (TB) | *HLA-DRB2*1501* and *HLA-DRB1*150 expressed HLA-Dr2HLA-DRB1*07* and *HLA-DQA1*0101, HLA-DQA1*0301* and *HLA-DQA1*0501HLA-DPB1*04, HLA-DRB1*1501* and *HLA-DQB1*0601* | Mehra et al. (2004), Rajalingam et al. (1996), Amirzargar et al. (2004), Ravikumar et al. (1999) |
| 3. | Malaria | *HLA-Bw53, HLA-DRB1*1302-DQB1*0501HLA-A3, HLA-B27*, and *HLA-B49HLA-DRB1*04* and *HLA-DRB1*0809*HLA-A19, HLA-A34, HLA-B18, and HLA-B37 and HLA-DQB1*0203HLA-DQB1*0301 and HLA-DQB1*03032 | Hill et al. (1991), Shankarkumar et al. (2002), Olivo-Díaz et al. (2004), Johnson et al. (2000), Shankarkumar and Sridharan (2011) |

deaths after being first encountered in the year 1918, hitting mainly Sub-Saharan Africa. Fortunately, because of different advanced preventive measures/interventions and the application of life-enhancing antiretroviral treatment therapies, many positive outcomes have been reported (Holmes et al., 2017). Among a few infections, the HIV/AIDS defines a definite and compatible HLA connection (Martin & Carrington, 2013). The interconnection between the HLA genes and HIV/AIDS disease progression/advancement has been widely studied because of the significant part of the HLA locus in the process of immune surveillance (Carrington et al., 1999).

The chief impact of HLA loci on the HIV respective to all other single human genetic variants is successfully reported utilizing various GWAS, pointing towards the significance of HLA locus in the differential control of HIV disease across humans (Martin & Carrington, 2013).

As there is a variable extent of immunity to HIV/AIDS, the genetic factors accounting for around one-fourth of this variation includes the genes of the HLA system which interact with CD8 + cytotoxic T and CD4 + helper T cells to aid in the process of an adaptive immune response initiation against the disease, providing HIV resistance (Saghazadeh & Rezaei, 2019). If the CD8 + T cells fail to detect the aberrant HLA I molecules, the cellular immune defense will not be provided against the virus (Weber, 2001).

On combining precisely with the innate myelomonocytic HLA class I receptors, the defensive HLA class I molecules limit the impact of HIV1-specific CD8 + T cells on the role of the dendritic cell. These bidirectional interplays between such two specific cell kinds add to natural HIV-1 immune control, pointing to the modulatory associations between innate and adaptive immune activities at the time of successive antiviral immunologic defense processes (Martin-Gayo & Xu, 2017; Pymm et al., 2017). A high degree of heterozygosity at the HLA loci has been observed and reviewed as an additional particular benefit against AIDS/HIV infections as it grants the host immune system with the capacity to and fight a broad array of pathogenic organisms, and hence in the case of heterozygous individuals in comparison to homozygous ones, the escape mutants took longer to emerge (Carrington et al., 1999). *HLA-B*35* and *HLA-Cw*04* have been usually connected with the advancement of AIDS-describing conditions. It has been reported that in the Caucasian patients, infected with HIV-1, the HLA heterozygosity of class I loci delayed the outbreak of AIDS, while the persons homozygous at one or more loci abruptly advanced to the infection and demises (Carrington et al., 1999). Multiple studies have given adequate evidential support in favor of the confirmation of *HLA-B*57* connected with a lower risk of HIV infection (Boulet et al., 2008; Catano et al., 2008; López-Larrea et al., 2005). *HLA-B*1503* was found to be linked with a poor prognosis after HIV-2 infection, and *HLA-B*0801* was linked with susceptibility to infection and also in the HIV-1-infected persons the presence of *HLA-B*w4* was reported to be linked with a lower risk of male-to-female HIV-1 transmission (Welzel et al., 2007; Yindom et al., 2010). Moreover, the KIR genes have also been ensnared in the process of providing resistance to HIV infection (Saghazadeh & Rezaei, 2019). Other potential candidate genes like MICA, MICB, TAP, etc., in addition, to the classical HLA molecules are also actively involved in terms of function in different immunomodulatory processes for strengthening the immunogenetic foundation of disease progression/advancement (Mehra et al., 2004). Meanwhile, there is a prominent concurrence among various *HLA* alleles connected with HIV as well as HCV infection clearance, specifically including *HLA-B*27*. Further associations with *HLA-B*57* and

*HLA-B*08* Dr3 are very little compatible and in a few subpopulations could relate to variations in viral sequences (Petrovic, Dempsey, Doherty, Kelleher, & Long, 2012).

## 8.6 Human leukocyte antigen system and tuberculosis

The bacterium *Mycobacterium tuberculosis*, commonly entering through the mucosal lung surface of the body, causes TB disease. Although pulmonary disease represents a major frequent clinical outcome, disseminated/miliary TB and tuberculosis meningitis represent other such important issues, with increasing death rates (Blackwell et al., 2009). A well-known chronic infectious disease TB has been regarded as a main global public health issue affecting mainly the lungs causing Pulmonary Tuberculosis disease condition. Around 1/3rd of the population of the world has been found in contact with the pathogenic organism *Mycobacterium tuberculosis*, however, about 90% of the infected individuals display no clinical symptoms (Dye, Williams, Espinal, & Raviglione, 2002).

The pathogen *Mycobacterium tuberculosis* is detected by the innate immune receptors viz: CLRs, TLRs, and NLRs followed by the adoption of various strategies/processes by which the immune cells such as macrophages, T cells, and different cytokines like IL-12, IFN-$\gamma$, IL-4, TNF-$\alpha$, TGF-$\beta$, etc., regulate diverse antimycobacterial functions/activities (Saghazadeh & Rezaei, 2019). The major effector cells are the Th1 CD4 + T cells producing gamma interferon, mainly in the primary infection stages (Blackwell et al., 2009). The class II HLA gene and the molecule's showing large polymorphism has been found to result in genetically controlled inter-individual differences in antigen-specific immune responsiveness, leading to disease expression/differential susceptibility to the infectious disease. In the course of mycobacterial infections, the cytolytic CD4 + Th1-like cells induction has been widely reported (Ab et al., 1990; Mutis, Cornelisse, & Ottenhoff, 1993). Some of the important cell types like CD8 + T cells, $\gamma\delta$ T cells, CD1-restricted T cells, and NK cells are recognized and reported to be significant gamma interferon producers against *M. tuberculosis* resistance (Ladel, Blum, Dreher, Reifenberg, & Kaufmann, 1995). It has been reported that in mice study, the deficiency in MHC class II impedes the acute infection response, while the deficiency in MHC class I display minute effect in the acute infection phase but stands critical during the chronic infection process (Ladel, Daugelat, & Kaufmann, 1995; Rolph et al., 2001; Sousa et al., 2000). The HLA-DRB2*1501 and HLA-DRB1*150 expressed HLA-Dr2 antigen has been found linked with the advancement of acute and multibacillary TB-forms, along with the higher frequency of forms resistant to drug therapy (Rajalingam, Mehra, Jain, Myneedu, & Pande, 1996). It has been reported that HLA-DRB1*07 and HLA-DQA1*0101 alleles are linked with higher susceptibility to pulmonary TB advancement and that the

HLA-DQA1*0301 and HLA-DQA1*0501 alleles have been found linked with protection against such infection (Amirzargar et al., 2004). Moreover, the heterozygote fusion of different HLA-Dr antigens has also been found to impact the tuberculin reaction in pulmonary TB (Selvaraj et al., 1996). Additionally, a study illustrated the connections of the HLA-DPB1*04 allele with TB protection, while HLA-DRB1*1501 and HLA-DQB1*0601 were found to be linked with susceptibility to TB (Ravikumar et al., 1999). Another study has reported the connection of the HLA-DRB1*1501 allele with susceptibility to TB, highlighting the significance of the HLA-DRB1*1501-DRB5*0101-DQA1*0103-DQB1*0601 haplotype (Mehra, Rajalingam, Mitra, Taneja, & Giphart, 1995). Further, meaningful studies should be done to find the significance of HLA and non-HLA genes in the determination of the heritable risk for *M. tuberculosis*-associated diseases/infections (Blackwell et al., 2009).

## 8.7 Human leukocyte antigen system and malaria

Malaria represents a global public health issue that is caused by *Plasmodium* parasites (Lima-Junior & Pratt-Riccio, 2016). The malarial disease imposes both micro/macroeconomic impacts in the affected areas/regions including reduced income, foreign investment, tourism, and growing expenditures of health, while the regions controlling the malarial disease display greater life expectancies and economic advances. The countries infected with the malarial disease suffer many consequences such as limited availability of medicine/health care resources, lower incomes, minimal health information, restricted education, poor hygienic conditions/system, etc. (Murphy, 2006). The disease has various clinical results, and a vast number of minute genetic consequences have been reported (Blackwell et al., 2009; Burgner, Jamieson, & Blackwell, 2006).

The genes situated in the HLA has been found to secure people in endemic areas against the acute malarial forms produced by the *Plasmodium* species viz: *Plasmodium falciparum* and *Plasmodium vivax*. During malarial infection, the antibody response produced is of peculiar notice, as the generation of particular IgG antibodies is necessary for the procurement of clinical immunity. Nevertheless, the genetic polymorphisms of the class II HLA genes could result in antibody response variations (Cardoso et al., 2014). The study was done by Hill et al. (1991) have reported the significance of HLA genes impacting malarial disease outcomes and demonstrated the effect of HLA genes in resistance against the intracellular pathogenic organism and the process of the evolution of HLA-genes polymorphism via the selection of pathogen-derived molecules. Their study done in West Africa with over 2000 children, reported that carriers of HLA Class I-Bw53 and HLA class II-DRB1*1302-DQB1*0501, usually arising in sub-Saharan Africa, were secured against the acute malarial disease (Hill et al., 1991). The rising

prevalence of the HLA-A3, HLA-B27, and HLA-B49 antigens and of the HLA-DRB1*04 and HLA-DRB1*0809 alleles has been reported in India and that in a similar population the HLA-A19, HLA-A34, HLA-B18, and HLA-B37 antigens and the HLA-DQB1*0203 allele were reported to be connected with protection (Olivo-Díaz et al., 2004; Shankarkumar et al., 2002). A study has reported that immunity against *Plasmodium falciparum* differs concerning the age of a person and that the HLA-DQB1*0301 and HLA-DQB1*03032 alleles only display age-related relation with antibody levels against the plasmodium's rRAP1 proteins in children from 5−15 years age group and after this age, the immunity against the disease is free of the two HLA- alleles. The study reported HLA impact on malarial disease progression concerning age, stimulated through various plasmodial antigens, and also the HLA complex can impact antibody quantities to be generated against the parasitic organism (Johnson et al., 2000).

Numerous research studies have reported the significance of ethnic background, which is required to be considered in the process of the development of a perfect vaccine for the malarial disease. Moreover, comparing the HLA-relations of different world populations, multiple studies indicate the respective significance of various HLA-alleles varying in diverse populaces (Hananantachai et al., 2005; Lima-Junior et al., 2012; Lulli et al., 2009; Osafo-Addo et al., 2008; Shankarkumar et al., 2002).

## 8.8 Conclusion

Millions of deaths yearly are caused by various infectious diseases, representing one of the leading global public health issues, in spite of the adoption of different safety, prevention, control, and treatment measures (Cohen, 2000). The connection of several microbial infections viz: bacterial-infections, fungal-infections, viral-infections, and parasitic infections with the host's HLA system is broadly explored and the immunogenetic analysis of disease susceptibility has markedly supported the identification of strong HLA links with multiple infections/diseases. The extremely polymorphic HLA system is involved in the process of immunity and within the human genome, it occurs in a disease-associated region. HLA genome area/regions how connections with multiple diseases, viz: cancer, infectious and autoimmune diseases. Due to its large linkage disequilibrium, the HLA complex is a major appealing and useful region uncovered in various useful and viable research studies (Gao et al., 2019). Understanding the genetics of the processes involved in providing protection/resistance against, and susceptibility to, infectious diseases is an essential step toward the control of such diseases in endemic areas (Alves et al., 2006).

In summary, this chapter provides a complete description of the idea and role of immunogenetics polymorphisms in different infectious diseases (HIV/AIDS, TB, and Malaria), their significance in the process of infection in a

way providing meaningful insights into the disease pathogenesis mechanisms and eventually aiding in the process of identification and development of novel molecular/immunogenic targets/strategies. The proper knowledge and comprehension of the relationship of the highly polymorphic HLA complex and infectious diseases will help to improve immunomodulatory therapeutics and the development of improved vaccines against the highly emerging infectious diseases.

## Acknowledgments

Science and Engineering Research Board, Department of Science and Technology (SERB-DST) Government of India, New Delhi; Project No: TAR/001213/2018 is highly appreciated for its financial assistance.

## Disclosure/conflict of interest

None declared.

## Author's contributions

Manzoor A. Mir designed, supervised, and edited the Chapter. Hafsa Qadri wrote the manuscript. Abdul Haseeb Shah and Manzoor A. Mir designed figures and tables. All the authors edited, read, and approved the final manuscript.

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Chapter 9

# MicroRNAs and their role in immunogenetic-dysregulation

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## 9.1 Introduction

MicroRNAs (miRNAs) are normally encoded in introns and are located near the genes they regulate. Although, most of the miRNAs and their target genes share the same promoter but this is not always the case. As a result, sequence-based prediction has played a major role in miRNA target identification. The miRNA database miRBase has a list of known miRNA and their target genes (Kozomara & Griffiths-Jones, 2011, 2014). RNA Pol II and probably viral Pol III transcribe miRNA from the genome, resulting in the development of primary miRNA which are around 1 kb in size (Lee et al., 2004). The transcript hairpin loop-like structure produced is immediately cleaved by enzyme Drosha (nuclear RNase III), resulting in the production of primary miRNA, a hairpin loop shaped around 65 nucleotides long (Lee et al., 2003). A complex is formed between the pre-miRNA, exportin 5 and GTP-binding nuclear protein RAN-GTP, which transports the pre-miRNA into the cytoplasm where the pre-miRNA is released after the GTP is hydrolyzed. The miRNA is normally cleaved by enzyme dicer and after that binds with the RNA-induced silencing complex (RISC) in the cytoplasm and then interacts with its target RNA. Long noncoding RNA expression, histone acetylation and deacetylation, DNA methylation, and microRNA (miRNA) expression are some genetic pathways used by cells to control gene expression (Netea et al., 2016). Regulation of the immune and nonimmune cells by miRNA has been a topic of investigation since from last few decades (Devasthanam & Tomasi, 2014; Maudet, Mano, & Eulalio, 2014).

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00005-1**
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A single miRNA is thought to be able to control hundreds to thousands of target genes, implying that miRNA regulates the majority of our human genes (Ambros & Chen, 2007; Lewis, Burge, & Bartel, 2005; Lewis, Shih, Jones-Rhoades, Bartel, & Burge, 2003). Over 100 miRNAs have been shown to influence the molecular pathways that regulate the production and function of both inborn and adaptive immune response. Many miRNAs have been found to get down regulated in many inflammatory autoimmune disorders and also have been found to act either as proinflammatory or anti-inflammatory regulators depending on their goal RNAs (Wightman, Ha, & Ruvkun, 1993). Some miRNA genes or miRNA target sites have mutations or single nuclear polymorphisms (SNPs) that are linked to the initiation and development of inflammatory autoimmune diseases (Lee, Feinbaum, & Ambros, 1993; Reinhart et al., 2000). MiRNA expression profiles are widely used for the diagnosis and prognosis of human autoimmune disorders, based on these results. MiRNAs are the main players and mediators in developmental timing, organogenesis, haematopoiesis, cell proliferation, apoptosis, and oncogenesis, in addition to their function in immune response and autoimmune disorders. In 2002 Croce's group confirmed two micro-RNAs, miR-15a, and miR-16−1, from within the commonly deleted region 13q14 to be down-regulated in the most of cases of lymphocytic leukemia suggesting a role in cancer by aberrant or failure of expression of miRNAs (Calin et al., 2002). The discovery of a genome-wide association between the genomic position of miRNAs and regions involved frequently in deletion, amplification, chromosomal breakpoint, and loss of heterozygozity in cancer pathogenesis provided further proof of a connection between miRNAs and cancer pathogenesis (Calin et al., 2004). In all cancer forms, miRNAs tend to play a direct role in oncogenesis and can function as both oncogenes (miR-155 and members of the miR-17 & miR-92 cluster) and tumor suppressors (miR-15a and miR-16−1). The concept of a cellular miRNA signature ('miRNome') has been shown to allow for more accurate diagnostic differentiation of normal from malignant tissue and subset definition within a tumor type than conventional gene expression analysis (Lu et al., 2005).

## 9.2 Genetic bases of immune response

The link between genetics and immune dysfunction was first made when Burton identified a gamma globulinemia, the first primary immunodeficiency, approximately 65 years ago (Ochs, & Hitzig, 2012). Several years later, the diagnosis of extreme combined immunodeficiency (SCID) was made. Over 240 unique DNA mutations that cause primary immunodeficiency have now been identified thanks to technical developments in molecular biology and human genome sequencing (Al-Herz et al., 2014; Hernandez-Trujillo, 2014; Raje & Dinakar, 2015). Gene defects impact a spectrum of immune functions from limiting or preventing development of

specific immune cells to mutations that affect immune cell functions, including some leading to increased susceptibility to specific organisms. Mutations causing primary immunodeficiencies are rare and most critically ill children do not have a primary immunodeficiency. However, studies of adoptees indicate that an individual's genetic make-up influences the outcome from infection (Sørensen, Nielsen, Andersen, & Teasdale, 1988). Adoptees with a biological parent who died due to infection before the age of 50 had a higher relative risk of death due to infection than the relative risk of death due to cardiovascular disease, cerebro-vascular disease or cancer for adoptees with a biologic parent who died early of the indicated diseases. This observation combined with data from the Human Genome and the HAPMAP projects suggest that common genetic variants might contribute to an individual's susceptibility to infection, disease severity, and/or response to treatment.

The role of genetic variants in disease is generally explored using either candidate gene (genes for proteins known to be involved in the disease) or genome wide association studies (GWAS). For sepsis and acute respiratory distress syndrome (ARDS) most studies have used a candidate gene approach, as GWAS requires hundreds to thousands of patients. There is a lack of consensus between many of the early genetic association studies stemming from the initial lack of understanding of the differences in frequency of genetic variants and in linkage disequilibrium between races and ethnicities, the inadequate power in many studies, and the failure to correct for multiple comparisons. In addition, the characteristics of cohorts of both sepsis and ARDS patients often vary between studies as both diseases have multiple triggers and manifestations and heterogeneity in phenotypes confound genetic association studies. It is now clear that observed association of genetic variants with disease needs to be replicated in different cohorts and that the variant should either result in a change in level or function of the encoded protein, or be in linkage disequilibrium with a variant that affects level or function. In addition, the protein encoded by the gene with the variant should be examined for biologically plausibility if it does not already have an established role in the pathologic process. Recently a number of common genetic variants associated with risk or outcome of sepsis or ARDS which meet the criteria described above have been reported in adult studies (Chung & Waterer, 2011; Meyer et al., 2013; Reddy & Kleeberger, 2009; Sapru & Quasney, 2011; Wong, 2012). Variants identified in genes related to the immune system in which multiple studies have suggested a role in infection, sepsis or ARDS are described below focusing on those variants either implicated in children, or identified recently. Common single nucleotide variants (SNVs) in the Toll-like receptor 1 gene (*TLR1*) have been implicated in differences in immune function in both adults and children. TLR1 forms a heterodimer with TLR2 and recognizes bacterial lipopeptides, lipoteichoic acid (from gram positive bacteria), and yeast (Opitz, van Laak, Eitel, & Suttorp, 2010). The *TLR1* gene has two variants, one in the

promoter region and one that results in an amino acid change, that account for 40% of the variability observed in ligand induced inflammatory cytokine production (Wurfel et al., 2008). The G allele at rs5743551 in the promoter and the isoleucine (Ile) variant at amino acid position 602 (rs5743618) are associated with greater TLR2/1 ligand induced cytokine production. There is a high level of linkage disequilibrium between these variants suggesting that they are often inherited together and that the observed affect may be due to either a single variant or a combined effect. The Ile variant has a higher level of cell surface expression on peripheral monocytes (Johnson et al., 2007; Wurfel et al., 2008), which may explain at least part of the increased response in individuals with this variant. Interestingly, the G allele in the promoter is associated with gram positive sepsis and ARDS and with increased mortality and organ dysfunction in adult patients with sepsis in two independent cohorts (Wurfel et al., 2008) and with increased mortality in trauma patients (Thompson et al., 2014). Together these studies indicate that the presence of the G allele and/or the Ile variant may result in a hyper-inflammatory response that contributes to worse outcomes. TLR1 is also found on neutrophils and the impact of *TLR1* variants on neutrophil function may be involved in the observed association of these variants with outcomes. The Ile variant in TLR1 is involved in variability in neutrophil response and priming (Whitmore et al., 2016). Priming using agonists for TLR2/1 in healthy individuals show marked differences in response with priming occurring in neutrophils from only half of the individuals tested. Neutrophils from individuals homozygous or heterozygous for Ile at amino acid 602 demonstrated TLR2/1 ligand induced priming whereas individuals without Ile showed little or no priming. Individuals with the Ile variant also express more TLR1 on the neutrophil cell surface (Whitmore et al., 2016). Interestingly, in children with septic shock and positive bacterial cultures, the Ile variant is associated with increased ICU length of stay (Whitmore et al., 2016). This is the first report indicating that a variant in *TLR1* may impact sepsis in children, though there is still work to be done to confirm whether multiple variants in TLR1 are associated with susceptibility to gram-positive infections and poor outcome in sepsis and whether such findings are also observed in other populations of critically ill children.

Genetic variants in the interleukin-1 receptor antagonist (IL-1ra) are also associated with variable outcomes in sepsis and ARDS. The IL-1ra gene, *IL1RN*, has multiple SNVs as well as a region of variable nucleotide repeats (VNTR). A multistage genetic association study in individuals of European descent using three different cohorts and examining $\sim$2000 genes identified a variant in *IL1RN*, rs315952C, which is associated with decreased risk of ARDS and increased levels of serum IL-1ra (Meyer et al., 2013). This variant is also associated with increased LPS stimulated IL-1ra levels in healthy adults of European ancestry, but not in healthy adults of African ancestry, and with improved survival from septic shock in adults of European ancestry

(Meyer et al., 2014). Genetic variants in IL1RN have to been shown to be associated with the severity of meningococcal disease (Read et al., 2003) and pneumonia (Patwari et al., 2008) in children. However, these studies were performed before differences in genetic structure between races and ethnicities were reported and before the complexity of the genetic variation within the IL1RN gene were appreciated. Recent work has begun to explore the role of genetic variants in less traditional components of the inflammatory response. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a regulatory molecule that inhibits clearance of endogenous lipid from the blood by decreasing hepatocyte LDL receptor density thereby decreasing LDL particle clearance; an important process for clearing lipid moieties from pathogens thereby decreasing the inflammatory response. Mouse models indicate that decreasing PCSK9 levels results in increased clearance of endotoxin, lower levels of proinflammatory cytokines and improved survival (Walley et al., 2014).

In humans loss of function variants of the *PCSK9* gene result in increased clearance, and gain of function variants result in decreased clearance of LDL cholesterol (Cohen, Boerwinkle, Mosley, & Hobbs, 2006; Horton, Cohen, & Hobbs, 2007; Kathiresan, 2008; Musunuru et al., 2010). In adults enrolled in the Vasopressin and Septic Shock Trial (VASST) (Russell et al., 2008) the presence of at least one loss of function variant was associated with increased survival at 28 days even after adjusting for covariates (Walley et al., 2014). These findings were replicated in a second independent cohort. Furthermore, patients with one loss of function allele had lower serum cytokine levels compared with those patients carrying the gain of function variant (Walley et al., 2014). These studies have not yet been replicated in children with sepsis.

Adiponectin, one of the most abundant gene products in adipose tissue is an adipokine secreted by adipocytes which exhibits an anti-inflammatory activity (Garaulet, Hernandez-Morante, de Heredia, & Tébar, 2007; MatsumotoIshikawa & Kajii, 2008; Swarbrick & Havel, 2008; Tilg & Moschen, 2008; Vendrell et al., 2004). Adiponectin inhibits NF-kappa-B signaling (Ouchi et al., 1999; Yokota et al., 2000), decreases TNF-$\alpha$ expression (Maeda et al., 2002), and increases the anti-inflammatory cytokines IL-10 and IL-1ra (Wolf, Wolf, Rumpold, Enrich, & Tilg, 2004). One variant in the 3' untranslated region (UTR) of adiponectin gene, *ADIPOQ*, results in increased levels of adiponectin (Dhillon et al., 2011; Siitonen et al., 2011), and is associated with increased mortality in ARDS (Walkey et al., 2010) supporting the previously described association between the higher levels of adiponectin and mortality in adults with ARDS (Walkey et al., 2010). Variants in several other genes related to the immune system have been reported to be associated with risk or outcome in sepsis or ARDS, including variants in Nrf2, a transcription factor involved in regulating antioxidant response. Several reports indicate that variants in Nrf2 are associated with

ARDS though the functional impact of the implicated variants is unknown (Acosta-Herrera et al., 2015; Marzec et al., 2007; O'Mahony et al., 2012). Variants in elafin (peptidase inhibitor 3), a proteinase inhibitor thought to protect against deleterious effects of proteinases during inflammation, and sphingosine 1-phosphate receptor 3, a protein implicated in lung inflammation, have been reported to be associated with ARDS (Tejera et al., 2014). Lastly, a SNV that changes an amino acid in the protein SVEP1, a cell surface protein involved in cell adhesion, is associated with increased mortality and organ dysfunction in individuals of European ancestry with septic shock (Nakada, Russell, Boyd, Thair, & Walley, 2015). Whether variants in elafin, sphingosine 1-phosphate and SVEP1 are involved in risk of ARDS or mortality in septic shock in children remains unknown.

## 9.3 miRNA regulating immune response

The motive of our immune system is to mobilize a response upon contacting with a pathogen, toxin, or allergen. This is only possible by discriminating self from nonself that is the fascinating property of our immune system. Both innate and adaptive arms of the immune system have the property of self-tolerance. The innate immune response is nonspecific like our complement proteins, creating pores into the pathogenic cell membranes, cytokines, interferons, etc. An adaptive immune response is highly specific, possessing immunological memory that is immune response is quick and strong on encountering the same pathogen next time. (Chaplin, 2010).

### 9.3.1 Innate immune response

Upon viral infection of both human and animal innate immune cells, miR-146a and miR-155 were found significantly upregulated via execution of TLR and inflammatory signaling. (Taganov, Boldin, Chang, & Baltimore, 2006; Wang et al., 2010; Zhou et al., 2010). First miR-146a expression is stimulated by an inflammatory insult, then its increased expression level profoundly suppresses the NF-kB and cytokine inflammatory signaling. Thus it acts as a negative feedback miRNA in inflammatory signaling. (Taganov et al., 2006; Wang et al., 2010). It has been found that downstream adapter proteins of TLR signaling such as TRAF-6, interferon regulatory factor-5, IRAK-1, and IRAK2 are directly targeted by miR-146a by base pairing to their 3prime end of the mRNA. Since the same proteins are downstream signaling mediators for type 1 interferon, miR-146a upregulation also negatively affects type1 interferon synthesis stimulated by TLR7 and the intracellular sensor retinoic acid-inducible gene-I pathway (Hou et al., 2009; Tang et al., 2009). It has also been observed in Akata cell line (Cameron et al., 2008) and lymphocytes that miR-146a blocks genes induced by type1-interferon signaling through STAT-1 transcription factor down expression

(Tang et al., 2009). The combined impact of miR-146a on type1 interferon synthesis and signaling is big, but its down expressing effect on the individual target is moderate. Apart from cell line-based studies, the miR-146a gene knockout mice model experienced a persistent NF-kB activation, and an autoimmune-like condition is seen in aged animals (Boldin et al., 2011; Zhao et al., 2011).

Apart from miR-146a, several other miRNAs also influence innate immune response. When TLR4 is initiated, miR-21 suppresses the activity of the tumor suppressor gene called PDCD4 (Sheedy et al., 2010). By target prediction analysis of miR-200a/b/c family members, it was found miR-200 family members target several downstream mediators of TLR4 signaling proteins. However, by reporter gene sequencing only MyD88 is down expressed by this miRNA which results in down expression of NF-Kb and TLR4-induced inflammatory cytokine signaling pathway. (Wendlandt, Graff, Gioannini, McCaffrey, & Wilson, 2012). It was found that TLR agonists promote miR-148 family expression that is associated with reduced expression of CaMKIIα. This leads to reduced release of APC function promoting cytokines that ultimately affects T-cell specific adaptive immune response (Liu et al., 2010). miR-23b regulates TAB2, TAB3, and IKKα in an interconnected way leads to inflammatory cytokine signaling (IL-17, TNFα/IL-1β) induced NF-kβ down expression (Zhu et al., 2012). It is found that TLR3/TLR4 agonist signaling causes down expression of miR-223 by releasing STAT3 factor from the repression that promotes IL-6, IL-1β release (Chen et al., 2012). When innate immune cells take up LPS antigen, it triggers miR-9 expression that directly NFkB1 transcription by mRNA degradation representing second important NF-kB feedback circuitry. (Bazzoni et al., 2009). miR-187 is considered an important anti-inflammatory miRNA as it drives the IL-10-driven anti-inflammatory response by blocking IκBζ transcription which is a potent regulator of proinflammatory cytokines like IL-6 and IL-12p40 (Rossato et al., 2012). This miRNA also directly interferes with the protein synthesis and blocks TNFα translation in monocytes. (Rossato et al., 2012). The TNFα mRNA also contains a binding spot for miR-125b located at its 3prime end. Down expression of this miRNA upon LPS exposure leads to stabilization of TNFα expression. Some miRNAs directly bind TLR mRNAs and block their transcription level (Bayraktar, Bertilaccio, & Calin, 2019). For example, Let-7e and miR-19a/b down express TLR4 and TLR2 respectively (Androulidaki et al., 2009; Philippe et al., 2012). Two studies recently found that miR-223 directly down expresses the NLRP3 transcription which in turn leads to a reduction of IL-1β proinflammatory cytokine release by NLRP3 inflammasome (Bauernfeind et al., 2012; Haneklaus et al., 2012).

miR-142−3p directly acts on IL-6 mRNA transcription that was confirmed since silencing of this miRNA leads to enhanced expression of IL-6 within immature DC cells and also following LPS exposure (Sun et al.,

2011). When NK cells were exposed to either in vivo or in vitro antigenic challenge, they experienced a down expression of miR-29 but an elevation of IFNγ production. On further research, a direct binding interaction was found between miR-29 and IFNγ that is further confirmed when the mutation in IFNγ 3'-UTR prevents its interaction with miR-29. By using an immunoprecipitation strategy, a significantly enhanced association of IFNγ with the Ago-RSIC complex was found upon cell transfection with miR-29a mimic. By using genetic engineering, it was further confirmed, transgenic expression of IFNγ sponge target leads to significant inhibition of miR-29 expression in animal models (Ma et al., 2011).

Releasing the NK cells from tumor-induced inhibition represents an important arm of antitumor activity of an innate immune system. miR-374b down-regulation is linked to poor prognosis as it is associated with cancer invasiveness and chemo resistance in several solid tumors of the gastrointestinal tract, including CRC, pancreatic, and gastric cancer (Sun et al., 2018; Xie et al., 2014). miRNA- 374b upregulation has a positive effect on the antitumor activity of NK cells since it reduces the expression of PD-1 receptor that is exploited by tumor cells to block its anticancer activity such as in hepatic tumors (Huang et al., 2018). Similarly, in the case of Renal cell carcinoma (RCC), both miR-133A and miR-133A when upregulated promote the activity of NK cells against the tumor cells (Jasinski-Bergner et al., 2015). It has been observed that interaction of B7-H3(CD276) protein on NK and T-cell surface with tumor cells compromises their antitumor activity such as in neuroblastoma and the same protein is targeted by miR-29a, miR-29, and miR-29c which promotes the tumor regressive activity of these immune cells (Nygren et al., 2014). miR-187 also prevents cell proliferation and survival by targeting the same protein that is, B7-H3 in RCC (Zhao et al., 2013). Several miRNAs such as miR-34a, miR-34c, miR-302c, miR-520c enhances the tumor-oriented NK cytotoxicity (Min, et al., 2013), and tumor-killing activity towards melanoma cells by directly targeting UL16 binding protein 2 (ULBP2) (Heinemann et al., 2012). Fig. 9.1 illustrates the role miR-146a and miR-155 in miRNAs in innate immune response induced inflammatory response.

### 9.3.2 Adaptive immune response

Development of Dicerfl/fl AID-Cre + /- mice model significantly affects journey of GC B-cells to plasma cells. After T-cell-dependent immunization in this model, there was a significant reduction of immature B-cells at multiple stages of development such as splenic GC B-cells, memory B-cells, and antigen-specific IgG1-positive antibody-secreting cells (ASCs) in the bone marrow. The next step was to identify the miRNAs that cause such developmental loopholes in B-cells (Xu, Guo, Zeng, Huo, & Lam, 2012). Zhang et al. discovered a high expression of miR-30 family members and miR-9

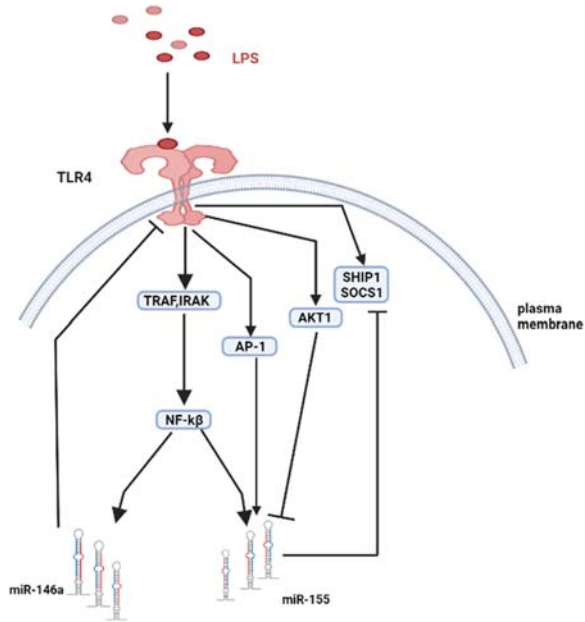


**FIGURE 9.1** Illustrates the role miR-146a and miR-155 in miRNAs in innate immune response induced inflammatory response. *Modified from (Mehta & Baltimore, 2016)*

compared to plasma B-cells by using an In silico target analysis of the above-mentioned miRNAs, found a binding site towards 3prime UTR of Prdm1-mRNA that encodes Blimp-1 protein. However, miR-30b and miR-30d transfection of U266 cell line reduced Blimp-1 level but not Prdm1-mRNA level which indicates they affect gene expression at the translational level (Zhang et al., 2009). Several studies reported that histone deacetylase inhibitors cause down-expression of Prdm1-mRNA via enhancing the expression of noncoding sequences that transcribes miR-30a, miR-23b, and miR-125b molecules in murine and human in vitro generated plasmablasts (Shen, Sanchez, Zan, & Casali, 2015; White et al., 2014). Atg5, Atg12, and Beclin 1 basically involved in cell autophagy are known as key factors in plasma cell differentiation and function and are also targeted by miR-30a and miR-23b (Conway et al., 2013). miR-125b that remained invariant during the journey of evolution is specifically expressed in GC centroblasts and discourages the plasma cell program by modulating Prdm1-mRNA and IRF4 mRNA levels (Gururajan et al., 2010). The human myeloma cell line (U266) experienced reduced levels of Blimp-1, CD138 and a reduced IgM-secretion in murine lymphoma cell line upon transfection with miR-125b mimic. miR-217 is another example of upregulated human and murine GC B-cell miRNA that has been found to enhance the GC reaction (De Yebenes et al., 2014). It has been found that increased proliferation of GC B-cells in lymph nodes of

miR-217 transgenic mice model along with the elevated frequency of mutations of JH4 intronic sequence of GC B-cells in the Peyer's patches upon T-cell dependent antigenic exposure. Endogenous miR-217 silencing by exogenous anti-miR-217 strategy via retroviral transduction of miR-217 sponge cell in mice model has shown opposite results upon immunization when compared to the transgenic model. This miRNA has been found to target proteins involved in DNA double-strand break repair mechanisms such as Bcl6 (de Yebenes et al., 2014). The GC reaction that takes place in the spleen and Peyer's patches is also modulated by miR-28 which has been found overexpressed in their GC B-cells (Bartolome-Izquierdo, de Yebenes, Alvarez-Prado, Mur, & del Olmo, 2017). The reduced proliferative power of murine splenic B-cells and consequently diminished frequencies of IgG1 + cells upon ectopic expression of miR-28 while grafting of miR-28 sponge construct expressing cells in chimeric mice resulted in significant improvement in the number of plasma cells thymus-dependent antigenic challenge. The miR-34a protein discourages the development of Pro-B to Pre-B- cell via down expressing Foxp1 factor and this finding is further strengthened since the loss of Foxp1 resulted in complete blockade of B-cell development expression (Danger, Braza, Giral, Soulillou, & Brouard, 2014). CD4-T-cells that have not yet encountered antigen-experienced c-MAF and IFN$\gamma$ repression by miR-155 and targets PU.1 and SHIP1 in B-cells. Anything that decreases the miR-155 expression weakens the adaptive antibody response to antigenic exposure and reduced antibody production and maturation as seen in a murine model (Danger et al., 2014).

The immune-signaling that T-cell activation has been functionally linked with several miRNAs such as miRNA-216, miRNA- 17−92, miRNA-214 clusters miRNA-29, miRNA-181 family, miRNA-146a, miRNA-155, let-7, miRNA-125 (Luan et al., 2018). The upregulation of miR-155 and miR-17−92 cluster has been linked to enhancement of Th1 cell differentiation (Baumjohann et al., 2013), while the latter one also represses IFN- $\gamma$ and differentiation of regulatory T (Treg) cells (Liu, Jiang, Li, Zhang, & Li, 2014). The overexpression of miRNA-326 has a positive effect on Th17 cell differentiation and IL-17 production via targeting ETS- 1 that is the negative regulator of Th17 differentiation. (Saki et al., 2015). Several miRNAs such as miR-10a, miR-146a, miR-155 played a dominant role in immune homeostasis and function by regulating Treg-cells that maintain immune cell response and discourages the development of autoimmunity (Hippen, Loschi, Nicholls, MacDonald, & Blazar, 2018). miRNA-10a is specifically expressed in Treg-cells and maintains their Treg phenotype by directly acting on repressor protein Bcl-6. Table 9.1 lists some of the miRNAs, their experimentally validated targets and their immunological manifestations.

**TABLE 9.1** Immunological significance of various miRNAs.

| microRNA | Experimentally validated target | Immunological significance | References |
|---|---|---|---|
| miR-146a | TRAF-6, IRAK1, IRAK2, IRAK5 | It acts as a negative feedback regulator of cytokine inflammatory signaling | Taganov et al. (2006), Wang et al. (2010) |
| miR-155 | PU.1, SHIP-1, Gab1, Foxp1, Jarid1 | Murine miR-155 knock-out model experiences diminished adaptive antibody response to antigenic exposure | Danger et al. (2014) |
| miR-21 | PDCD4 | MiR-21 decides T-helper cell differentiation into Th1 and Th2 type mediated through IFN-$\gamma$ and IL-2 signaling regulation | Lu et al. (2011) |
| miR-200a/b/c | MyD88 | MyD88 is down expressed by this miRNA which results in down expression of NF-Kb and TLR4-induced inflammatory cytokine signaling pathway | Wendlandt et al. (2012) |
| miR-9 | NFkB1, CTLA-4 | miR-9 discourages inflammation triggered by LPS stimulation while promoting cy-toxic T-cell activity by targeting CTLA-4 | Bazzoni et al. (2009), Jebbawi et al. (2014) |
| miR-187 | I$\kappa$B$\zeta$, B7-H3 | miR-187 targets I$\kappa$B$\zeta$ transcription which is a potent regulator of proinflammatory cytokines like IL-6 and IL-12p40 | Rossato et al. (2012), Zhao et al. (2013) |
| miR-125b | Prdm1, IRF4, TNF$\alpha$ | miR-125b down-express TNF$\alpha$ transcription and discourages the plasma cell program by modulating Prdm1-mRNA and IRF4 mRNA levels | Gururajan et al. (2010), Tili et al. (2007) |
| miR-223 | STAT3, NLRP3 | TLR3/TLR4 agonist signaling causes down expression of miR-223 by releasing STAT3 factor from the repression that promotes IL-6, IL-1$\beta$ release | Bauernfeind et al. (2012), Chen et al. (2012), Haneklaus et al. (2012) |
| miR-142−3p | IL-6, GARP,PD-L1 | miR-142−5p is linked with T cell proliferation (CD4 + CD25 + ) via down-expressing GARP (Glycoprotein A repetitions predominant) mRNA transcription | Jia et al. (2017), Sun et al. (2011), Zhou et al. (2013) |
| miR-29a, miR-29b, miR-29c | IFN$\gamma$, CD276, T-bet, Eomes, Ifnar1 | miR-29 knock-out promotes IFNAR expression, STAT1 phosphorylation that indicates miR-29 governs the IFN-$\alpha$ signaling sensitivity in mTECs | Ma et al. (2011), Kneitz et al. (2014), Nygren et al. (2014), Papadopoulou et al. (2012). |

(*Continued*)

**TABLE 9.1** (Continued)

| microRNA | Experimentally validated target | Immunological significance | References |
|---|---|---|---|
| miR-374b | PD-1 | miR-374b discourages the expression of immune-check point protein which cause enhancement of NK cell anti-tumor activity | Huang et al. (2018), Xie et al. (2014), Sun et al. (2018) |
| miR-30a/b/d | Prdm1, Atg5, Atg12, Beclin1 | Histone deacetylase inhibitors cause down-expression of Prdm1-mRNA via enhancing the expression of noncoding sequences that transcribes miR-30a, miR-23b, in murine and human in vitro generated plasma blasts | White et al. (2014), Shen et al. (2015), Conway et al. (2013) |
| miR-217 | Bcl6 | This miRNA has been found to target proteins involved in DNA double-strand break repair mechanisms such as Bcl6 | De Yebenes et al. (2014), de Yebenes et al. (2014), Küppers (2014) |
| miR-34a | Foxp1, PD-L1 | P53 which is the well-known tumor suppressor gene directly promotes the transcription of miR-34a thereby discourages the PD-L1 mediated CD8 + T-cell exhaustion | Rao et al. (2010), Berry et al. (2014), Cortez et al. (2015), Danger et al. (2014) |
| miR-326 | ETS-1 | This miRNA promotes Th17 differentiation and IL-17 production via targeting ETS-1 | Saki et al. (2015) |
| miR-17−92 | Bim, Pten, Ikzf4, PKB | miR-17−92 upregulation in T lymphocytes triggers the generation of antigenome autoantibodies, inflammatory response in multiple organs, enlargement of spleen, and malfunctioning of lymphoid organs | Baumjohann et al. (2013), Koralov et al. (2008), Ventura et al. (2008), Xu et al. (2014), Baumjohann et al. (2013), Kang et al. (2013) |
| miR-28 | | | Bartolome-Izquierdo et al. (2017) |
| miR-181 | PTPN22, SHIP-2, DUSP5, DUSP6, PTEN | miR-181 plays a key role in T-cell education and maturation via fine-tuning TCR signaling | Xiao et al. (2008), Ebert et al. (2009) |

### 9.3.3 miRNAs in T-cell immune response

Upon the arrival of antigenic stimuli, naïve T-cells vigorously multiply into effector cells, and memory T-cells. Broadly speaking, effector T- cells are CD4 + T-cells and CD8 + T-cells based on the presence of specific receptors. In addition, to CD4 + and CD8 + receptors, other receptors like CCR7, CD45RA, CD70, and CD27 are also present on the surface of these cells (Romero et al., 2007; Salaun et al., 2011). T-cells mainly play a critical role in adaptive immune response and liberates diverse interleukins (IL-1, IL-2, IL-17, IL-9) which activates other defensive cells in the body (Baumjohann et al., 2013; Harrington et al., 2005). microRNAs are important functional players of T-cell activity (Jindra, Bagley, Godwin, & Iacomini, 2010; Neilson, Zheng, Burge, & Sharp, 2007). The microRNA expression pattern differs among naïve T-cells, memory T-cells, and effector T-cells. Down expression of certain miRNAs such as Let-7f, miR-15b, miR-15b, miR-16, and miR-142 has been observed in effector T-cells. However, on antigenic stimulation, miR-155, miR-146a, and miR-21 are enhanced in cytotoxic T-cells. 14. Mouse memory T-cells residing in thymus experiences KChiP1 translation regulation by miR-150 (Almanza et al., 2010) while miR-142−5p is linked with T-cell proliferation (CD4 + CD25 + ) via down-expressing GARP (Glycoprotein A repetitions predominant) mRNA transcription (Zhou et al., 2013).MiR-21 decides T-helper cell differentiation into Th1 and Th2 type mediated through IFN-γ and IL-2 signaling regulation (Lu et al., 2011). The silencing of miR-21 in mice leads to repressed lung eosinophilia on allergic exposure while enhancing cytokine IFN-γ, IL-12 release from Th1 and dendritic cells respectively. Mice exposed to Listeria monocytogenes or bacillus Calmette-Guérin (BCG) experienced a drop in miR-29 expression in cytotoxic T-cells, T-helper cells and natural killer cells. Further, it has been experimentally found that miR-29 discourages IFN-γ release via directly regulating Eomes, IFN-γ, and T-bet mRNA levels (Ma et al., 2011; Steiner et al., 2011). IL-23 also participates in Th17 responses. One study showed that miRNA let-7f inhibits the expression of IL-23 reporter in CD4 + T cells (Li, Wu, Brant, & Kwon, 2011); this result indicated the function of these miRNAs in Th17 responses. MiR-125p is transfected into naive T-cells, which terminate differentiation from naive T-cells to effector cells (Rossi et al., 2011). Fig. 9.2 illustrates role of miRNAs in T-cell development.

## 9.4 miRNA and immune tolerance

A diverse range of immunological processes is taken place that prevents potentially harmful immunological responses within that host. These processes educate our immune system to tolerate self-antigens and trigger a response for nonself antigen. A better understanding of these processes helps

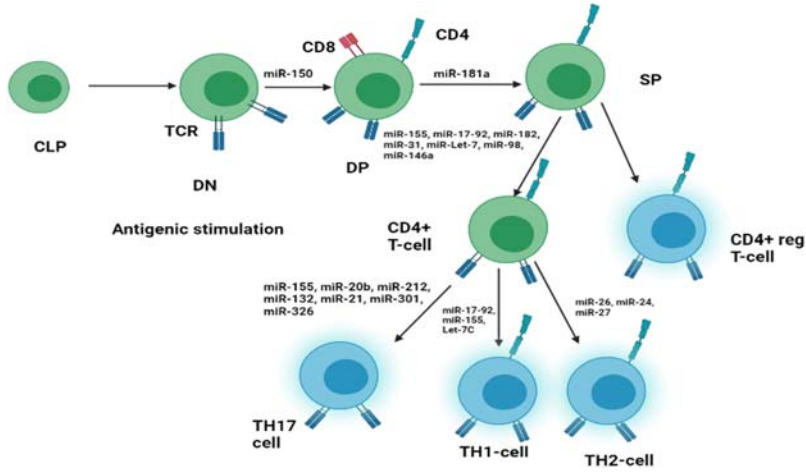


**FIGURE 9.2** This figure illustrates the role of miRNA in T-cell differentiation and development in primary lymphoid organ. *Modified from (Mehta & Baltimore, 2016)*

us to develop such therapeutic strategies that are successful in managing autoimmune diseases (Waldmann, 2010).

### 9.4.1 Central tolerance

This is the process of negative selection that takes place in the medullary region of the thymus where immature T-cell receptor or B-cell receptor binds to MHC-peptide complex on antigen-presenting cells along with co-stimulatory signaling. This develops strong signaling within the T-cells that causes apoptosis (negative selection) of T-cells. This prevents the development of self-reactive T-cells or B-cells and protects us from autoimmunity (Sprent & Kishimoto, 2001). miRNAs play a key role in the development of self nonreactive lymphocytes via regulating apoptosis, antigen receptor signaling that has been found associated with lymphocyte selection. The global miRNA biogenesis is essential for lymphocyte development as silencing of DICER has been found associated with the complete slowdown of pro- to pre-B cell transition (Koralov et al., 2008). miRNAs play an important role in the process of V(D)J recombination since Dicer deficiency has been found to affect BCR gene-encoded functional antigen receptor and regulates the fate of pro-B survival via targeting proapoptotic BIM protein encoded by Bcl2111 (Koralov et al., 2008). Another miRNA-17−92 cluster is composed of six miRNA members (miR-17, miR-18, miR-19, and miR-92) and the rest of the members are transcribed from other two similar genomic locations. They have been found involved in B-cell proliferation and development (Ventura et al., 2008). The miR-17∼92 cluster enhances the survival of

nonself reactive developing B lymphocytes via upregulating the expression of several downstream mediators of PI3K signaling pathway. The Bim proapoptotic protein expression is cooperatively retarded by PI3K signaling and several miR-17∼92 cluster miRNAs (Koralov et al., 2008; Ventura et al., 2008). MiR-17−92 also has been found with the indirect enhancement of PI3K signaling by down expression of its well-known negative regulator; PTEN mRNA. It has been found that any kind of imbalance in miR- 17∼92 expression (upregulation or down expression) in developing B-cells either leads to lymphomas and autoimmunity or apoptosis and B-cell deficiency (Xiao et al., 2008).

The TCR signaling is fine-tuned by miR-181 via induced expression changes in multiple phosphatase targets (PTPN22, SHIP-2, DUSP5, DUSP6) (Li et al., 2007) as well as PTEN (Xiao et al., 2008). MiR-181 plays a key role in the maturation and education of T-cells as it enhances the sensitivity of TCR signaling and starts decreasing as the T lymphocytes approach toward double positive and single positive stages. Disturbing the miR-181 expression affects TCR signaling that protects self-reactive T-lymphocytes that would have been eliminated by apoptosis. These findings indicate that miR-181 may have a key role in the molecular pathophysiology of autoimmune diseases (Ebert, Jiang, Xie, Li, & Davis, 2009). MiR-29a has been found overexpressed in mTEC and directly targets Ifnar1 mRNA. The Ifnar mRNA translates into IFN-α receptor protein and knock-out of miR-29 promotes IFNAR expression, STAT1 phosphorylation that indicates miR-29 governs the IFN-α signaling sensitivity in mTECs (Papadopoulou et al., 2012). These findings indicate that miRNAs are effective modulators of lymphocyte development and education. MiRNAs regulate immune cell survival and sensitivity of immune-signaling pathways and developmental checkpoints in diverse immune cells.

### 9.4.2 Peripheral tolerance

Sometimes, central tolerance fails to grip on self-reactive mature lymphocytes that may trigger autoimmunity within an individual. Fortunately, there is a second line of defense that specifically blocks these self-reactive mature lymphocytes, termed peripheral tolerance. Peripheral tolerance targets self-reactive antibody production by B-cells and tissue inflammation, toxicity caused by self-reactive T-cells. Many studies proved that all these processes are affected by miRNA expression and serve as a key turning point in lymphocyte fate and autoimmunity. It has been found by in-vivo cell expression studies miR-185 expression in MZ (marginal zone) cells is lower while as significantly higher in FO B (follicular B-cells) which are involved in antigen-antibody interaction in peripheral lymph nodes. MiR-185 directly targets BTK in FO B-cells. BTK is a kinase that is a key mediator of BCR signaling which includes self-reactive B-cells (Belver, de Yébenes, &

Ramiro, 2010). Thus miR-146a affects B-cell decision to apoptosis or survival. It has been found through Dicer knockout/knockdown experiments in peripheral B-cells that global miRNA down expression also changes BCR number and diversity via elevated class-switch recombination along with enhanced specific JH element and basic amino acids usage that predisposes B-cells to autoreactivity. This was further confirmed when Female Dicer knockout mice develop self-reactive antibodies which spontaneously aggregate into antigen-antibody complexes within kidneys (Belver et al., 2010). Thus these studies clearly indicate the optimum miRNA expression is an essential factor for governing B-cell receptor signaling to discourage self-reactivity of B-cells and autoimmunity. It has been observed that miR-150 expression has a good prognostic outcome in CLL (chronic lymphocytic leukemia) since it directly targets PI3K signaling mediator such as Gab1 which helps in recruitment of PI3K to the plasma membrane upon mitogen stimulation and also targets Foxp1, a protein factor that controls PI3K signaling via negative feedback mechanism (Mraz et al., 2014). miR-34a is another miRNA with Idd9.3 locus that retards self-reactive B-cell development via targeting Foxp1 and found a protective effect in mice suffering from Type 1 diabetes (TID) (Berry, Budgeon, Cooper, Chris- tensen, & Waldner, 2014; Rao et al., 2010). PTEN, a tumor suppressor acts as the same target for multiple miRNAs producing diverse effects depending on the immune cell type (Baumjohann et al., 2013; Henao-Mejia et al., 2013; Khan, Penny, Yuzefpolskiy, Sarkar, & Kalia, 2013). It has been also observed that a group of B-cells involved in CLL promotion produces a significantly high level of miR-22 which of course, blocks PTEN expression resulting in uncontrolled PI3K signaling (Palacios et al., 2015). Mice carrying a mutated version of Pten gene, are more prone to splenomegaly, lymphadenopathy, and experienced kidney, lung inflammation, maybe due to autoreactive antibodies like anti-ssDNA antibodies (Di Cristofano et al., 1999). Systemic lupus erythometoses individuals also experienced a significant depression in Pten level along with the enhanced expression of miR-22, miR-21, and miR-7 (Wu et al., 2014). miR-155 knock-out mice are less prone to develop experimentally induced autoimmune encephalomyelitis (EAE). Further investigation found a significant drop in IL-17A which has been found as a critical factor in EAE pathogeneses (O'Connell et al., 2010). miR-155 knockout mice have been found immune to collagen-triggered arthritis and experienced CD-4 + -Th17 differentiation impairment (BlümL et al., 2011). Recently, it has been observed that miR-155 encourages CD4 + cell type to Th17 differentiation via targeting Jarid2 (jumonji, AT-rich interactive domain 2), an effective cofactor of the polycomb repressive complex (PRC-2) (Escobar et al., 2014). MiR-210 shows opposite properties compared to miR-155 since it limits IL-17 release by Th17 cells and also acts as a stimulating factor in the mouse model of inflammatory bowel disease (Wang et al., 2014). MiR-210 drops autoantibody response strength found in elderly mice by discouraging the

class switch mechanism that confines autoimmune response development induced by B- and T-cells (Mok., et al., 2013). The miR-17∼92 promotes Th17 cell development and activity by decreasing PTEN and IKZF4 (IKAROS family Zinc finger 4) gene expression and its knockout retards Th17 differentiation and thus alleviating EAE pathogenesis (Liu et al., 2014). MiR-20b is an exceptional family member since it represses IL-17 releasing activity of Th-17 cell cultures through down expressing RORC (RAR related orphan receptor C) and STAT3 (signal transducer and activator of transcription 3) gene products (Zhu, Wang et al., 2014; Zhu, Chen et al., 2014). miR-17−92 upregulation in T lymphocytes triggers the generation of antigenome autoantibodies, inflammatory response in multiple organs, enlargement of spleen, and malfunctioning of lymphoid organs (Xu et al., 2014). MiR-17−92 enhances FO Th cell polarization, partly via down expressing PTEN and PKB phosphatase (Ph1pp2) (Baumjohann et al., 2013; Kang et al., 2013).

## 9.5 miRNA and immune checkpoint proteins

There are some proteins termed as immune checkpoint inhibitory proteins present on the surface of T-cells especially Treg- cells. These proteins interact with their ligands present on APCs (Antigen-presenting cells). This interaction stimulates a kind of negative functional effect within the T-cells. This phenomenon is the product of evolution that prevents immune system overstimulation and protects our cells from inflammation and autoimmunity. Upon T-cell activation, CTLA4 and PD-1 surface proteins are upregulated which binds to CD80/CD 86 and PD-L1 present on APC cell membrane respectively. It brings a dominant T-cell immune response mitigation (Zhang, Lu, Wang, Sheng, & Zhang, 2019).

### 9.5.1 PD-1

This immune checkpoint protein interaction is hijacked by cancer cells to escape from immune surveillance of T-cells. The miR-138 has been found to directly target CTLA4 and PD-1 transcription and block their expression in the mouse Glioma model (Wei et al., 2016). In addition, to miR-138, miR-28 also shows the PD-1 expression repression (Li et al., 2016). Thus both enhance the immune response of tumor surveillance. In addition, to above mentioned immune checkpoint proteins, TIM-3 and B- and T- lymphocyte attenuater (BTLA) are immune checkpoint proteins actively expressed by cytotoxic CD8 + T-cells which discourage the tumor cell immune surveillance. The interaction of TIM-3 (T-cell immunoglobulin and mucin-domain containing-3) and BTLA present on active cytotoxic T-cells with CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) and HVEM (Herpesvirus Entry Mediator) respectively prevents the cytotoxic

killing activity of the T-cells and leads to tumor immune tolerance. No doubt BTLA expression is down expressed during cytotoxic T-cell differentiation but it has been observed that BTLA protein is dominantly expressed on CD8 + cells residing in the tumor microenvironment (Paulos and June, 2010). MiRNA-28 serves as a tumor suppressor miRNA as it discourages tumor immune tolerance by repressing the expression of both BTLA and TIM-3 gene targets (Das, Zhu, & Kuchroo, 2017).

The tumor cells express ligands of immune checkpoint proteins only to corrupt cytotoxic T-cells such as PD-L1 and CEACAM1. The 3prime UTR region of PD-L1 mRNA has a binding affinity to multiple miRNAs and generally, tumor cells express more miRNAs compared to healthy cells (Adams, Kasinski, & Slack, 2014). Gastric cancer becomes more aggressive when an SNP occurs at the 3′-UTR of the PD-L1 gene which aborts the miR-570 binding and PD-L1 upregulation (Wang et al., 2012). By the time, many miRNAs have been revealed such as miR-570, 34a, 200, 21, and 197 that have a binding affinity for PD-L1 mRNA (Cortez et al., 2015; Fujita et al., 2015; Wang et al., 2012; Zhu, Wang et al., 2014; Zhu, Chen et al., 2014). No doubt, these miRNA down-regulate PD-L1 mediated CD8 + function inhibition, however, their significantly low expression in aggressive tumors is the disadvantage. P53 which is the well-known tumor suppressor gene directly promotes the transcription of miR-34a thereby discourages the PD-L1 mediated CD8 + T-cell exhaustion. Thus miR-34a has an important intermediary role in the tumor suppressor properties of p53 (Cortez et al., 2015).

Both miR-138−5p and miR-142−5p interacts with 3prime UTR of PD-L1, suppress its expression in CRC (colorectal cancer) (Zhao et al., 2016) and pancreatic cancer (Jia et al., 2017) respectively, and linked to poor clinical outcome. The tumors of the thyroid, ovary and head, and neck also experienced a reduced expression of miR-138−5p (Liu et al., 2009; Yeh, Chuang, Chao, & Wang, 2013). MiR-138−5p was recently identified as one of the key miRNAs dysregulated in RCC (Ying et al., 2018). The transfection of RCC (renal cell carcinoma) with the synthetic miR-138−5p oligonucleotides significantly discourages their proliferative and invasive power (Xiong, Zhang, & Song, 2019). Also, lung epithelial cells exposed to benzo-a-pyrene in smokers experienced the down-expression of miR-138−5p (Jiang et al., 2018). Thus it seems that miR-138−5p mimics may play an important role in modulating immunity against lung carcinoma and renal cell carcinoma. It has been observed that miR-513 plays a tumor-suppressive role in many solid cancers by discouraging tumor evasion from immune surveillance and suppressing inflammation. The persons with the cholangitis show decreased levels of miR-513 (Lazaridis, Strazzabosco, & Larusso, 2004) along with an elevated level of IFR-$\gamma$ mediated PD-L1 markers on biliary epithelium (Gong et al., 2009). Also, miR-513 suppresses PD-L1 expression in retinoblastoma (Wu, Chen, Zhang, & Xing, 2012) as well as discourages

primary tumor cell migration in endometrial carcinoma (Dong, Xiong, Yue, Hanley, & Watari, 2018). MiR-570 is another inhibitor of PD-L1 expression, which is commonly downregulated in hepatocellular carcinoma (HCC) (Guo et al., 2015). It was experimentally proved that PD-L1 is directly targeted by miR-152 and plays an antagonistic role against proliferation, metastasis in cervical cancer, colorectal cancer, NSCLC, and gastric cancer. miR-152 directly targets immune checkpoint protein that is expressed on tumor cells; PDL1(Subudhi, Alegre, & Fu, 2005). This miRNA discourages proliferation and metastasis via targeting kruppel-like factor 5 transcription activator (Zhang, Chang et al., 2019) and ERK signaling in colorectal and cervical carcinoma (Ghazanchaei, Mansoori, Mohammadi, Biglari, & Baradaran, 2018). The diminished expression of miR-152 is associated with NSCLC proliferation via upregulation of fibroblast growth factor gene expression (Cheng, Ma, Tan, & Zhang, 2014) and predisposes gastric cancer patients to advanced disease with poor disease outcomes (Wang et al., 2017). Another miRNA family called miR-34 which constitutes miR-34a, miR-34b, and miR-34c directly lowers PD-L1 expression in tumor tissue (Wang et al., 2015) and its lower expression is linked to overexpression of PD-L1 (Cortez et al., 2016). The guardian of genome stability called p53 which frequently mutated in cancer promotes the expression of this miR-34a expression and certainly lowered expression of miR-34a found in cancers such as NSCLC and acute lymphocytic leukemia (Chen et al., 2014).

### 9.5.2 CTLA-4

CTLA-4 expressed by CD8 + (cytotoxic T-cells) cause their autoinhibition on interacting with specific receptor located on APCs (antigen-presenting cells). CTLA-4 (CD152) was observed first by Brunet et al. (1987). Since due to its structural homology with CD28, research community felt that it may have an enhancive effect on T-cell activity. However, CTLA-4 was experimentally proved in 1995 as a negative factor for T-cell proliferation and activity (Krummel and Allison, 1995). Since 1995, several miRNAs have been discovered as down regulators of CTLA-4 expression. The mutant CTLA-4 (G replaced with A; rs56102377) is downregulated by miR-105 compared to the wild type version. It has been found that persons with rs56102377 SNP experienced an aggressive type of periodontitis compared to wild type version, which maybe due to reduced expression of CTLA-4 in them by miR-105 action. miR-105 behaves as a dual player, promotes or inhibits cancer governed by tumor stage like initiation, progression, and metastasis (Li et al., 2019). It has been observed that miR-487a-3p directly downregulates CTLA-4 expression. Interestingly, miR-487a expression is seen elevated in blood mononuclear white blood cells of type1 diabetes individuals, probably which may lead to autoimmune response via overactivation of T-cells (Zurawek et al., 2018). Also, miR-487a is found overexpressed in

prostate carcinoma while as down expressed in other kinds of carcinomas (Chang et al., 2017; Wang et al., 2020). MiR-9 is an essential miRNA that controls the Treg cell activity by directly targeting CTLA-4 and its down expression is important for Treg cell activity (Jebbawi et al., 2014). However, miR-9 in cancer is not straightforward as it is showing either tumor enhancer or suppressor nature in variable experimental models (Khafaei et al., 2019). Apart from miRNA direct regulation, many studies show indirect regulation of CTLA-4 via down expressing its transcription activators. For instance, FOXP3 (forkhead box P3) which is a transcription activator of CTLA-4 is directly targeted by miRNAs.

## 9.6 Conclusion

Micro-RNAs are capable of regulating the expression of target genes and cellular activities posttranscriptionally by epigenetic mechanisms. Micro-RNAs play an important role in the mechanisms of immune regulation and stimulation of T-cell responses, inflammatory reaction, and the expression of inflammatory agents. Dysregulation in the expression of micro-RNAs may lead to a range of immune disorders including cancer. The wider perceptive of the role of micro-RNAs can provide novel molecular mechanistic insights of human ailments and disease biomarkers and therapeutics.

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Chapter 10

# Immunogenetic causes of infertility

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## 10.1 Introduction

Fertilization success at the gamete-level is highly reliant on the biochemical interactions mediated by the various chemical signals that are released by female gamete into the reproductive tract (Eisenbach & Giojalas, 2006; Kekäläinen & Evans, 2018). The female releases signals that trigger a number of alterations in the physiology of the sperm, like capacitation, acrosome reaction, and hyperactivation and then finally directs the sperm toward an unfertilized egg (Brown et al., 2017; Cramer et al., 2016; Flegel et al., 2016; Kekäläinen & Evans, 2017; Yoshida, Kawano, & Yoshida, 2008). Out of the large number of sperm released, only a small number of ejaculated spermatozoa are capable of entering the female oviducts, among these only a few can finally reach the egg(s) (Eisenbach & Giojalas, 2006; Holt & Fazeli, 2015; Robertson, 2005). Various studies in humans strongly suggest the role of cellular-level signaling and immunological factors or immunological factor encoding genes and their regulatory mechanisms as a cause of reproductive failures that lead to infertility (Kekäläinen & Evans, 2018). The term immunogenetics constitutes all the processes that are controlled both by genes and immunological defense mechanisms of an organism. Infertility is a major medical problem faced by 10%−15% of couples worldwide (Gnoth et al., 2005). According to the current definition of the World Health Organization (WHO), infertility is a disease of the reproductive system, manifested by the inability to achieve pregnancy after 12 months of unprotected sexual intercourse (Zegers-Hochschild et al., 2009). A large number of factors contributes to the reproductive failure in humans viz sperm DNA damage, cervical incompetence (CI), cystic fibrosis, Klinefelter

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00013-0**
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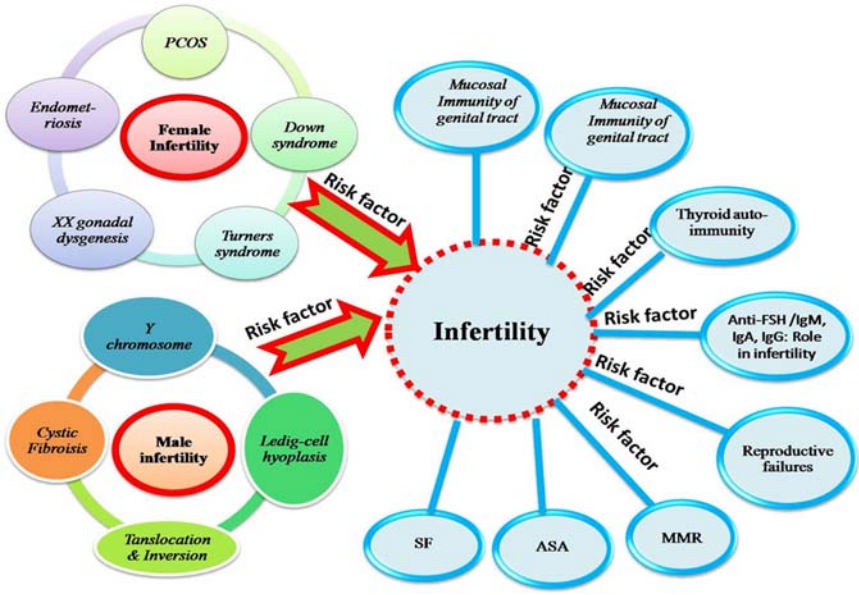


**FIGURE 10.1** The representative diagram depicts various immunogenetic factors as risk factors for infertility.

syndrome, and Kallmann KAL syndrome (McDonald, 1987; Molnar, Terasaki, & Amory, 2010; Pedersen-White, Chorich, Bick, Sherins, & Layman, 2008; Zegers-Hochschild et al., 2009; Zhao, Mu, Ma, Dai, & Jiao, 2019). The immunogenetic factors include various protein encoding genes that are controlling expression levels at various reproductive stages. Various testing methodologies like sequencing, cloning, and expression analysis are used for studying various immunogenetic factors and mechanisms involved in infertility. Nowadays, animal models are being used for investigating direct influence of genes and their encode proteins in pregnancy. Knockout mice have provided promising results in studying the mechanisms involved in immunogenetic triggered infertility. In this chapter we will discuss the involvement of various immunogenetic factors in causing infertility (Fig. 10.1).

## 10.2 Immunogenetic factors as a cause of infertility

### 10.2.1 Role of immune system in infertility

#### *10.2.1.1 Human leukocyte antigen*

The Human leukocyte antigen (HLA) region is spanning about ~4 Mb of DNA on the p-arm (Short arm) of 6$^{th}$ human chromosome (Browning & McMichael, 1996). HLA region contains about 150 protein encoding gene

loci, those are found to control cell-cell communications and immune responses by presenting its encoded peptides to T cells for generating immune complex proficient of T cell stimulation (Browning & McMichael, 1996; Ober & van der Ven, 1997). The immunogenetic investigations have altogether focused on the involvement of HLA encoding MHC (Major histocompatability complex) genes in reproduction (Sorokowska et al., 2018). In humans the mate selection is also influenced by the HLA antigens (Roberts, Gosling, Carter, & Petrie, 2008; Sorokowska et al., 2018). The MHC complex is found to play twin role in the reproduction process, firstly by influencing the mate selection, secondly by controlling various stages of reproduction (Fernandez, Cooper, & Sprinks, 1999; Grob, Knapp, Martin, & Anzenberger, 1998). The MHC (HLA) is comprised of three distinct regions viz class I, class II, and class III, with highest number of allelic polymorphism in the first two classes among all mammalian functional genes (Apple & Erlich, 1996; Ober & van der Ven, 1997). The class I HLA genes are located on the telomeric region, while as the class II HLA genes that act as risk factors for numerous autoimmune disorders are located on the peroximal-centromeric regions (Barcellos, Sawcer, & Ramsay, 2006; Graham, Ortmann, & Langefeld, 2002). The class I MHC molecules present the endogenous or exogenous derived peptides to the CD8 cytotoxic cells, while the class II HLA that is HLA -DP, -DQ, and -Dr molecules are comprised of a and b chain heterodimers encoded by HLA which function by presenting exogenous or endogenous peptides to the CD4 helper cells (Apple & Erlich, 1996). The HLA class II antigens are believed to act as the predisposed factors of recurrent spontaneous abortions (RSA), occurring due to autoimmune destruction of trophoblast (Barcellos et al., 2006). Among the HLA class II antigens the HLA-Dr (That identifies DRB1*01 gene products) is mainly considered as the reason for unsolved recurrent miscarriage (Christiansen et al., 1995). The particular amino acids of HLA-Dr and HLA DRB1*01 may create an immunologic response thereby triggering a maternal reproductive autoimmune failure and consequent loss of fetus (Sarra, Fekih-Mrissa, Rachdi, & Gritli, 2012; Sasaki, Yamadaa, & Kate, 1997). The lymphocytes of women suffering from RSA release TNF-a (Tumor necrosis factor), interferons (interferon-g), TNF-b upon stimulation by the trophoblast specific antigens, but no cytokine production occurs in the women with normal fecundity (Hill, Polgar, & Anderson, 1995). The hypersecretion of lymphocytes are further linked with the genetic cause (Santamaria, Gehrz, Bryan, & Barbosa, 1989). In individuals carrying DRB1*01 allele TNF-a secreation potential of mononuclear cells was found to be higher than individuals carrying rest of the HLA-Dr alleles (Pocoit, Briant, & Jongeneel, 1993). In Danish women with RSA, DRB1*01 allele was found to a risk factor (Christiansen et al., 1995; Christiansen, Rasmussen, Jersild, & Grunett, 1994; Kruse, Steffensen, Varming, & Christiansen, 2004). At the maternal and fetal interface the cells of fetus

and mother are in direct contact, particularly at the implantation site. Generally first trimester trophoblast cells are found to release more class I mRNA and encoded proteins than term trophobalst cells (Kovats, Main, & Librach, 1990; Wei & Orr, 1990).

#### *10.2.1.2 Reproduction immune failures*

The female fertility is tightly controlled by a sequence of extremely coordinated and harmonized interactions in the ovarian hypothalamicpituitary-axis. Thus the female fertility is affected by the disorders/diseases/dysfunctions of immune system, neuroendocrine system, reproductive tract, or by any other severe medical condition. The association between autoimmune failures and infertility was described much earlier by Gleicher et al. in infertile females with endometriosis and raised autoantibodies (Gleicher, El-Roeiy, Confino, & Friberg, 1987). Infertility disorders viz PCOD (polycystic ovary syndrome), UI (unexplained infertility), and frequently unsuccessful IVF attempts in women are triggered by increased production of auto-antibodies, thus may act as a risk factor of spontaneous abortions or preeclampsia (Matarese, Nikas, & Alviggi, 2003; Reimand et al., 2001). In 10%−20% of Western infertile women endocrine dysfunctioning was reported (Crosignani & Rubin, 2000). POF a secondary amenorrhea condition affecting 1%−2% women (below 40 years of age) is a common cause for hypergonadotropic hypogonadism. POF is having close association with autoimmune disorders, with nearly half cases showing autoimmune reaction to ovarian tissue (Baird, Benagiano, & Cohen, 2002; Fenichel et al., 1997). Normogonadotropic anovulation constitutes about 50% females having endocrine disorders as a cause of infertility and mostly contain patients with PCOS which affects 4%−10% of women in their reproductive age (Knochenhauer et al., 1998). Infertility in PCOS is linked with the autoimmune mechanism, exhibiting raised organ- and non organ specific antibodies and increased occurrence of AOA (Antiovarian Autoantibodies) (Forges, Monnier-Barbarino, Faure, & Bene, 2004; Matarese et al., 2003). Tubal factor infertility occurs in 85% developing nations and in 10%−30% of developed nations. In this condition their occurs damaged ovum transport because of adnexal adhesions or/and fimbrial damage (Land & Evers, 2002). The tubal destruction in Chlamydia trachomatis infection occurs due to the autoimmune destruction of tubal cells by excess production of HSPs that shares sequence homology with amino acid sequence of microbial HSPs (Heat shock proteins) that elicit a strong immune reaction (Rodgers, Wang, & Zhang, 2010; Sarapik et al., 2010). Endometriosis another reproductive disorder affects 10%−20% of women in their reproductive ages and is characterized by the pelvic pain, infertility, dyspareunia, dysmenorrheal, impaired implantation, ovulatory abnormalities, pelvic mass and endocrine dysfunctioning (Genital & Wheeler, 1989). In addition, to cell mediated and humoral immune reactions, endometriosis is linked with the "autoimmune syndrome" identified by polyclonal activation

and release of different antibodies mostly smooth muscle autoantibodies (SMA), anti-endometrial antibodies, anti-phospholipid antibodies, and AOA (Gleicher et al., 1987; Lang & Yeaman, 2001; Lebovic, Mueller, & Taylor, 2001; Sarapik et al., 2010).

#### 10.2.1.3 Anti-FSH/IgM, IgA, IgG: role in infertility

Follicle stimulating hormone is a gonadotrophin hormone released by the pituitary gland. In controls the gonad formation by targeting the granulosa cell receptors and ovule follicle maturation in females (Themmen & Huhtaniemi, 2000). FSH controls the growth of antral follicles, thus making the preovulatory follicle competent to ovulate and development of corpus luteum in reaction to LH surge (Thomas & Vanderhyden, 2006). The FSH plays a central role in the reproductive functioning and its mutation causes altered ovarian response to released hormones, thus decreasing the normal reproductive functioning (Levallet, Pakarinen, & Huhtaniemi, 1999). The β-subunit of FSH determines its specificity and receptor binding, further the haplotype analysis determined the two common fecundity triggering forms of FSHB gene (HAP1 and HAP13) (Fox, Dias, & van Roey, 2001; Grigorova, Rull, & Laan, 2007). However, the direct association of auto-immune reaction with FSH has not fully elucidated, but the role of anti-FSH antibodies has been well elucidated. The levels of antibodies viz IgG, IgM, IgA against FSH was found to be higher in infertile women (Haller et al., 2005). The anti-FSH antibodies are not acting as markers for auto-immune reaction against FSH but, are naturally occurring antibodies in the sera. The increased levels of serum IgG levels in women with fertility failure signify the immune-pathological reaction. A number of studies depict the reactivity of female sera towards sperm antigens. The female sera is found to exhibit a strong immune reaction against the proteins (35, 40, 47, 65 kDa) extracted from the sperm (Brazdova et al., 2012). The studies of Kutteh (1995) provided the distribution of immunoglobulin A of antisperm antibodies in the infertile female's sera and cervical mucus. Their occurs increased release of FSH IgM antibodies in patients with tubal and male factor infertility (Haller, Salumets, & Grigorova, 2007). The FSH enters the genital tract of female as a constituent of male semen which gets recognized by the immune system of female by a phenomenon called "seminal priming," it's appropriate functioning facilitates pregnancy (Luboshitzky, Kaplan-Zverling, Shen-Orr, Nave, & Herer, 2002; Robertson, Bromfield, & Tremellen, 2003). During partner-specific tolerance both specific and nonspecific immune reactions are commenced together with the production of specific antibodies against the maternal and semen-specific antigens like anti-FSH (Hegde, Ranpura, D'Souza, & Raghavan, 2001; Robertson et al., 2003). In this way the anti-FSH IgA observed in the female circulation are believed be alloantibodies that are initiated by semen. The release of anti-FSH IgA is linked with the occurrence of DQB1*03 allele (Haller, Sikut, Karro, Uibo, & Salumets, 2008).

After antigenic exposure, the levels of IgM antibodies dominates in the early phases, along with the secreation of IgE in genetically disposed individuals as a response to certain allergens. In the later phases of repeated antigenic exposure, the antibodies display class switching into IgG and IgA (Aalberse, van der Gaag, & van Leeuwen, 1983; Nakagawa, 1986; Sondergaard, Poulsen, Osterballe, & Weeke, 1991). under subsequent exposure the IgG1 and IgG4 levels become highly predominant classes of IgG isotype. The levels of IgG1/$IgG_4$ predominates in anti-seminal antibodies, while IgG4/IgG1levels predominate in ASA (Brazdova et al., 2015).

#### *10.2.1.4 Thyroid Auto-immunity*

The thyroid auto-immunity is highly prevalent in infertile patients and in females with increasing spontaneous miscarriages, positive thyroglobulin (Tg) and thyroid peroxidase (TPO) antibodies (Dendrinos et al., 2000; Poppe et al., 2002; PDe Carolis et al., 2004; rummel & Wiersinga, 2004). The presence of circulating anti-thyroid antibodies are causing 3−5 times increment in miscarriage rate in comparison to the TAI-negative women, even in the absence of thyroid abnormalities (De Carolis et al., 2004; Prummel & Wiersinga, 2004; Stagnaro-Green et al., 1990). The thyroid auto-immunity causes recurrent failure in the embryo implantation and anti-thyroid antibodies are believed to act as independent markers for the collapse of assisted reproductive procedures (Bellver et al., 2008; Bussen, Steck, & Dietl, 2010). The thyroid failure at the initial phase of pregnancy are supposed to act as a factor responsible for miscarriages in euthyroid females with thyroid autoimmune disorders (Prummel & Wiersinga, 2004). It is hypothesized that during IVF (in vitro fertilization) response to the gonadotropin the estradiol level increases which causes an increment in the thyroxin-binding-globulin production. Thus decreasing the free thyroxin, putting pressure on the hypothalamus-pituitary-thyroid Axis (Poppe, Velkeniers, Glinoer, & Medscape, 2008) resulting in subclinical hypothyroidism and might cause embryo implantation failure (Muller, Verhoeff, Mantel, De Jong, & Berghout, 2000; Poppe et al., 2008). The development and maturation of secondary follicle in females is characterized by the formation perifollicular mesh of blood vessels (Mattioli et al., 2001). That permits the uptake of growth factors and nutrients for follicular growth. The occurrence of anti-thyroid antibodies may proceed to antibody mediated cytotoxicity in the developing ovarian follicle, thus harms the maturing oocyte and hampering its developmental potential and quality. The existence of anti-thyroid antibodies reduces the oocyte fertilization potential and hence embryo implantation (Monteleone et al., 2011). The anti-thyroid antibodies are located on the surface of embryo before implantation (Lee et al., 2009). The antigens in humans are recognized in the murine thyroid tissue by anti-zona pellucida antibodies (Kelkar, Meherji, Kadam, Gupta, & Nandedkar, 2005), thus zona-pellucida may serve as a target for anti-thyroid antibodies.

#### 10.2.1.5 Mucosal immunity of genital tract

The mucosal tissue of the female genital tract is characterized by the lymphoid tissue that works on the local levels in the external secretory glands and mucosa. It reduce the exposure to the environmental antigens that are significantly regulating the fertility potential, further restricts the penetration of antigens into the systemic compartment. The genital tissue and its secretions provides protection that varies from the systemic reaction provided by cellular immunity and the antibodies, however, the early antigen contact to mucosae produces the systemic T cell hypo-responsiveness (Mestecky, Russell, & Elson, 2007; Woof & Mestecky, 2005). The immunity provided by the mucosae of the genital tract is subjective to the levels of various antibodies, hormones and cytokines. The humoral immunity exhibited by the surface mucosal tissue present and release the antibodies of class IgA, IgG, IgM isotypess, depending on the menstrual cycle and hormones. The IgG and IgA, levels reach the extreme concentrations ahead of ovulation, that is coupled to the raised level of IL-1β (interleukin 1β). The estrogen levels are found to raise the selective expression and transport of IgA (S-IgA) antibody. Thus regulating distribution and transportation of antigen-presenting cells and Ig possessing cells, in addition, to the cells (CD4 + and CD8 + cells) involved in MHC response in the female fallopian tubes, uterus and vagina (Kutteh, Prince, Hammond, Kutteh, & Mestecky, 1996). The levels of IgG and IgA in the female cervical mucus levels are further influenced by the oral contraceptives (Franklin & Kutteh, 1999). The female uterine cervix contributes in the local immune responses due to the occurrence of Ig (immunoglobulin) secreting cells in complex form termed as fluid/mucus or plasma that contains up to 80% water and is surrounding the cervix internally and externally. The composition of cervix mucus mainly depends on the glycoprotein mesh containing electrolytes (sodium, calcium and potassium), trace elements (Cu, Fe, Se, Zn) sugars (glucose, fructose), immuno-competent proteins, amino acids, certain complement proteins (C3 and C4) and cytokines viz Th1 and Th2. Any alteration in balance of its various components proceeds to the spontaneous abortion and immune infertility (Cibulka et al., 2005; Moghissi, 1972; Schumacher, 1988; Ulcova-Gallova, 2010). At the time of ovulation the pH generally remain alkaline so as to allow sperm survival, but after menstruation cervical mucus becomes acidic (Gruberova, Bikova, Ulcova-Gallova, Reischig, & Rokyta, 2006).

#### 10.2.1.6 Role of antiserum antibodies in pregnancy

The association of ASA (antiserum antibodies) with the infertility exhibits inconsistent results, but in male infertility the association is positive. The successful pregnancy not only depends on the maternal-fetal exchanges/interactions but also on the sequence of immune reactions occurring in the female

body, which needs to get adapted to these reactions (Bronson, 2000). Still certain studies provide evidence regarding the association of abortions (recurrent or missed) with ASA (Berger, Smith-Harrison, & Sandlow, 2019; Robertson & Sharkey, 2016). Many studies suggest the involvement of higher ASA and spontaneous abortions (Heng, Huang, Zhong, Yin, & Tong, 2015; Simopoulou et al., 2019). The studies of Wang and Zhu (2011) showed no association between recurrent abortions and ASAs, but ASAs are responsible for infertility (Wang & Zhu, 2011). The studies also suggest that the role circulating ASAs as infertility causing factors in PCOD (Chernyshov, Radysh, Gura, Tatarchuk, & Khominskaya, 2001; Singla, Gupta, Khemani, & Aggarwal, 2015). Along with the anti-phospholipids auto-antibodies the ASAs are reported in the infertile PCOD females (Malinowski et al., 2000). Yang, Zhu, and Jing (2012) correlated the presence of ASAs with the pathogenesis of PCOS.

#### *10.2.1.7 Role of seminal fluid in female immune infertility*

The seminal fluid (SF) constitutes a part of the semen that possess a wide array of inorganic and organic constituents like fructose, prostaglandins, hyaluronidase, zinc, carnitine, selenium and α-glucosidase that are essential for physiological processes of sperm. SF acts as a buffering, nutritive and protective medium for maintaining the proper functioning of the sperms and their viability. The transportation of sperm in female genital tract is assisted by the presence of raised content of TGFβ and PGE that are inhibiting the functioning of the NK-cells, neutrophils occurring in the upper superficial layer of cervix tissue. The TGFβ is dependent on the testosterone hormone for its synthesis in prostrate. The is a glyco-protein belonging to the class of cell secreted molecules and its 75% occurs in SF as dormant form (Denison, Grant, Calder, & Kelly, 1999; Robertson, Ingman, O'Leary, Sharkey, & Tremellen, 2002). It is there after activated in the female genital tract by the enzymes of either make or female source upon coming in contact with the acidic vaginal pH or via conformational alteration after communicating with epithelial cells. The active proportion (25%) of TGFβ may result in the immune-tolerance of SF antigens. The deviating member of TGFβ family called GDF15 (growth/differentiation factor 15) promotes pro-inflammatory-immune reaction and is exhibiting anti-tumorigenic activity. The increased levels of GDF15 (i.e., expressed in the placenta) in females promotes frequent abortions due to occurrence of fetal seminal antigens that facilitates the TGFβ promoted female immune intolerance hence abortions (Robertson, 2005). Cathepsin D other seminal constituent also degrade vaginally exposed proteins that might be involved in the antibody synthesis connected to immune infertility (Pardesi, Dandekar, Jamdar, & Harikuma, 2004). Seminal ZAG a noval adipokine is an IgG-binding protein and a major player in successful fertilization, antigen binding and lipid mobilization. In addition, it

takes part in the immune response expression in females as it is having fold similarity with the MHC1 molecules occurring on the APC (antigen presenting cells). ZAG IgG- binding protein is having a protective function by blocking the female ASA (Brazdova, 2014; Brazdova, Vermachova, Zidkova, Ulcova-Gallova, & Peltre, 2013; Hassan, Waheed, Yadav, Singh, & Ahmad, 2008). The SF mediates the rare Ig-E mediated immune reaction to the semen causing hypersensitivity to the semen—a condition called Human seminal plasma allergy (HSPA) showing systemic or local symptoms like vaginal itching, swelling, and redness. after exposure to SF (James, 1945; Weidinger, Mayerhofer, Raemsch, Ring, & Kohn, 2006). The symptoms of HSPA further includes nausea, diarrhea angioedema (of tongue throat, face, lip), dyspnea, vomiting, chest apun cough, etc. and can even cause a life threatening anaphylactic shock. The patients suffering from HAS usually fail to conceive (Bernstein, 2011; Shah & Panjabi, 2004; Weidinger et al., 2006;).

#### *10.2.1.8 Role of mismatch repair*

Infertility worldwide is faced by 10%−15% couples, out of which 40%−50% cases are due to male factor infertility (De Kretser & Baker, 1999; Gnoth et al., 2005) but the cases of impaired male fertility are also reported (Dohle et al., 2005). The sperm DNA damage in male germ cells is currently being looked at as the cause of male infertility (Sakkas & Alvarez, 2010). Sperm DNA damages causes several adverse clinical results like poor semen quality, impaired pre-implantation, miscarriages, low fertilization rates and increased death rate of fetus (Borini et al., 2006). Their occurs deficiency in the DNA repair mechanism during spermatogenesis that is having adverse effects on sperm DNA integrity (Paul, Melton, & Saunders, 2008). The certain genetic polymorphisms in DNA repair genes impair the development of sperm DNA and results in male infertility (Gu et al., 2010). The DNA mismatch repair (MMR) plays a crucial role in genetic integrity and its failure can cause various type of mammalian cancers (Buermeyer, Deschenes, Baker, & Liskay, 1999; Harfe & Jinks-Robertson, 2000; Kunkel & Erie, 2005). The several members of mismatch repair family take part in the process of meiotic recombination and gametogenesis (Baarends, van der Laan, & Grootegoed, 2001; Kolas & Cohen, 2004). Among the MMR family genes MutS homologs (MSH4 and MSH5) and MutL homologs (MLH1, MLH3, and PMS2) are important candidates causing infertility (Carrell & Aston, 2011; Nuti & Krausz, 2008; Tuttelmann, Meyts, Nieschlag, & Simoni, 2007). The two homologs of MutL family that is MLH1 and PMS2 form a heterodimer MutLa, that directs the restoration of mismatched DNA via the exonuclease mediated DNA degradation (Sancar, 1999). It is likely that polymorphism of PMS2 Ser775Asn is interfering with the interactional integrity between MLH1 and PMS2 (Guixiang et al., 2012).

## 10.2.2 Role of genetics in infertility

### *10.2.2.1 Genetic causes of female infertility*

About 10% females of reproductive age fail to conceive, and female infertility factors alone make 35% of all the cases. The female infertility is an outcome of multifactorial events that affect the oocyte maturation, ovary development and fertilization ability. The large number of genetic factors like chromosomal abnormalities, deletions, duplications etc is linked with the female infertility (Table 10.1). The common genetic causes of infertility are discussed as below:

#### 10.2.2.1.1 Polycystic ovary syndrome

The Polycystic ovary syndrome (PCOS) is a heterogeneous complex endocrine disorder affecting 5%−10% of women. The PCOS exhibit features like insulin resistance, hyperandrogenism, chronic anovulation, and hyperinsulinemia (Hossain, Cao, & Wang, 2013; Walters, Allan, & Handelsman, 2012). The clinico-pathological features involve follicle growth arrest at antral stage, low proliferative rate of granulose, irregular menstruation, and hyperthecosis (Dunaif, 1997). A number of endocrine, genetic, environmental, and metabolic factors are linked with PCOS (De Leo et al., 2016). The PCOS linked genes are likely involved in sex hormone regulation, steroid synthesis, and insulin sensitivity (Simoni, Tempfer, Destenaves, & Fauser, 2008). The genes showing relation with PCOS are: CYP17 (CYP17-cytochrome), HSD17B1−3 (17-β hydroxysteroid dehydrogenase type 1−3), StAR (steroidogenic acute regulatory protein), ACTR1 (activin receptor I), CYP11A (CYP11A-cytochrome), LHCGR (luteinizing hormone/chorionic gonadotropin receptor), INHBA (inhibin β-A), ACTR2A−B (activin receptor 2A and 2B), INHC (inhibin C) (Jakubowski, 2005). The mutations that modulate androgen synthesis its transportation, its maintenance and regulation are the evident candidates for PCOS. The evident mutations in LHR (LH receptor), FBN3 (fibrillin-3) have been observed in Chinese and Europena PCOS patients (De Leo et al., 2016). The FBN3 is encoding an extracellular fibrillin protein that cooperates and regulates TGF-β binding proteins, in absence of FBN3 the unregulated TGF-β promotes collagen and fibroblast production in ovarian cortex (Prodoehl, Hatzirodos, & Irving-Rodgers, 2009). The LHR variants cause PCOS via activating mutations to increase the pituitary LH effects on ovarian thecal cells (De Leo et al., 2016).

#### 10.2.2.1.2 Endometriosis

The endometriosis occurs in 30%−40% females suffering from infertility and pelvic pain. These are ectopic endometrial or estrogen-dependent stomal glands (Barnhart, Dunsmoor-Su, & Coutifaris, 2002). The retrograde

**TABLE 10.1** Various fertility disorders, their characteristic features and genes responsible.

| Fertility disorder | Gene responsible | Clinical manifestation | References |
|---|---|---|---|
| Polycystic ovary syndrome | ACTR2A-B, SHBG, StAR, VDR, CYP11, HSD3B1−2, CYP19GF1 | The PCOS exhibit features like insulin resistance, hyperandrogenism, chronic anovulation and hyperinsulinemia | Jakubowski (2005). |
| XX, gonadal dysgenesis | PSMC3IP,NR5A1FSHR, HSD17B4, NR5A1 | Heterogeneous, but externally recognized by occurrence of gonadal streaks, absence of spontaneous pubertal development, uterine hypoplasia, primary amenorrhea and hypergonadotropic hypogonadism | Fogli and Boespflug-Tanguy (2006), Ledig et al. (2008), Lourenço et al. (2009), Zangen et al. (2011) |
| Premature ovarian failure | ADAMTS16, FOXO3A, FSHFMR1, TSHB | Onset of menopause in women under the age of 40 years | Shelling (2010) |
| Endometriosis | HOXA11 and A10 | Ectopic endometrial tissue implant on peritoneal cavity sites | Ranney (1971) |
| Spermatogenic failure | RBYM1A1, BPY2, DBX3Y, TAF4B, SMYD3, DMRT1USP9Y, DAZ1, HSFY1, TSPY1PIWIL2 | Teratospermia, oligospermia, azoospermiaAsthenozoospermia | Massart et al. (2012) |
| Leydig cell hypoplasia | LHCGR | Leydig cells fail to produce testosterone hormone due to their unresponsive behavior towards HCG 0 | Salameh et al. (2005), Richter-Unruh et al. (2005) |
| XY, gonadal dysgenesis | SUPT3H, PRKACG, DMRT1, MAMLD1SUPT3H, PRKACG | Presence of few tubular structures or fibrous tissues, or may occur as streaks | Ledig, Hiort, Wünsch, and Wieacker (2012), Kalfa, Fukami, and Philibert (2012) |
| Cystic fibrosis | W1282X, R117H & F508del in CFTR | Congenital bilateral deficiency of male vas deferens or may also cause sperm anomalies like azoospermia, oligospermia and teratospermia | Stuppia et al. (2005), Schulz et al. (2006) |

menstruation theory is the extensively accepted mechanism responsible for causing endometriosis (Ranney, 1971). The involvement of genetics in endometriosis is well established due to occurrence of this disease in first or second degree relatives (Ranney, 1971). The HOX gene family is found to have association with endometriosis. The important homeobox genes responsible for endometriosis are HOXA11 and A10 (HOXA10). The uterine-based mechanism is involved in lowering the reproductive tendency of women with endometriosis (Taylor, Bagot, Kardana, Olive, & Arici, 1999). The role is epigenetics in causing female infertility and endometriosis is also well established (Kulp, Mamillapalli, & Taylor, 2016). In females with endometriosis the hypo-methylation has been analyzed in HOXA10 genes CpG site (Kulp et al., 2016). SNPs in certain set of genes are also having direct relation with endometriosis and infertility.

#### 10.2.2.1.3 XX gonadal dysgenesis

The mammalian fetal sex determination is manifested in germ cell development, their movement towards urogenital ridge and formation of either ovaries or testis from the bipotential gonad (Zangen, Kaufman, & Zeligson, 2011). This course of sex determination is directed by the complex array of molecular signaling mechanisms, whose deformities can proceeds to gonadal dysgenesis like XX female gonadal dysgenesis (XX-GD). XX-GD is genetically mixed/heterogeneous, but externally recognized by occurrence of gonadal streaks, absence of spontaneous pubertal development, uterine hypoplasia, primary amenorrhea and hypergonadotropic hypogonadism (Zangen et al., 2011). In XX-GD ovarian insufficiency is observed where in no puberty development occurs until menopause prior to the period of 40 years. Several gene mutation have been reported in XX-GD persons viz HSD17B4, FSHR, NR5A1, HARS2, BMP15, EIF2B5, and EIF2B2 (Fogli & Boespflug-Tanguy, 2006; Ledig, Röpke, Haeusler, Hinney, & Wieacker, 2008; Lourenço, Brauner, & Lin, 2009; Zangen et al., 2011). The mutations in PSMC3IP (pGlu201del) has been found to cause impairment in estrogen-driven transcription that leads into gonadal dysgenesis (Zangen et al., 2011). Both isolated and syndromic (Perrault syndrome, Ovarioleukodystrophy) cases of XX-GD has been reported.

#### 10.2.2.1.4 Down syndrome (trisomy 21) and turner syndrome (45, X)

The trisomy of chromosome number 21 causes mental retardation and infertility in humans: a condition termed Down syndrome. It is estimated to occur in one out of 700 births. The affected females are fertile in rare cases. The absence of X chromosome (45, X) is the distinctive karyotype in individuals with Turner syndrome. The turners syndrome occurs in nearly one in 5000 to one in 10,000 (and occurs in about 55% of cases) (Steven, Gersen, & Keagle, 1999). The XY homologous genes are playing a crucial role in the

gonad development and hyplo-insufficiency of these XY gens cause gonad deformities like streak gonads and complete sterility (Witters et al., 2001). In 75% of the cases the single X chromosome is having maternal origin. Thus the spermatozoa having a missing sex chromosome and due to paternal meiotic nondisjunction are responsible for turners disease. (Hassold et al., 1988). Further, the turners syndrome may also occur due to paternal double anaphase lag of X or Y chromosome in a XX or XY zygote (Jacobs et al., 1997).

#### *10.2.2.2 The genetic causes of male infertility*

The male infertility is a multifactor disorder responsible for 50% of all infertility cases. Male infertility is evaluated by medical history, hormone measurements, semen analysis (its density, morphology and motility) and physical examinations. Various genetic factors are responsible for male infertility, out of which the 10%−15% infertility cases are due to single gene mutations, and chromosomal abnormalities (Table 10.1) (Poongothai, Gopenath, & Manonayaki, 2009).

##### 10.2.2.2.1 Chromosome genes as a cause of Spermatogenic failure

The major cause of male infertility is Spermatogenic breakdown (Massart, Lissens, Tournaye, & Stouffs, 2012). The failure of spermatogenesis proceeds to Teratospermia (abnormal sperm morphology), oligospermia (fewer sperm count), azoospermia (absence of sperms), asthenozoospermia (decreased sperm motility). The Y chromosome (the smallest human chromosome) is an important candidate involved in genetic infertility. Male Y chromosome carries the genes responsible for sperm formation. Y chromosome genes are very prone to errors due to the occurrence of palindromic sequences (Rives, 2014; Vogt, 2005). The micro-deletion in the long arm (Yq) of Y chromosome is identified in males with spermatogenic breakdown (Tiepolo & Zuffardi, 1976). The deletions in azoospermia (AZF) factor regions associated with spermatogenic breakdown are believed to be an outcome of nonallelic homologous recombination. The deletion of ~792 kb and ~98 kb of AZFa region proceeds to the complete deficiency of spermatozoa (Kuroda-Kawaguchi, Skaletsky, & Brown, 2001). The Sertoli cell-only syndrome (condition in which only sertoli cells are lining the seminiferous tubules) cells is exhibited in the males with AZAa region deletion (Krausz, Quintana-Murci, & McElreavey, 2005). The deletion of second region that is AZFb arrests the Spermatogenesis (Krausz et al., 2005). The deficiency of AZFb genes (RBM 1 and 2) cause severe defects in the processing and metabolism of RNA (Ma, Inglis, et al., 1993; Ma, Simpson, et al., 1993). The RNA binding protein that is RBMY is localized and confined to the Y chromosome germ cell nucleus throughout the sperm formation process except elongation step (Elliott, Millar, & Oghene, 1997). The loss of AZFc region causes oligospermia or azoospermia (Stouffs, Lissens, Tournaye, Van

Steirteghem, & Liebaers, 2005). The number of AZF-related candidate genes RBYM1A1 (RNA binding motif protein, Y-linked, family 1, member A1), DBX3Y (DEAD [Asp-Glu-Ala-Asp] box helicase 3, Y-linked), HSFY1 (heat shock transcription factor, Y-linked 1), and TSPY1 (testis specific protein, Y-linked 1) have been studied and tested for spermatozoa defects (Massart et al., 2012). The DAZ gene located in the AZFc region contains an amino-terminal RNA binding motif and a sequence of amino-acids RNA-binding protein. The DAZ gene is deleted in the condition of azoospermia (Reijo, Lee, & Salo, 1995). DAZ gene is important for successful spermatogenesis and its deletion is associated with male infertility (Ferlin et al., 1999a).

#### 10.2.2.2.2 Chromosomal alterations as a cause of male infertility: translocation and inversion

The structural alteration in chromosomes is also responsible for male infertility. The anomalies responsible for infertility are translocation, deletion of Y chromosome, and inversion. The intensity of structural deformity is dependent on the size and location of particular anomaly and occurrence of inter-chromosomal effects at the time of meiotic recombination (Tempest et al., 2010). The translocation constitutes 1.8%−3.3% of the autosomal anomalies among infertile men. The translocation is vital in the preservation of genetic material that is involved in the growth, and functional maturity of spermatozoa along with inheritance of genetic material to embryo (Yatsenko, Yatsenko, & Weedin, 2010). The balanced translocation is rarely associated with the infertility. The meiotic errors occur at elevated rates in the spermatozoa (29%−81%) as a consequence of translocation having breakpoints within a particular gene or its controlling/regulating region, that proceeds into disomy or unbalanced translocation producing aneuploid embryos (Sarrate et al., 2005; Tempest, 2011). The male sterility via disruption of sperm development/Spermatogenesis also occurs due to translocation event between the autosomes and X chromosome (Madan, 1983). The male Y chromosomal and autosomal rearrangement also causes sterility if the breakpoint lies in the Y chromosomes pseudoautosomal area (as this contains fertility and meiotic controlling genes). The meiotic flaw of sperms occurs due to the Robertsonian translocations between the acrocentric chromosomes (van Assche, Bonduelle, & Tournaye, 1996). The Robertsonian translocations between chromosome 13, 14, 15, 21, and 22 that is in acrocentric chromosomes occur as eight five-fold enhanced in infertile males. The chromosomal inversion produce unbalanced gametes at higher incidence (0.7%−54.3%) and are 8 times more recurrent in infertile men (van Assche et al., 1996). The inversion caused deformity in sperms is dependent on the size and position of inversion (Sarrate et al., 2005). The inversion in the chromosome 1 carrying the most of genetic material has an elevated risk for male infertility (van Assche et al., 1996).

#### 10.2.2.2.3 Leydig cell hypoplasia as a cause of male infertility

In humans the male genital organs start developing approximately around the ninth week of gestation. The male sex hormone that is testosterone which is responsible for genitalia formation is secreted from leydig cells of fetus under the action of Human chorionic gonadotropin (HCG) that later on is replaced by another luteinizing hormone (Salameh, Choucair, & Guo, 2005). The Leydig cell hypoplasia is an uncommon recessive autosomal medical condition, in which the fetal Leydig cells fail to produce testosterone hormone due to their unresponsive behavior towards HCG. This condition occurs due to the autosomal inactivating mutations in LHCGR gene (having 11 exons) and results in feminization of exterior genitals and fractional development of micropenis (Richter-Unruh et al., 2005; Salameh et al., 2005). The eleventh exon of LHCGR gene codes for intracellular and 7-transmembrane domains of the receptor and its frame shift mutation (c. A589fs) and nonsense mutation (c. T1836G) has been reported in a Turkish patient and 22-year-old Bedouin female born of consanguineous marriage (Richter-Unruh et al., 2005; Salameh et al., 2005). The mitogen-activated protein kinase (MAPK) signaling cascadein postnatal Leydig cells gets activated once the LH binds with LHCGR (Shiraishi & Ascoli, 2007). In the experimental mice the Leydig cell-specific deletion of MAPK 1/2 causes hypoplasia of leydig cells and the activation of MAPK signaling pathway generates steroids (Martinelle, Holst Söder, & Svechnikov, 2004; Yamashita, Tai, Charron, Ko, & Ascoli, 2011).

#### 10.2.2.2.4 Cystic fibrosis as a cause of male infertility

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cyclic adenosine monophosphate (cAMP) activated ion conducting channel in epithelial cells. The activated CFTR channel is carrying conduction the Cl- and HCO3- ions. (Quinton, 2007). The mutation in CFTR is responsible for cystic fibrosis (CF)- a systemic disorder occurring in multiple organs. The infertility is also linked with CF, the CFTR mutations in males results in congenital bilateral deficiency of male vas deferens or may also cause sperm anomalies like azoospermia, oligospermia and teratospermia (Chen, Ruan, Xu, Chen, & Chan, 2012; Welsh & Fick, 1987). In the CFTR the mutations viz W1282X, R117H, and F508del have been observed in the infertile males (Schulz et al., 2006; Stuppia, Antonucci, & Binni, 2005). The phosphorylation and production of cAMP response element-binding protein induced by FSH is crucial for sperm formation. The defect in CFTR is responsible for inadequate activation of FSH-triggered signal transduction. (Chen et al., 2012). The $HCO_3$-, ions secreted by oviduct and endometrial are crucial for the capacitation process of sperms (Wang, Zhou, & Shi, 2003). The fluid control in the female reproductive tracts is also under the influence of CFTR occurring in the ovary, cervix, uterus and oviduct. The menstrual

abnormalities like irregular cycles, amenorrhea, is also associated with CF (Ajonuma, Ng, & Chow, 2005; Kent, Oliver, & Foskett, 1996). The CF is frequently occurring critical autosomal recessive condition in Caucasians, with the incidence rate of one in 25 and carrier rate of 1 in 2400 (Hargreave, 2000). Male infertility in CFTR mutations is mainly due to obstructive azoospermia (Sheynkin, 2000). In more than 95% CF cases, the abnormalities occurs in structures originated from Wolffian duct.

#### 10.2.2.2.5 Down syndrome and klinefelter syndrome (47, XXY)

The females with Down syndrome are fertile in certain rare cases; however, the affected males always sterile. In males the Down syndrome is marked by certain external phenotypic changes like decline in germ cells count, spermatogenic arrest and hylinized tubule. The possible mechanism, by which trisomy affects male fertility is believed to be the decline in proliferation of primordial germ cells during relocation/migration to the appropriate gonadal ridge, possibly correlated with the increased rate of cell death apoptosis (Patrizio & Broomfield, 1999).

The Klinefelter syndrome occurs at the rate of one in 1000 males. It is marked by the genotype 47, XXY occurring in all cells or may occurs as mosaic form. It is marked by spermatogenic failure, so males are usually sterile unless mosaic (Rives et al., 2000). The males with Klinefelter syndrome when reproduce give birth to aneuploid offspring (Mroz, Carrel, & Hunt, 1999). The one extra X chromosome in >50% of male parents arise in meiosis I non-disjunction and about 40% of female parents arise in meiosis I or II in XY bivalents, and in rest occurs post-zygotically (Hassold et al., 1996). The chances of Klinefelter syndrome increases with maternal age (Hargreave, 2000). The older men similar to older females have more likelihood to produce XXY offspring (Griffin et al., 1995)

## 10.3 Conclusion

Successful fertilization and reproduction involves a dynamic interaction between immunogenetic and immunological factors. The failure of any of these events proceeds to infertility. The infertility is an outcome of multiple factors and is used to describe a wide range of varied phenotypes. The genetic factors like single chromosomal aberrations, single or polygenic mutations usually affect germ cells. However, The idiopathic infertility is associated with the immune aspects like levels of immunoglobins, natural tolerance, and antibodies specific to local and systemic secretions. The Isoimmunization is also linked with female immune infertility. The reproduction is a subject of intense research. There is a need for the new drug therapies and improvising the existing ones. There are no precisely defined genes that could be used for the genetic testing of sterility and infertility. Further the

immunological factors responsible for infertility needs proper validation for complete treatment and prevention of infertility. The studies of immunological, phenotypic, and genotypic correlations and micro-array based GWS (genome −wide studies) will also help in understanding the disease causing factors. Expression analysis, Bisulfite sequencing, affinity purification sequencing, and methylated DNA immunoprecipitation sequencing may also add to the research. The animal models may also be used as substitutes for human studies so as to avoid the various difficulties that are being faced during human reproduction. This chapter highlights the significance of immunegenetic factors and the disruption of their normal interacting networks as a cause of reproductive failure.

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Chapter 11

# Immunopharmacogenomics: clinical applications, challenges, and future prospects

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## 11.1 Introduction

For the last two decades immunotherapy has revolutionized the therapeutic approach of several diseases like cancer and autoimmune diseases, via initiating, boosting of suppressing the immune system (Kulwal & Sawarkar, 2021). IPG helps to personalize and create an accurate treatment by analyzing the molecular basis of the disease. Immunopharmacogenomics (IPG) integrates immunogenomics and pharmacogenomics to investigate the immune responses to treatment in various diseases. IPG evaluates the genetic effect of the diseases during treatment, including the efficacy and adverse reactions of the immune response, to predict the possible outcome of immunotherapy. IPG helps the better understanding of immune responses in numerous biological and pathological conditions, including cancer, autoimmune disease, infectious disease, drug-induced toxicity, food allergy, and post organ transplant rejection (Fig. 11.1) (Zewde et al., 2018). IPG analyzes the molecular mechanisms and functional aspects for various cancer treatments. The alterations of several checkpoint receptors and tumor vaccines are considered the most powerful tools in fighting against cancer. The targeted immune-oncology is considered an essential prospect of IPG that has a crucial role in cancer cell destruction by TCRs and killer T cells. Cancer immunotherapy may be divided either as active immunotherapy or passive immunotherapy. For passive immunotherapy, tumor cells are directly targeted via injection of immune reactive agents, such as antibodies or T cells, whereas, for active immunotherapy, the immune system of the host is activated to fight against the malignancy.

Recently, advancement in cancer research has brought considerable attention toward the IPG, especially to the immune checkpoint antibodies, such as

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00004-X**
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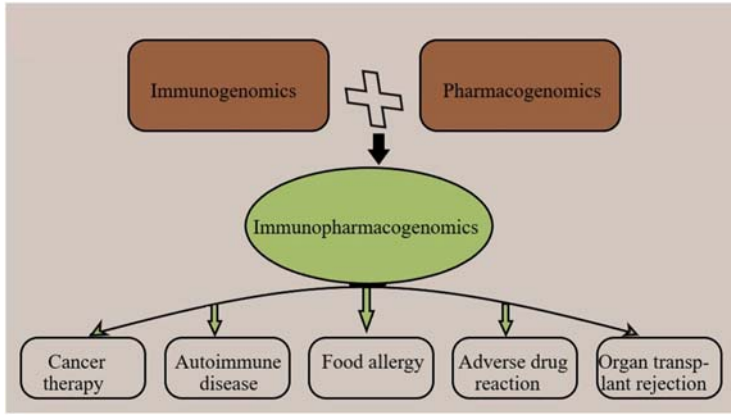


**FIGURE 11.1** Immunopharmacogenomics is involved in evaluating the immune response to predict the possible outcome of immunotherapy in several diseases, including cancer, autoimmune disease, infectious disease, drug-induced toxicity, food allergy, and post organ transplant rejection.

lymphocyte activation gene-3 (LAG-3), cytotoxic T- lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), its ligand (PD-L1) (Fig. 11.2) (Kiyotani, Chan, & Nakamura, 2018). CTLA-4, PD-1, PD-L1 have revolutionized cancer immunotherapy via focusing on effective diagnostic, prognostic, and therapeutic aspects of cancer therapy (D'Andréa, Lassalle, Guevara, Mograbi, & Hofman, 2021). These antibodies are designated to target the suppressive molecules, which prohibit the immune system of the host cell against the malignant growth. The suppression of these molecules activates the defense system that consequently helps in eliminating the cancerous cells. Apart from the suppression of the immune system, the weak immunogenicity of the cancer cells is an important reason for their immune escape. Neoantigens are tumor-specific antigens (TSA) with stronger immunogenicity, which could be used to target the cancer cells efficiently in immunotherapy. Neoantigens are recognized as foreign antigens that increases the immune response against cancer cells by efficiently triggering the T cell response. It reduces the risk of autoimmunity in immunotherapy (Peng et al., 2019). Neoantigen vaccine is emerging as effective antitumor immunotherapy and various studies have reported that neoantigens improve the survival period and quality of life among cancer patients.

In an autoimmune disorder, antibodies are produced due to the overactivation of the immune system against the body's own normal cells or organs. The unregulated activation of T and B cells exhibits autotoxic effects in several organs, including the brain, lungs, pancreas, endocrine organ, gastrointestinal tract (Lee et al., 2020). In order to avoid the unnecessary autoimmune response, the immune system maintains a proper balance between

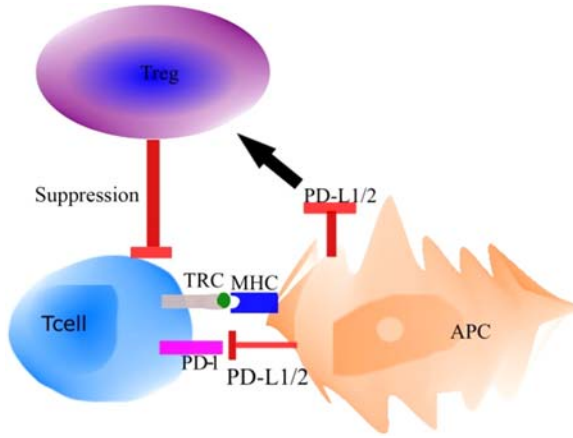


**FIGURE 11.2** PD-1, an inhibitory checkpoint receptor interacts with PD-L1/PD-L2 ligands to inhibit the activity of T- cells. PD-L1/PD-L2 boosts the growth and developments of Tregs that are involved in reducing the antigen specific T-cell response. *Adapted and modified from He X. S. Gershwin M. E., & Ansari A. A. (2017). Checkpoint-based immunotherapy for autoimmune diseases—Opportunities and challenges.* Journal of Autoimmunity, *79, 1–3.*

stimulatory cytokines and antiinflammatory factors. The abnormal immune response reduces the efficiency defense mechanisms against various infections and increases the vulnerability of various immune-mediated diseases, like autoimmunity. The genetic alterations regulating the levels of inflammatory molecules have become an important approach for the treatment of autoimmune disorders. IPG plays has essential role in monitoring the adverse reactions of autoimmunity by identifying the activated T cells or biomarkers associated with immune activation (Immunotherapy for autoimmune disease, 2019).

Allergy is a systemic disease that causes sensitization due to the exposure of allergens in the airways, gastrointestinal tract, body fluids, or skin. Immunotherapy targets the main mechanisms of the immune system in the allergic disease that induces immunological tolerance (Larsen, Broge, & Jacobi, 2016). Food allergy is an abnormal immune response against the food proteins and addresses a breakdown of oral tolerance (Kamdar & Bryce, 2010). As such, there is no approved treatment available to date to cure food allergies. Immunotherapy induces the desensitization or tolerance toward the allergens that can trigger adverse immune reactions while moving into the gastrointestinal tract (Kamdar & Bryce, 2010; Mia, 2015). The IgE-mediated stimulation is the common therapeutic approach that leads to the desensitization of immune cells (Kamdar & Bryce, 2010). IPG helps the better understanding of molecular mechanisms that is very essential for designing an effective drug.

Immunomodulatory drugs have revolutionized the treatment of several diseases by targeting the signaling pathways of the immune system that increases or decreases the production of antibodies for clinical benefits, including the overall survival rate. However, these drugs could have immune-related adverse effects on organs or systems of the body, especially the gastrointestinal system, liver, skin, endocrine glands (Morgado, Plácido, Morgado, & Roque, 2020). Although the exact mechanism of adverse reactions to the immune system is not known, however, studies have reported the association of T cells, antibodies, and cytokines to patients with adverse drug reactions. These adverse reactions could occur at any time within the first week to months after initiating the treatment. Generally, the adverse reactions of the immune system are mild to moderate in nature, but they can cause serious life-threatening.

Furthermore, IPG also plays a role in organ transplantation and helps to determine the overall success of transplantation and disease relapse during the organ transplantation. For food allergy, IPG can help in understanding the differential outcome of allergic reaction among the patients in terms of their genetic makeup. IPG also helps to monitor and identify the appropriate immunotherapy in potentially allergic patients. In this article we discussed the important perspective on understanding IPG in the field of immunotherapy. The main focus of the study was to investigate the therapeutic role of IPG in fatal diseases, including cancer. The clinical application, challenges and future prospect of IPG were also evaluated for the therapeutic approach in various immune diseases.

## 11.2 Immunopharmacogenomics in cancer therapy

Recent advancements in IPG immunotherapeutic techniques, including immune target checkpoint antibodies, have successfully revolutionized cancer treatment for a better prognosis of the disease. Immune checkpoint antibodies target those molecules that suppress the natural immune response of the host toward tumor cells, and efficiently triggers the immune defense mechanisms against the malignant cells (Choudhury & Nakamura, 2016). During an immune response, T cell checkpoints act as molecular breaks to maintain the balance of the immune system. Recent studies have reported that cancer cells efficiently express checkpoint inhibitors, in order to escape successfully from the immune defense system (Peng et al., 2019). Recently, various immune target checkpoints have been identified, such as LAG-3, CTLA-4, PD-1, PD-L1, which are considered an essential accomplishment in immune checkpoint inhibitors, and have demonstrated that immune system could efficiently exterminate the tumor cells (Qin, Xu, & Yi, 2019). These immune target checkpoints play an essential role in suppressing the immune response against the T cell arbitrated anticancer immune response (Hugo et al., 2016; Kiyotani et al., 2018).

In 2018 Allison and Honjo reported that CTLA-4 and PD-1 negatively regulate the immune system and blocks the anticancer immune response. They further endorsed that antibody-mediated blockade of the immune checkpoints could successfully induce the antitumor on malignant cells (Peng et al., 2019). CTLA-4 has a crucial role in preventing autoimmunity and sustain self-tolerance (Waterhouse et al., 1995). They are expressed on the surface of T cells and controls the magnitude of the T cell response. Antigens exhibited on MHC of Antigen-presenting cells (APC) are presented to the T cell receptors and the co-stimulatory signal is transmitted via binding of CD28 to CD80 and CD86 on APC for efficacious T cell response. CTLA-4 competes for the binding site of CD80 and CD86 with CD28 and suppresses the TCR downstream signaling (Lenschow et al., 1996). Several clinical trials have reported that inhibition of CTLA-4 can be used in cancer therapy in humans due to its anticancer properties (Fig. 11.3).

In 2015 Hung et al., evaluates the combined effect of Lag-3 and PD-1 pathway blockade in ovarian cancer and found both pathways

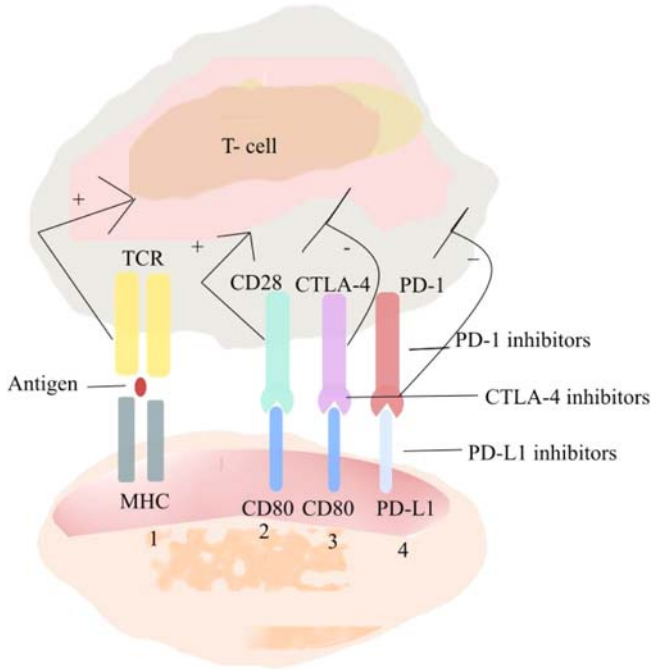


**FIGURE 11.3** Immune Checkpoint mechanisms (1) MHC present the antigen to T cell receptor to activate T cells. (2) CD80 on tumors cells interacts with CD28 present on T cells to deactivate the T cells. (3) CTLA4 competes with CD80 to represses the T cells. PD-L1 interacts with PD-1 receptors on T cells to suppress T cells. *Adapted and modified from Sambi M., Bagheri L., & Szewczuk M. R. (2019). Curent challenges in cancer immunotherapy: Multimodal approach to improve efficacy and patient response rates.* Journal of Oncology. *Article ID 4508794 12 pages.*

interdependently boost anticancer activity. The malignant growth could be suppressed either by increasing CD8 + tumor-infiltrating T cells or by reducing the Tregs in the tumor microenvironment (Huang et al., 2015). Furthermore, the over-expression of PD-L1 and high invasion of immune cells are supposed to have a significant correlation with treatment response to anti-PD-1 therapy (Kiyotani et al., 2018; Topalian, Drake, & Pardoll, 2012). However, various studies have reported that patients who lacked the PD-L1 expression in tumor tissues exhibited satisfactory results from anti-PD-1 therapy (Brahmer et al., 2012; Kiyotani et al., 2018). PD-L2 ligand also inhibits T-cells cytotoxic activity in cancer cells, so the elevated expression of could be associated with a positive clinical response (Kiyotani et al., 2018; Sharpe, Wherry, Ahmed, & Freeman, 2007). PD1 that are expressed on various immune cells, such as activated T cells, B cells, and natural killer cells, binds with either PD-L1 or PD-L2 ligand, found on the surface of APC and cancer cells (Choudhury & Nakamura, 2016; Freeman et al., 2000). The interaction of PD-1 to PD-L1/2 inhibits the kinase activity of Cdk2 and results in T cell activation (Choudhury & Nakamura, 2016; Patsoukis, Sari, & Boussiotis, 2012). In tumor cells, the functional disruption of T cell leads to over-expression of PD-1, which could be reversed by suppressing the binding of PD-1/PD-L1 (Barber et al., 2006; Choudhury & Nakamura, 2016). The increased PD-L1 expression and high invasion of immune cells respond positively to anti-PD-1 cancer therapy (Kiyotani et al., 2018).

Recently, researchers have explored a new group of antigens, neoantigens, that has the potential to be used for cancer therapy (Choudhury & Nakamura, 2016; Peng et al., 2019). Neoantigens are TSA, which are expressed only in tumor cells. Neoantigens exhibit strong immunogenicity and higher affinity toward MHC, are unaffected by central immunological tolerance (Peng et al., 2019). Neoantigens have a greater individual-specificity and are generated due to the nonsynonymous mutations in the tumor cell leading to the altered amino acid sequence of proteins that is processed into short peptides (Blum, Wearsch, & Cresswell, 2013; Peng et al., 2019). These peptides, recognized as foreign-antigen, are exposed to MHC molecules that activate the immune response against the cancer cells.

The efficiency of tumor vaccines depends on the degree of expressional variations between the cancer cells and normal cells. Neoantigens increase the antitumor response and decreases the autoimmunity risk (Peng et al., 2019). Therefore neoantigens-mediated T cells could produce highly efficient T cells, whose TCRs exhibit greater affinity for MHC-neoantigen-peptide complexes and escape the clearance via central immune tolerance (Peng et al., 2019; Stone, Harris, & Kranz, 2015). Various clinical trials have reported that T cells of the host efficiently recognized neoantigens of the tumor tissue and initiates the anticancer immune response. Neoantigens are recognized by CD8 + and CD4 + T cell in malignant tissues and efficiently initiates the anticancer response (Linnemann et al., 2015; Peng et al., 2019;

Robbins et al., 2013). Studies have reported that a combination of tumor vaccine along with immunosuppressive therapy is more efficient in fighting against the malignancy as compared to monotherapy (Karyampudi et al., 2014; Peng et al., 2019). Several studies have reported that neoantigen vaccines have exhibited the expected results in strict phase I clinical trials, especially the vaccines based on dendritic cells, synthetic long peptides, and RNAs have exhibited outstanding safety and immunogenicity.

Cancer vaccines are used either for preventive or for therapeutic treatments. The vaccine treatment against the oncoviruses, including HPV, HBV, HCV, EBV decreases the cancer risk (Li, Franceschi, Howell-Jones, Snijders, & Clifford, 2011). The vaccine Cervarix and Gardasil significantly reduce HPV-associated cervical cancer in young women (Giarelli, 2007). Peptide vaccines are more effective, has short-time manufacturing period, low cost and stable under varying storage condition (Liu & Kline, 2015). They efficiently trigger T cell response and the peptide-specific T cell response can be observed directly. The main purpose of tumor-specific peptide vaccines is to enhance the antitumor response against the cancer cells and several immune cells including $CD8^{+}$, T cells, and cytokines can be

**TABLE 11.1** Types of cancer vaccines in different phases of clinical trials.

| S. no | Type of vaccine | Type of cancer |
|---|---|---|
| 1. | Whole-cell or lysate cancer vaccine | Melanoma, leukemia, brain, cervical, myeloma, gastrointestinal, renal cell carcinoma |
| 2. | Gene modified cancer cells | Lung cancer, myeloma, pancreatic, breast, ovarian renal cell carcinoma |
| 3. | Peptides | Leukemia, melanoma, myeloma, lung, prostate, ovarian, cervical, pancreases, hepatocellular carcinoma |
| 4. | Proteins | Non-Hodgkin's lymphoma, ocular melanoma |
| 5. | Heat shock proteins | Renal cell carcinoma, melanoma, gastric, breast, gastrointestinal, pancreatic, sarcoma |
| 6. | Immunocytokines | Epithelial, melanoma |
| 7. | Naked DNA | Prostate, melanoma |
| 8. | Viral vectors | Gastrointestinal, melanoma, prostate |
| 9. | Lymphocytes | CNS, melanoma, renal cell carcinoma, prostate, breast, ovary, cervical, lung, gastrointestinal cancer |
| 10. | Dendritic cells | CNS, Melanoma, prostate, breast, ovarian, cervical, lung, gastrointestinal cancer |

used as biomarkers and could help to observe the therapy efficiency of the patients. The different types of cancer vaccines that are in different phases of clinical trial is given in Table 11.1.

## 11.3 Immunopharmacogenomics in autoimmunity

An autoimmune disease is a clinical condition resulting from inappropriate activation of T cells, B cells, or both against the body's own tissues or organs. Generally, the self-reactive T and B cells are eradicated in central immune tolerance, but some cells escape the process and enter the blood and tissues, where they are monitored by immune tolerance mechanisms (Smilek, Ehlers, & Nepom, 2014). The disruption in the normal functioning of the immune system leads to autoimmunity disorder and tolerance restoration is the center of focus in autoimmune therapy. IPG immunotherapies have been considered an efficacious tool in cancer therapy, allergy treatments, suppressing immune response during organ transplant, and autoimmunity. Chimeric antigen receptor (CAR)-T cells targets autoantigens to induce immune suppression and reduce inflammation. Patients are monitored closely during the IPG treatments to avoid any adverse effects such as infections, activation, or inhibition of immune pathways via monitoring the immune processes or biomarkers that are associated with immune system activation or repression. A study conducted by Kwong et al. reported that in mice the urinary nanosensor helps to detect acute rejection of transplanted skin allograft during its early stage (Kwong et al., 2013).

Cell-based immune therapy signifies an essential step in establishing a personalized therapeutic approach for the treatment of autoimmune disease due to its high specificity, self-renewing, and self-regulatory mechanisms (Smilek et al., 2014). The functional loss of regulatory T cells (Tregs) could result autoimmunity, whereas overexpression of Treg could trigger tumorigenesis (Dees, Ganesan, Singh, & Grewal, 2021). Several studies have used the manipulated immune cells (such as T cells, B cells) for autoimmune treatment in animal models. Ex vivo experiments have reported that expansion and transfer of regulatory T cells into the nonobese diabetic mice forestalled and switched diabetics (Bluestone & Tang, 2004; Jaeckel, von Boehmer, & Manns, 2005; Smilek et al., 2014; Tang et al., 2004). The transfer of regulatory T-cells (Tregs) in a murine model of myelin-peptide-induced experimental autoimmune encephalomyelitis (EAE), prevents the progression of central nervous system inflammation and its other clinical symptoms (Kohm, Carpentier, Anger, & Miller, 2002; Smilek et al., 2014). Further, in an experimental murine model of inflammatory bowel disease, the transferred regulatory T cells suppress the infiltrated inflammation of lamina propia inflammation and heals the intestinal inflammation (Mottet, Uhlig, & Powrie, 2003; Smilek et al., 2014). Furthermore, to target the autoimmunity-relevant factors, the drugs are designed to activate or inhibit

the factors involved in immune suppression. The inflammatory responses in autoimmune diseases are regulated by balancing the cytokines and antiinflammatory molecules. In MRL/lpr/lpr mice with Systemic Lupus Erythematosus (SLE), the survival rate increases after injecting plasmid cDNA inducing TGF-β injection, whereas, intramuscular injection of plasmid cDNA forming IL-2 reduces survival rates (Lee et al., 2020).

The autoimmune response of the activated T cells can be reduced by blocking the costimulatory signal of T cells. In MRL/lpr/lpr mice, the soluble CTLA4/IgG complex interacts with CD80 or CD86 and decreases autoimmune diseases (Takiguchi et al., 1999). Furthermore, when recombinant adenovirus vector containing the CTLA4/IgG gene was inserted in MRL/lpr/lpr mice, the serum concentration of CTLA4/IgG increases that leads to the reduction of autoantibodies and sustained the renal function (Takiguchi et al., 2000). SLE has a positive correlation with serum interferon-γ (IF-γ) and impaired IF-γ levels or IF-γ receptor significantly reduces the severity of disease (Haas, Ryffel, & Le Hir, 1998; Hooks et al., 1979; Lawson et al., 2000). The intramuscular injections of plasmid cDNA inducing IF-γ receptor/Fc prevents the development of SLE, increases the survival rate of MRL/lpr/lpr mice, and decrease the glomerulonephritis formation (Lawson et al., 2000).

Synthetic oligonucleotides (ODNs) are the sequence-specific short nucleotides that regulate gene expression. Over the last two decades, scientists have made tremendous progress in the development of synthetic ODN therapeutics. Synthetic ODNs are designed to target the complementary sequences on the gene and mimics the interaction of natural nucleotide sequences regulating gene expression. ODNs are designed to inhibit the induction of the pro-inflammatory cytokine (Lawson et al., 2000). The repeated doses of synthetic ODNs delay the initiation and progression of glomerulonephritis, whereas, IL-12, IFN-γ anti-dsDNA autoantibodies level decreases in BWF1 mice (Dong, Ito, Ishii, & Klinman, 2005). It has been reported that there was no immune response against the RNA containing antigen in mice lacking toll-like receptor 7 (TLR7) were absent. The loss of TLR7 increases several disease risks and has low serum IgG (Christensen et al., 2006). In clinical treatment, the expression of pro-inflammatory cytokines is successfully reduced either by siRNA or by inhibiting cytokines that efficiently regulate the autoimmunity (Venkatesha, Dudics, Acharya, & Moudgil, 2014).

## 11.4 Immunopharmacogenomics in food allergy

Food allergies are a major health issue in developed countries and the cause is due to oral intolerance. The incidence could vary according to different ethical backgrounds and geographical location. Studies have reported that Asian populations are the least susceptible to food sensitizations compared to

African and western populations (Mia, 2015). Food sensitization induces the formation of specific IgE antibodies, and exposure to a particular antigen in the future might trigger the acute or delayed-type of the immune response (Wang et al., 2010). In the acute immune response, IgE binds to IgE receptor (FceRI) present on mast cells and basophils, this interaction triggers the release of histamine, platelet-activating factor (PAF), and 5-hydroxytryptamine (5-HT). Histamine, PAF, and 5-HT increase arteriolar vasodilation and vascular permeability that leads to cardiac contraction (Strait, Morris, Yang, Qu, & Finkelman, 2002). In mice, the IgE receptor knockdown exhibited no response to antigens, and in acute allergies, the blockage of FceRI receptors are used in immune therapy to stop the immune response (Lin et al., 2000; Mancardi et al., 2008; Wechsler, Schroeder, Byrne, Chien, & Bryce, 2013). In a delayed response, inflammatory cells like T cells, B cells, basophils are involved (Galli, Tsai, & Piliponsky, 2008).

Food allergy exhibits a significant association with the loss of TGF-β expression within the intestinal mucosa (Kamdar & Bryce, 2010). The oral supplementation of TGF-β increases the oral tolerance and decreases the antigen-specific IgE concentration (Ando & Shima, 2007; Kamdar & Bryce, 2010). Furthermore, studies have reported that the intestinal mucosa of children with food allergies have a low circulating number of IL-10 and TGF-β producing cells (Kamdar & Bryce, 2010; Pérez-Machado et al., 2003; Scott-Taylor, Hourihane, Harper, & Strobel, 2005). The immunotherapeutic approach depends on the response of T cells or antibodies to the allergens. The T cell response for allergens can be modified via T help cells or Tregs. Tregs reduce the immune response by inducing the secretion of IL-10 and TGF-β cytokines (Kamdar & Bryce, 2010).

Allergen immunotherapy is an effective approach for IgE-mediated allergy that induces long-term clinical tolerance via $IgG_4$ response. $IgG_4$ inhibits the IgE-mediated allergic response by acting as a blocking antibody via intercepting cross-linking of IgE receptors (James & Till, 2016; Kamdar & Bryce, 2010). $IgG_4$ has an essential role in immunotherapy, especially in long-term treatment. IgE-mediated stimulation could desensitize the mast cells and basophils and resists their immune response (Kamdar & Bryce, 2010). The low-dose exposure to antigens during the initial phase of immunotherapy endorses the local desensitization, which gradually increases the tolerance for high doses (Kamdar & Bryce, 2010).

The IPG therapeutic approach to food allergy can be delivered sublingually and orally, or epidermally (Mia, 2015). In oral immunotherapy (OIT), the desensitization is induced by swallowing gradually the increased amount of allergic food. The success rate of OIT is usually around 70%−80%. The food-specific IgE levels were found low in children who received OIT against milk, with an increased level of $IgG_4$ (Kamdar & Bryce, 2010). In another study, OIT for hen's egg exhibited low levels of egg-specific IgE

antibodies and high levels of TGF-$\beta_1$ among the children. Jones et al. reported that out of 29 children who received OIT against peanut, the secretion of IL-10, IL-13, IL-5, IFN-$\gamma$, TNF-$\alpha$, and $IgG_4$ was found high, while the IgE levels were reduced (Jones, Pons, & Roberts, 2009; Kamdar & Bryce, 2010; Mia, 2015). Sublingual immunotherapy (SLIT) is similar in mechanism to that of OIT, except the small doses of allergen extract are put under the tongue. SLIT is effective in food hypersensitivity treatment, including hazelnut, cow's milk, apple, wheat, egg, (Kamdar & Bryce, 2010). For hazelnut SILT, the patients showed a significant tolerance after 3 months of therapy, and around 50% of patients were able to tolerate the highest dose of 20 g of hazelnuts. The increased levels of IL-10 and $IgG_4$ was also reported in patients who received SLIT against hazelnut (Kamdar & Bryce, 2010). SLIT appears to have a less therapeutic effect for peanut allergy, although it has several advanced compared to OIT (Kamdar & Bryce, 2010; Narisety et al., 2015). In epicutaneous therapy (EPIT), the food allergens were administrated into the skin through patch application and result in the activation of Langerhans cells. The activated Langerhans cells move to lymph nodes and decrease the allergic response of cells (Luckashenak, Wahe, Breit, Brakebusch, & Brocker, 2013; Mia, 2015; Nakajima et al., 2012). EPIT has certain advantages over OIT and SLIT and exhibits mild to moderate reactions at the site of its application that are generally self-healed. EPIT has a long-lasting effect as compared to OIT and SLIT (Jones, Burks, & Dupont, 2014; Mia, 2015). In the milk-allergy mouse model, EPIT is effective in reducing the Th2 cytokine levels that would consequently reduce the immune response for milk-allergy (Mia, 2015).

## 11.5 Immunopharmacogenomics and adverse drug reactions

IPG biomarkers are essential to recognize the potentially allergic patients and identify the appropriate treatments (Karnes, Miller, White, Konvinse, & Pavlos, 2019; Muraro, Lemanske, Castells, Torres, & Khan, 2017). HLA alleles are effective in the identification and prevention of previously unknown diseases (Karnes et al., 2019). Genotyping of HLA alleles has an essential role in personalizing medicine during drug treatment and prevent IM-ADRs (Karnes et al., 2019; Leckband, Kelsoe, Dunnenberger, George, & Tran, 2013; Mallal, Phillips, Carosi, Molina, & Workman, 2008; Saag, Balu, Phillips, Brachman, & Martorell, 2008). IPG investigates the effect of immune-specific variation on drug disposition to develop effective immunotherapy against diseases. IPG increases the efficiency to predict and prevent immune-mediated-adverse drug reactions (IM-ADR) via screening of immunotherapy. In immediate ADR, the onset of drug reaction occurs within one hour of drug dose and includes both dose-independent IgE-mediated mast cell reactions and dose-dependent non-IgE-mediated mast cell reactions. IgE-mediated reactions are consistent in their onset that intensifies into severest

form with time (Karnes et al., 2019). Studies have reported the strong association of human leukocyte antigens (HLA) with various immunologic diseases including IM-ADRs and HLA screening is used as a preventive strategy (Daly, Donaldson, Bhatnagar, Shen, & Pe'er, 2009; Karnes et al., 2019; Kindmark et al., 2007).

Severe cutaneous adverse reactions (SCARs) are classified as the most severe form of IM-ADRs that include Stevens−Johnsons syndrome (SJS), toxic epidermal necrosis (TEN), and eosinophilia and systemic symptoms (DRESS). The SJS/TEN involves the epidermis, in which the drug binds to HLA protein on keratinocytes and result in CD8 + cytotoxic T cells activation. As drug-specific CD8 + cytotoxic T cells are accumulated within blisters and perforin and granzyme B are released, besides inducing other potent cytotoxic cells to secrete granulysin to kill keratinocytes. It has been reported as the most severe form of IM-ADR with a mortality rate of around 50% (Karnes et al., 2019; Pirmohamed, Aithal, Behr, Daly, & Roden, 2011). The characteristics of SJS/TEN include prodrome fever, sore throat, blister formation, epidermal necrosis, while long-term complications include blindness and psychiatric illness (Karnes et al., 2019; White, Abe, Ardern-Jones, Beachkofsky, & Bouchard, 2018). DRESS involves the lymphocytic infiltrate of T cells into the dermis and release TNF-$\alpha$ and IFN-$\gamma$ cytokines. In DRESS, the mortality rate is around 10% and clinical features of are similar to that of viral illness, such as lymphadenopathy, pneumonitis, myocarditis, and nephritis (Karnes et al., 2019).

## 11.6 Organ transplant rejection and immunopharmacogenomics

In organ failure and high-risk hematological malignancies, solid organ transplantation (SOT) and hematopoietic stem organ transplantation are the only available treatments that have a curative approach and increase the survival rate among patients. Organ rejection and disease relapse are the major complications that determine the overall success of transplantation. Immune tolerance inhibits the immune response of some antigens for the proper functioning of the transplanted. Recently, the efficacious immunosuppressive drugs along with better medical care decrease the possible chance of rejection and increase the survival rate by 1 year (Suthanthiran & Strom, 1994; Alachkar, 2015). Organ rejection is mediated by T lymphocytes that become unresponsive either by central tolerance or by peripheral tolerance. In central tolerance, T cells and T cell receptors that interact with antigen are depleted, while in peripheral tolerance, the mature T cells are suppressed or eliminated (Muhlberger, Perco, Fechete, Mayer, & Oberbauer, 2009; Alachkar, 2015). Furthermore, MHC has and essential role in immune system response on acceptance and rejection of grafts, therefore MHC can be modified to improve the outcome of transplantation.

## 11.7 Challenges of immunopharmacogenomics

IPG has revolutionized the field of immunotherapy which has become an important approach in the treatment of various disease conditions. IPG aims to evaluate the possible role of specific regulatory molecules in immune therapy. However, in IPG there are still numerous challenges that increase the complexities of immunotherapy in patients during the treatment.

### 11.7.1 Selection and monitoring of patients

In IPG, individual patients exhibit different immune responses and a significant amount of diversity and complexity in the data pose new challenges for analysis and handling data. Data sorting and analysis that is collected from millions of patients in the different areas of diseases like cancer, autoimmunity, and food allergy is a very difficult task. The patient selection for the appropriate response during the treatment of disease is quite very challenging due to the personalization of the immunotherapy approach. Furthermore, to collect the immunogenomics information for the selection of better responders to immunotherapy, several studies compare the immunological difference among the patients before and after treatment. But this attempt is very challenging due to the different immunological background of patients.

### 11.7.2 Efficacy is generally unpredictable

The major challenge in IPG is to develop an efficient therapeutic approach that is directed toward customized molecular alterations and patient-specific, in order to be highly effective. However, the prediction of the accurate patient-specific therapeutic result is very difficult. The increased risks of the adverse reactions are hard to identify and it is even worst for life-threatening conditions. Immunotherapy depends on the activation and amplification of immune response and every patient exhibits a different immune response that offers a great challenge to the effectiveness of immunotherapy. The outcome of clinical trials may not be appropriate for every individual due to the varying response rates toward the treatment of immunotherapy.

### 11.7.3 Check-point based immunotherapy

Check-point based immunotherapy is a promising therapeutic strategy in IPG that regulates the response of T cells via directly blocking the activation or inhibition pathways of autoreactive T cells. However, in the development of checkpoint-based immunotherapy major challenge is to recognize the appropriate pathways with high efficiency and minimum immune-induces adverse reactions (He, Gershwin, & Ansari, 2017). Furthermore, in immune checkpoint blockage, the biological response of immune-suppressive molecules

leads to the activation of T-lymphocytes that increases the secretion of cytokines. The cytokine causes the inflammation of tumor at the early stages of treatment before reducing the size of the tumor (Choudhury & Nakamura, 2016). Therefore proper strategies are required to monitor and evaluate the patient's response to immunotherapy in an appropriate time frame.

### 11.7.4 Lack of target specificity

The lack of selective targeting in Treg is a major challenge leading to inadequate efficacy of IPG. The lack of target specificity affects the antitumor effector T cells and their number usually decreases during Treg therapy (Dees et al., 2021).

### 11.7.5 Mutational landscape

The development of next-generation sequencing (NGS) revolutionized the therapeutic approach to identify the biomarkers by exploiting the molecular mutations with target therapies. NGS analyses the particular genetic mutation in the patients and provides a better opportunity to have their treatment according to the presence of mutation (Bernicker, 2016). Although nowadays sequencing analysis is not too expensive, however, bioinformatic analysis could be a challenging due to complexity of all parameters (Choudhury & Nakamura, 2016). Furthermore, immune repertoire sequencing encounter challenges due to the complexity of the development of B and T cells (Jang et al., 2015).

### 11.7.6 Development resistance

The microenvironment affects the efficacy of immunotherapy by offering resistance to the therapeutic agents (Sambi, Bagheri, & Szewczuk, 2019). The development of resistance to treatment is related to genetic modifications that include epigenetic changes or reactivation of alternating pathways to compensate for the alteration in their immediate environment (Sambi et al., 2019). Drug resistance also possess a great challenge in IPG. Studies have reported that the presence of bacteria in the microenvironment of pancreatic cancer exhibits strong resistance to chemotherapy (Geller, Barzily-Rokni, & Danino, 2017; Sambi et al., 2019).

### 11.7.7 Gut microbiota

The gut microbiota constitutes trillions of bacteria, viruses, and fungi of the intestine that offers natural protection against the various types of infections (Sambi et al., 2019). Studies have relieved that microbiota affects the efficacy of chemotherapies and immunotherapies (Gopalakrishnan, et al., 2018; Iida, et al., 2013; Sambi et al., 2019; York, 2018). Recent studies have

reported that gut microbiota has a significant impact on the immune response to anti-PD-1 immunotherapy among melanoma patients (Gopalakrishnan et al., 2018). Researchers have investigated the influence of different gut microbiota on the effectiveness of PD-1 immunotherapy and found the previous exposure to antibiotics had a weaker response to PD-1 inhibition immunotherapy as compared to those that had not been exposed to antibiotics (Routy et al., 2018; Sambi et al., 2019). Furthermore, it has been observed that patients with a diverse microbiota composition responded well to immunotherapy and exhibited reduced tumor size than the patients with less diverse composition (Sambi et al., 2019).

### 11.7.8 Immunotherapy drug are expensive

The economic sustainability of IPG has become a global concern. Although the development of immunotherapy drugs and molecular agents have improved the survival rate and life quality. The cost of targeted therapy varies among different types of diseases. These molecules are very expensive and the effect of their costs are needed to be considered carefully.

## 11.8 Future direction

Although, the IPG approach has played an essential role in understanding the different aspects of various diseases, especially the immune response in patients. IPG investigates the comprehensive effect of immunotherapy and the detailed immune response in several diseases. IPG field is expected to progress rapidly in the coming years, in order to enhance the efficiency, and reduce the toxicity of immunotherapies. Although in recent years, IPG has advanced rapidly, there is still a lot to be done needed to done for prediction, prevention, and understanding of the immune response.

### 11.8.1 Identification of additional biomarkers

Identification of these biomarkers would help to develop effective immunotherapy to several diseases, including cancer, autoimmunity, adverse drug reactions that target a particular sequence without damaging the healthy cells. For example, in cancer patients, there are several biomolecules called TSA or neoantigens that are expressed only on the surface of tumor cells. However, several TSA is also found on healthy cells that could impact the efficacy of immunotherapy during treatment noncancerous cells get damaged.

### 11.8.2 Overcoming resistance to immunotherapy

Although immunotherapy has revolutionized the outlook of therapy and significantly increases the overall survival rate of patients. However, after a

definite period of treatment, the majority of the patients do not respond to immunotherapy and develop resistance. In cancer, drug resistance is one of the biggest challenges of immunotherapy that relies on various factors like genes, metabolism, inflammation, etc. Although the mechanism of resistance is complex but comprehensive analysis revealed the alteration of various signaling pathways might have a significant role in immune resistance. For example, in tumor cells, any alteration in signaling pathways in tumor cells against the antitumor immune response has the ability to induce immunotherapy resistance (Bai, Chen, & Li, 2020). In order to overcome the immunotherapy resistance, various methods could be developed that can alter or inhibit the activity of those biomolecules that promotes the immune resistance during the treatment.

### 11.8.3 Administration of immunotherapy

Immunotherapy is generally given in advanced stages of diseases as second-line treatment along with chemotherapy that may have a significant impact on the efficacy of immunotherapy especially in patients with a weak immune system. Therefore it is recommended to give immunotherapy treatment during the initial stages of diseases to boost the host immune system.

### 11.8.4 Personalized approach to overcome molecular and physical barriers

The approved immunotherapy drugs are generally designed for a wide range of cancer that identifies the specific molecular profile. However, IPG focuses on personalizing the immunotherapy by characterizing the patient-specific immune responses and identify the additional targetable biomarkers to increase the efficacy of immunotherapy.

### 11.8.5 Accurate prediction of immunotherapy prediction

The main focus of IPG is to assess the accurate outcome of targeted therapy to improve the efficacy and specificity of the immunotherapy. Recently, several predictive methods have been used to access the appropriateness of specific immunotherapy and improve the efficiency of the treatment and response rate as a whole.

### 11.8.6 Gut microbiome

Recent research has reported that gut microbiota has a key role in regulating the effects of immunotherapies. Both probiotic and prebiotic enhances the immune response of the host. To increase the immune response against immunotherapy, probiotic and prebiotic supplements are given to the

patients, in order to target and regulate the composition of gut microbiota (Sambi et al., 2019).

### 11.8.7 Application of nanotechnology

Nanotechnology mainly focuses to improve the diagnosis and efficiency of therapeutic approaches. The therapeutic drugs are mainly encapsulating into nanomaterial-based carrier systems to overcome physical and biological barriers, such as hydrophobicity, short half-lives. In cancer immunotherapy, nanoparticles are delivered with drugs, immunomodulatory substances, and oligonucleotides to regulate the T cell proliferation (Sambi et al., 2019).

## 11.9 Conclusion

IPG is known to contribute a better understanding of the pathogenesis of various disease conditions. IPG plays a significant role in increasing the efficiency of immunotherapy but also helps to develop highly effective molecular targeting drugs for the treatment of several diseases like cancer, autoimmunity, food allergy, and adverse drug reaction. Despite progress from the last two decades, IPG had certain limitations that affect the therapeutic efficacy of IPG. To improve the efficacy of IPG, a multimodal approach is needed that reveals the complexity of the immune system in order to characterize the contributing factors in various disease conditions, and further research is needed for a better understanding of the molecular basis of disease for its successful treatment.

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Chapter 12

# Immunopharmacology of Alzheimer's disease

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## 12.1 Introduction

Aging is the innate and natural progression that begins with the birth and stops with the ultimate death of an individual. It is sometimes counted from the date of conception until birth when related to embryological studies, but actually it is counted from birth till the death of the individual. Aging on one side makes addition to the experiences, but from another perspective it declines the normal body functioning. One of the major health related issues as an outcome of aging is a neurodegenerative disorder referred to as dementia which increases with age (Fig. 12.1). Dementia is a major and destructive neurological turmoil. Elderly people are mainly affected by dementia, although there has been a surge in the incidence of dementia cases that start before the age of 65. After the age of 65, its possibilities of occurrence nearly doubles after every 5 years. The distinct symptoms and distinctive microscopic brain anomalies are associated with each distinct type of dementia *viz* Alzheimer's disease (AD), Creutzfeld-Jacob disease, post-stroke dementia, normal pressure hydrocephalus, dementia with Lewy bodies, Parkinson's disease, etc. In medical literature Alzheimer's disease is also referred as SDAT (Senile Dementia of the Alzheimer Type). The disease was named after Alios Alzheimer, a German psychiatrist and neuropathologist, who was the first to describe it in 1906. AD is the commonest form of dementia, commonly diagnosed in the age group $>60-65$ years, but early

**Immunogenetics: A Molecular and Clinical Overview. DOI:** https://doi.org/10.1016/B978-0-323-90053-9.00014-2
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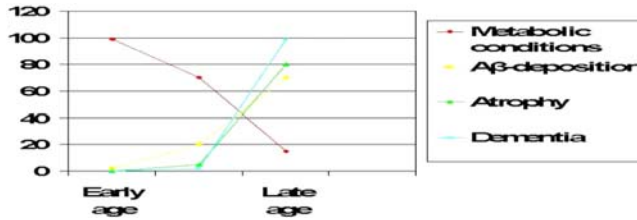


**FIGURE 12.1** Advancement of age precipitating Alzheimer causing agents.

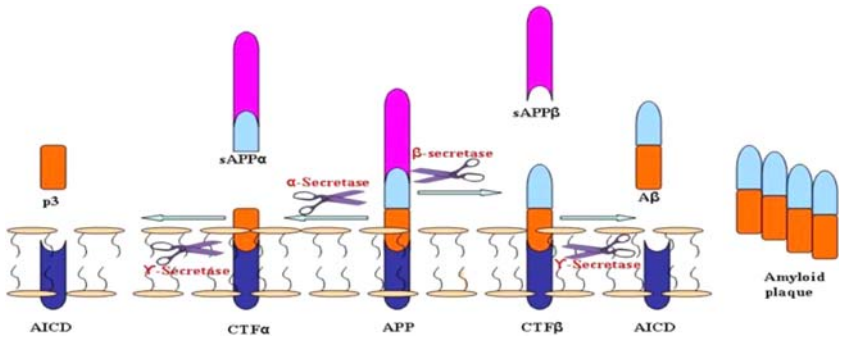


**FIGURE 12.2** Processing of amyloid precursor protein (APP). Left half of APP represents non-amyloid processing of APP, whereas, right half represents amyloidogenesis.

onset AD also occurs in population at low frequency. The AD pathogenesis is an outcome of multifactorial causes (both genetic and environmental). Its sporadic form constitutes more than 90% among the reported prevalent cases (Yu et al., 2010). Genetic heritability of AD (and memory components thereof) on a review basis of twin and family studies, range from 49% to 79% (Gatz, Reynolds, & Fratiglioni, 2006; Wilson et al., 2011). Both the sex-linked and sex-limited factors are associated with AD, about 0.1% of AD cases are familial forms (i.e., genetic) of nonsex linked or autosomal dominant inheritance showing disease appearance before the age of 65 years (Blennow, de Leon, & Zetterberg, 2006). This form of the AD is known as early onset familial Alzheimer disease. The autosomal dominant and familial majority of autosomal dominant familial SDAT or AD is linked with the mutations in either of the three genes *viz*: presenilins 1 and 2, APP (amyloid precursor protein) gene, presenilins1 and 2 (Waring & Rosenberg, 2008). The AD is noncurable disease described by gradual memory loss, poorer cognition, calculations and worsened comprehension and orientation (time and place). The decline in cognitive power is occasionally succeeded by deteriorated emotional, motivational and social behavior (World Health Organization, 1992; World Health Organization, 2008). A well known hypothesis called Amyloid hypothesis has been given according to which the extracellular amyloid beta ($A_\beta$) deposits are the fundamental cause of the disease (Fig. 12.2) (Hardy, 1991; Mudher & Lovestone, 2002). The various

evidences in favor of this hypothesis is derived from analyzing the exact location of gene on 21 chromosome for the amyloid precursor protein (APP), along with the evidences and facts regarding the close associations of AD symptoms with Down's syndrome (trisomic for chromosome 21) (Lott & Head, 2005; Nistor, Don, & Parekh, 2007).

The subtypes are indistinct and mixed forms coexist (World Alzheimer Report, 2009). Medical history, history from close ones, and behavioral observations are usually used to diagnose Alzheimer's disease (Endez, 2006; Klafki, Staufenbiel, Kornhuber, & Wiltfang, 2006), single positron emission tomography (SPECT) can be helpful in excluding other cerebral pathology (ies) or subtype(s) of dementia (Dementia: Quick Reference Guide, 2006). The progression and effects of AD is dependent on the patient's personality and health condition. The Dementia can be categorized into three stages *viz*:

1. early stage dementia—initial one or two years from disease diagnosis;
2. middle stage dementia—the progression of disease from second to third or fifth year; and
3. late stage dementia—the progression of disease from fifth year onwards.

The periods of disease progression are generalized on approximate basis (Jotheeswaran, Williams, & Prince, 2010). The dementia is further categorized on the basis of its severity as: mild, moderate or severe. The different stages are associated with distinguished characteristics *viz:*

1. Early stage: It is characterized by:
   a. The events of forgetfulness
   b. Some confusion and misunderstanding in unfamiliar situations
2. Middle stage: It is characterized by:
   a. Freshly learnt knowledge/information memorized with difficulty
   b. Confusion is deepened
   c. Disturbances of Sleep/sleep disorders
   d. Not properly oriented
3. Late stage: It is characterized by:
   a. Least ability to think properly
   b. Not in a condition to speak or conversate
   c. Same conversion is repeated by patients
   d. Patients are more violent in this stage

These various distinctive stages of AD are relying on progressive pattern of functional and cognitive impairment. It would be very helpful, if one would be able to differentiate between signs of Alzheimer's dementia and typical-age related changes (Table 12.1).

## 12.2 Innate immunity and Alzheimer's disease

It has been noted that after Aβ deposition immune activation occurs, but as per clinical research, inflammatory changes occur much earlier (Brosseron,

**TABLE 12.1 Signs of Alzheimer's dementia and typical-age related changes.**

| Signs of Alzheimer's disease | Typical-age related changes |
|---|---|
| *Loss of memory*, especially forgets recently learned information.*Problem in executing plan(s) and solving problem(s)*, which they used to execute and solve easily in earlier times.*Difficulty in completing day to day work* at home, at office etc. even have difficulty in driving to a known location.*Not oriented*, with time and/or place.*Trouble in understanding* visual images and spatial relationships.*Problem with writing and/or speaking*, stop in the middle of the conversation, struggle with vocabulary, problem in finding the exact word or call things by wrong name (e.g., calling a watch a "hand clock").*Placing things* at unusual places and are unable to track back their steps to find them back. Over the passage of time, it happens frequently.*Have reduced or poor* judgment in dealing with money. They also pay less attention in keeping themselves clean or grooming.*Social withdrawal* and/or withdrawal from work.*Personality and mood changes*. Become confused, suspicious, depressed, fearful and/or anxious. | *Occasionally* forgetting names or appointments, but able to recall them later.*Occasional errors*, when balancing a checkbook.*Occasionally* need help. *May get* confused about the day of week, but finally figure it out.*Vision changes* related to cataracts, glaucoma & age related molecular degeneration. *Sometimes* do have problem in finding the exact word.*Misplace* things, but are able to track them back.*Sometimes* makes poor decision.*Sometimes* feel burdened & thus try to avoid work and society.*Become* irritable when their specific routine of doing work is disrupted. |

Krauthausen, Kummer, & Heneka, 2014; Tarkowski, Andreasen, Tarkowski, & Blennow, 2003). An Alzheimer disease phenotype as well as Aβ-plaques, neurofibrillary tangles (NFT) and gliosis resulted, in mice with dsRNA analog, because of systemic immune challenge in mice, indicating that immune challenge(s) can initiate Alzheimer's disease (Krstic et al., 2012).

The immune system's direct association in AD pathology has been suggested by a number of studies that have shown the participation of modulating immune system specific proteins on AD pathology. On identification of pathogenic invasion, innate immune system gets activated, because of pattern recognition receptor (PRR) expressing cells (Akira, Uematsu, & Takeuchi, 2006; Suresh & Mosser, 2013). In case of Alzheimer disease neuroinflammation is initiated by neurofibrillary tangles (NFTs) and Aβ-plaques. The AD progression occurs with the activation of PRRs, Aβ-plaques and NFTs that provides differential and distinct signaling patterns to the concerned cells.

Cellular activation by DAMP's (Danger associated molecular patterns) is mediated by various PRRs *viz* CD36, Leucine rich repeat and Pyrin domain containing (NLRP)s, TLRs, RAGE and Nucleotide binding oligomerization domain (Cribbs et al., 2012). This whole incident eventually directs to NLRP3 inflammasome assembly, and NFkB reliant proinflammatory gene transcription that proceeds to generation of pro-inflammatory cytokines (Latz, Xiao, & Stutz, 2013; Liu, Zhang, Joo, & Sun, 2017). The cytokines are manufactured and secreted by nearly all cells, they act locally by both the endocrine and paracrine (Zhang & An, 2007). Chemokines, interferons (IFNs), Interleukins (ILs), transforming growth factors (TGFs) and tumor necrosis factors (TNFs), are different classes of cytokines (Feghali & Wright, 1997; Zhang & An, 2007). All processes-like release of neurotransmitter, apoptosis, cellular migration, cell proliferation, and gliogenesis involve cytokine signaling (Borsini, Zunszain, Thuret, & Pariante, 2015; Boulanger, 2009). Adaptive immune cells and innate nonspecific immune cells are attracted to the position of injury, by chemokines. The Cytokines released are either proinflammatory or antiinflammatory, and equilibrium between them help in elimination of a pathogen and protection of nearby tissue from extreme harm (Szelényi, 2001), whereas, imbalance could add to AD advancement. Cytokines work by means of their attachment to receptors, the expression of whom is strongly regulated spatially as well as temporally, for controlling inflammatory reaction (Szelényi, 2001). The AD risk is associated with 23 polymorphic forms occurring in 13 different cytokines (Zheng, Zhou, & Wang, 2016). Studies have demonstrated that in Alzheimer's disease the normal expression of several interleukins like IFN-$\alpha$, IL-1$\beta$, IFN- $\gamma$, IL-18. IL-10 is hampered (Griffin et al., 1989; Hull, Berger, Volk, & Bauer, 1996; van der Wal, Gómez-Pinilla, & Cotman, 1993). However, certain intelukins like IL-10, TGF- $\beta$, and IL-1ra no doubt occur at higher concentrations in AD patients but genetically aren't associated with disease (Zheng et al., 2016). polymorphic forms of certain ILs *viz* IL-23, IL-4, and IL-12 no doubt have connection with AD, but whether they alter expression pattern or not that is not known.

## 12.3 Alzheimer's disease and interferons

IFN are a class of cytokines having wide spectrum of immunomodulatory roles (de Weerd et al., 2013; Lindenmann, 1982). IFN are categorized into three subtypes on the basis of their cognate receptor, these are type-I IFN, type-II IFN, type-III interferon having pro-inflammatory properties in common and trigger several signaling pathways (de Weerd et al., 2013). The common signaling pathways triggered by these interfrrons is JAK-STAT [Janus-associated kinase (JAK) and signal transducer and activator of transcription (STAT) pathway] (Platanias, 2005). Once phosphorylated, STATs migrate into the cell nucleus and stimulate transcription of IRGs (interferon-

regulated genes) (Platanias, 2005). Interferon response is further modified, if IRFs (IFN-regulated factors) unite to STATs earlier to nuclear translocation (Honda et al., 2005). There are more than 2000 genes which are together known as ISGs (interferon-stimulated genes), may be regulated by type-I IFN (Schreiber & Piehler, 2015). To stop autoimmune attack on myelin in multiple sclerosis IFNα and IFNβ are used as therapeutic agents (Wingerchuk & Carter, 2014). It has been found in murine autoimmune encephalitis, that IFNβ is mainly responsible for microglial phagocytosis of myelin (Kocur et al., 2015).

However, it has been proven by an epidemiological study that multiple sclerosis patients treated with IFNβ developed PD (Parkinson's disease)-like features, like errors in cognition and damaged motor function, with extensive IFN type-I treatment (Manouchehrinia & Constantinescu, 2012). Interferon signaling and its regulation may be advantageous, especially within the CNS and in the periphery. The interferon abnormal functioning may be unfavorable and add to the development of many neurodegenerative diseases. For emphasis of this contradiction, IFNβ-/- develop Lewy body and Parkinson's disease like symptoms (Ejlerskov et al., 2015), but in MPTP model of Parkinson's disease pharmacological and genetic inhibition of IFN (type-I interferons) are neuroprotective (Main et al., 2017). The implication of cytokines and chemokines in Alzheimer disease pathology progression serves to show up immune activation complexity in disease. As is evident from different studies that marking any particular cytokine, without proper knowing and understanding its downstream influence on the immune system will not be successful treatment against AD. Depending on the cell category involved and cellular context, cytokines may cause different effects, so the association among different types of cells in AD should be explored.

## 12.4 Alzheimer's disease and glial cells

Microglia, oligodendrocytes, astrocytes, ependymal cells, and satellite cells are different types of glial cells. In CNS the most profuse mononuclear phagocytes are microglial cells and are mainly studied for their function in immune supervision and surveillance, neuronal development, synaptic plasticity and phagocytosis and synaptic plasticity. whereas, maintenance, security and supply of various nutrients to diverse neurons and to the vascular system is provided by astrocytes (Allen & Eroglu, 2017; Li & Barres, 2018). Myelin coating of axons of nerve cells which aid in propagation of action potential is formed by oligodendrocytes, while as Schwann cells act analogously in the PNS (Lassmann, 1998). Central canal of spinal cord and ventricles of central nervous system are lined by ependymal cells and also regulate CSF (Johanson et al., 2008). In the tripartite synapse neurons, microglia and astrocytes play a critical role and therefore all of them are important synaptic transmission, recalling, and learning (Araque, Parpura, Sanzgiri, & Haydon,

1999). Glia play an important role in cytokine secretion and phagocytosis of cellular debris (Leyns & Holtzman, 2017).

## 12.5 Alzheimer's dementia and microglia

Microglia have receptors for neurotransmitter, hormone, cytokines, chemokines, and PRRs and therefore play a significant part in response to array of inflammatory mediators associated in Alzheimer's dementia, incorporating Aβ (Dewapriya, Li, Himaya, Pangestuti, & Kim, 2013; Liu et al., 2005; Rangaraju et al., 2018a; 2018b; Smith, Das, Ray, & Banik, 2012). The activation of microglial cells is linked with AD advancement, but it is yet to be established whether this is harmful, protective or both. Alzheimer's disease pathology may be reduced by decreasing Aβ buildup. Aβ is removed from CNS circulation directly by activated microglial cells (Michaud et al., 2013). Dysfunctional synapses are recognized and phagocytosed by microglia and by releasing BDNF (brain derived neurotrophic factor) stimulate synapse formation (Parkhurst et al., 2013; Tremblay, Lowery, & Majewska, 2010; Wake, Moorhouse, Jinno, Kohsaka, & Nabekura, 2009). Neurotoxic effects of microglial cytokines add to cognitive decline (Calsolaro & Edison, 2016; Heneka et al., 2015; Heppner, Ransohoff, & Becher, 2015; Kettenmann, Hanisch, Noda, & Verkhratsky, 2011). Neuroprotection and homeostatic maintenance is a well established character of microglial cells, but some recent studies have shown their (microglial) contribution to dysregulated phagocytosis. It is thought that impaired microglial Aβ clearance play a major part in the SAD advancement than anomalous Aβ production (Mawuenyega et al., 2010). It has been well demonstrated that Aβ clearance is diminished in Alzheimer patients in comparison to controls of same age group, therefore establishing the valid evidence that impaired clearance properties of microglial cells or astrocytes may act as AD causative agents (Mawuenyega et al., 2010). Disease and environmental factors can have impact on phagocytic activity, leading to range of phagocytic reactions/responses. Phagocytosis can be sequentially explained in three steps: 1. find-me, 2. eat-me, and 3. digest-me (Sierra, Abiega, Shahraz, & Neumann, 2013; Wolf, Boddeke, & Kettenmann, 2017). The step of finding is commenced when receptors (target recognizing receptors) of microglia are bound by specific molecules. Different signaling pathways are triggered by different pathways, stimulating phagosome development/formation (Arcuri, Mecca, Bianchi, Giambanco, & Donato, 2017). In case of Alzheimer's dementia NFTs and Aβ-plaques are identified by TLRs which leads to pro-inflammatory cytokine storm that is correlated with discharge of TNF, NO, and IL-1, whereas, detection of cellular debris or dystrophic neuritis by receptors of microglial cells (TREM2 receptors) is correlated with phagocytic reaction besides increment in TGFB and UL10 signaling (Neumann & Takahashi, 2007; Neumann, Kotter, & Franklin, 2009; Takahashi, Prinz, Stagi, Chechneva, & Neumann, 2007).

In next step of phagocytosis that is eating, target molecules are enclosed in extended plasma membrane forming vesicular phagosome, which finally fuses with lysosome(s) leading to formation of phagolysosme.

The final step of phagocytosis is digestion, where target molecule is digested within phagolysosme. After this, byproducts should either be piled up and stored or recycled by phagocytic cell(s) (Flannagan, Jaumouille, & Grinstein, 2012).

Within brain, an inflammatory response is triggered by Aβ, via stimulation of microglia to remove Aβ from the brain and thus prevent the development of plaques (Colton et al., 2006; Jekabsone, Mander, Tickler, Sharpe, & Brown, 2006; Morgan, 2006; Zhao, Hu, Tsai, Li, & Gan, 2017). Aβ after being swallowed are directed for degradation by lysosomes. Alternatively, Aβ can also be cleared by reactive astrocytes and proteolytic degradation. Metalloprotease neprilysin can also cleave Aβ (Takaki et al., 2000), therefore Aβ load has been found to increase in mice treated with neprilysin inhibitors (Marr et al., 2003). During neuronal activity microglial cells come in contact direct with the synapses and dendritic spines. Vital role of microglia in synaptic pruning is well established, but evidences suggest that in case of developed brain they enhance synaptic plasticity by following a similar method (Hong et al., 2016; Sominsky, De Luca, & Spencer, 2018). The phagocytic feature of microglial cells appear frequently in the hippocampus (Paolicelli et al., 2011). Complement protein and receptor expression are playing a significant role in identifying the appropriate synapses for the phagocytosis (Schafer et al., 2012). During synaptic stripping (a neuroprotective process) microglia remove damaged synapses (Trapp et al., 2007; Yamada et al., 2008). Once Aβ is phagocytosed by microglia NLRP3 inflammasome gets activated leading to activation of caspase-1 and IL-1β maturation and discharge, which could be dangerous to immediate tissue (Halle et al., 2008). The experimental findings in APPPS1 mice showed the decline in Aβ deposition, diminished IL-1β discharge and improvement in cognition, in genetically deleted NLRP3 and caspase-1 (Heneka et al., 2013). Toxic proinflammatory cytokines can be secreted by activated microglia (Colonna & Butovsky, 2017; Kinney et al., 2018) and directs the release of chemical mediators that provoke astrocytes to produce a neurotoxic agents (Liddelow et al., 2017) eventually adding to neurodegeneration. Contrary to this some of the microglial cytokines have physiological role in neurogenesis and synaptic flexibility (Albensi & Mattson, 2000; Bernardino et al., 2008; Morris, Clark, Zinn, & Vissel, 2013). For example, TNF, IL-1β, complement cascade proteins and IL-6 are all concerned with memory consolidation (Boulanger, 2009; Goshen et al., 2007; Yirmiya & Goshen, 2011). Microglial activity and appropriate cytokine secretion in conclusion are important for the average cognitive processes, but, in other perspective are neurotoxic.

## 12.6 Astrocytes and Alzheimer's disease

Astrocytes are another type of glial cells within CNS. Further more astrocytes protect CNS against damage and are important for tissue repair (nerve tissue repair). The process of neuroprotection is known as astrogliosis, this process supplements the separation of injured tissue *via* the glial scars formation and consolidation. Brain inflammatory response is mainly regulated by astrocytes and are competent enough of secreting and counteracting a wide array of immune mediators (Farina, Aloisi, & Meinl, 2007). In AD samples of human brain and transgenic AD mouse models numerous cytokines are found to be over expressed (Abbas et al., 2002; Apelt & Schliebs, 2001; Benzing et al., 1999; Tan et al., 2002), especially TNFα, TGFβ, IL-1β, IFNγ, and IL-6 (Constam et al., 1992; Hu, Akama, Krafft, Chromy, & Van Eldik, 1998; Johnstone, Gearing, & Miller, 1999; McGeer & McGeer, 1995) and have capability of Aβ secretion (Frost & Li, 2017). Like that of microglia, astrocytes go through considerable alteration in reply to particular stimulus and may be activated or resting in accordance to the cellular environment (Mrak, 2012). Throughout AD the astrocytes number is considered to stay fixed, but some cells occur in close proximity to Aβ-plaques, turn out to be reactive, conversely degenerating large number of astrocytes, referred as astrodegeration (Rodríguez et al., 2014). Differential gene expression of structural proteins, synaptic modulators and vascular, transcriptional and inflammatory responses are result of astrogliosis. The characteristic feature of "astrogliosis" is that the astrocytes display hypertrophy of methods, represented by amplified expression of intermediary filament especially GFAP (glial fibrillary acidic protein) (Kamphuis et al., 2012; Wilhelmsson et al., 2006). Astrocytes can turn on number of synapses on one occasion, therefore facilitating synchronization of neuronal network (Ben Haim & Rowitch, 2017). Connection of astrocytes and cognition is undeniable, but more studies are needed to be done in order to understand the underlying mechanism and how they can add to the disease.

## 12.7 Alzheimer's disease and oligodendrocytes

Most frequently, Alzheimer's investigations have been confined to gray matter, but the anomaly of white matter has been recognized in Alzheimer's disease. In FAD patients hyper-intensity amount of white matter is found to be elevated about two decades before the appearance of sign/symptoms, thus can be used to expect AD prevalence and reduction in cognition rate in SAD cases as well (Lee et al., 2018; Nasrabady, Rizvi, Goldman, & Brickman, 2018). It (white matter) is vital for learning and recalling and are the areas with high level of neuroinflammation and microgliosis (Raj et al., 2017). Myelin in white matter is produced by oligodendrocytes. In addition, to this, neurotrophic factors which influence neural connectivity is produced by

oligodendrocytes (Schwab, 1990), and may also react to immune response within brain.

## 12.8 Alzheimer's dementia and glia barriers

Injury or disease that leads to damage of CNS, leads to immune response. In response to this injury, glial barriers also called as glial scars, are formed. This is created by both glial and nonglial cells surrounding the damaged position (Adams & Gallo, 2018). It is the extensively studied phenomenon in the situation of traumatic brain injury. In ischemic stroke and neurodegenerative disease such as multiple sclerosis, glial scars were also found (Woodcock & Morganti-Kossmann, 2013).

Heterogeneity of glial scar development and disease category have been reported by many studies, advancement of glial barrier generally commences with the reactive astrocyte proliferation next to injured tissue, and the astrocytes, microglia, and oligodendrocytes gets orchestrated in order to build a rigid wall around the damaged tissues (Sofroniew, 2009; Wanner et al., 2013). Microglial and astrocyte presence is the basic defining feature of senile plaques (Itagaki, McGeer, Akiyama, Zhu, & Selkoe, 1989). Reactive astrocyte presence tracks amyloid deposition in the track of AD (Vehmas, Kawas, Stewart, & Troncoso, 2003). Glial barrier neighboring plaques are protective (neuroprotective) in two different ways: (1) It (Glia), especially microglia can rupture down and engulf amyloid plaques, (2) Spread of toxic material is prevented by astrocytes and therefore decreases collateral breakdown (Anderson et al., 2016; Faulkner et al., 2004). The decreased ability of formation of glial barriers have been found to worsen Alzheimer disease pathology. Mouse model of AD with GFAP knockout showed as marked increment in plaque-related dystrophic neuritis (Kraft et al., 2013). Glial barriers may assist in axonal re-growth and restoration in those areas of brain where barrier firmness halts neuronal growth are more extensively affected (Bollmann et al., 2015; Yu & Bellamkonda, 2001). Neurons are postmitotic, thus nondividing and making glial scarring recovery more difficult (Herrup & Yang, 2007).

Immune therapy(ies) for amending the glial scar development in Alzheimer's disease might enhance advantageous re-growth of neuronal connections in the central nervous system (CNS)

## 12.9 Alzheimer's disease and current immune-related therapies

Presently Alzheimer's disease treatments are confined to inhibitors of memantine and cholinesterase altering diffusion of glutamate & acetylcholine respectively (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016; Thomas & Grossberg, 2009). These drugs only slow down symptoms in case of AD

with severity ranging from mild to moderate, and no therapeutic treatments are available that significantly slow down progression of Alzheimer's disease (Burns et al., 1999; Reisberg et al., 2006). It have been reported that people taking NSAIDs for different unceasing inflammatory conditions have lesser incidence of AD (de Craen, Gussekloo, Vrijsen, & Westendorp, 2005). These findings have lead to chain of experimental trials that test the effect of celecoxib or naproxen on Alzheimer disease development. Naproxen acts by reversibly inhibiting enzymes *viz* COX-1 and COX-2, which causes decline in prostaglandin production. These (prostaglandins) are proinflammatory, and thus naproxen functions as a nonselective inhibitor of provocative retort (Robinson, 1983). Naproxen was at first thought to soothe the pathology of Alzheimer's disease by attacking the immune system, but outcome of INTREPAD work shows its nullifying clinical benefits, as seen in elderly patients, who took naproxen for 2 years didn't confirm reduction in cognitive destruction or decline in markers of AD in CSF (Pellicano, 2014).

One of the most effective approach to tackle AD is immunotherapy, because of its specificity in clearing toxic proteins of NFT and Aβ (Valera & Masliah, 2013). A therapeutic plan that was developed against Aβ is vaccine development (Folch et al., 2016). Active immunotherapy lasts for longer time, but has high risk of developing autoimmune reaction and can be restricted by epitope specificity & availability (Bard et al., 2003; Winblad, Graf, Riviere, Andreasen, & Ryan, 2014). As far passive immunotherapy is concerned, it may have high specificity but shorter effect, so, requires uninterrupted antibody treatment (Ward, Himmelstein, Lancia, & Binder, 2012).

During one of the initial trials of Aβ immunotherapy utilizing AN1792 (a synthetic Aβ peptide) that induced antibodies against Aβ by active immunotherapy, 6% of the recruited patients developed meningoencephalitis in 2nd stage of the clinical trials (Cao, Hou, Ping, & Cai, 2018; Gilman et al., 2005). A follow-up study that was done after 4 years of immunization established the facts of improvement in cognition in 68% of patients. In follow-up studies, done 3.6−4.6 years, significant improvement in brain capacity and cognitive decrease was observed. Although, among responders (Ab-responders) only 68% which constitute <20% of total immunized patients showed improvements (Vellas et al., 2009). Use of CAD106 as an active immunotherapy has been found to be safe, standing well for clinical trials is still in developmental phase (Farlow et al., 2015).

Bapineuzumab and Solanezumab are two passive immunotherapeutic mediators that were on clinical trials, later on bapineuzumab (targets N-terminus of Aβ) failed because of reduced safety contour in AD patients with disease severity ranging from mild to moderate (although some of the AD patients were APOE carriers) (Doody et al., 2014, Liu et al., 2015; Vandenberghe et al., 2016). Solanezumab which often attacks soluble mnomeric Aβ, is basically a humanized IgG1 monoclonal antibody with a positive safety profile which passed stage 1st and stage 2nd trials, but didn't

exhibit efficacy in stage 3 and was stopped until it again enters clinical trials for FAD patients (Doody et al., 2014; Siemers et al., 2016). Another monoclonal antibody, "Aducanumab" showed very promising results in phase 1st, decreasing soluble fibrillar Aβ forms and slowing down cognitive deterioration after treating patients for 54 weeks in a dosage dependent manner (Sevigny et al., 2016). But, in March 2019 phase 3 trials of Aducanumab were stopped, following an announcement by autonomous data examining committee, that trails would not meet the satisfactory primary end results. Despite this fact, Biogen Inc., declared continuation of clinical trials for subsequent analysis of phase 3rd data (Biogen, 2019).

## 12.10 Conclusion

It has been found that molecular, cellular, and genetic changes that are tightly linked with AD provide a firm support regarding contribution of innate immunity in Alzheimer disease pathology (Kim et al., 2018). We can suggest that there is fragile equilibrium involved in the nonspecific innate immune response to Alzheimer's disease, it may be beneficial or harmful. Inflammation in some aspects is beneficial and prevents Alzheimer disease pathology. The benefits provided by immune response to AD, if harnessed, may prove fruitful for AD treatment. Substantial evidences suggest that acquired immunity also play a role in pathology (Sommer, Winner, & Prots, 2017). Microgliosis involves phagocytosis of dysfunctional synapses and amyloid plaques and enhanced complement protein expression (for neuronal pruning) and trophic factor release for cell growth and plasticity. In contrast to this, it (microgliosis) increases chemokine and cytokine quantities, which may become toxic to neuronal cells. Transmission of calcium ion currents for increasing signal transduction and enhance restoration and protection is the beneficial effect of astrogliosis, but excessive astrogliosis may raise toxic entities. Even though we have seen a number of agents responsible for pathogenesis of Alzheimer's disease, but more specific perceptive is the call of the hour for developing effective therapeutics against Alzheimer's disease.

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Chapter 13

# miRNAs: the genetic regulators of immunity

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## 13.1 Introduction

The development and function of immune cells are vital for host defense against external and internal threats and for immune resolution following the elimination of threats, which requires rapid changes in the transcriptome and proteome. These changes are tightly regulated at both the transcriptional and posttranscriptional levels, where microRNAs (miRNAs) have been demonstrated to be crucial molecular players. miRNAs are small noncoding RNAs (about 18−22 nucleotides in length) produced by a multistep process involving a series of enzymes and proteins (Pillai, Bhattacharyya, & Filipowicz, 2007). The genes encoding miRNAs are transcribed by either RNA polymerase II or III as primary miRNAs (pri-miRNAs) containing a cap structure at the 50 end and polyadenylation at the 30 end (Lee et al., 2004). The nuclear microprocessor complex, composed of the ribonuclease (RNase) III enzyme Drosha and the RNA-binding protein DGCR8, processes pri-miRNAs into the precursor miRNAs (pre-miRNAs) which are exported into the cytoplasm facilitated by Exportin 5 and the GTP-binding nuclear binding protein RAN-GTP (Bohnsack, Czaplinski, & Gorlich, 2004; Denli, Tops, Plasterk, Ketting, & Hannon, 2004; Gregory et al., 2004; Lee et al., 2003; Okada et al., 2009). RNase III enzyme Dicer then cleaves pre-miRNAs into mature miRNA duplexes through binding to the two-nucleotide overhang at their 30 end generated by Drosha (Gottesman, 2004; Macrae et al., 2006). One strand of the miRNA duplex is usually incorporated into the miRNA-induced silencing complexes (mRISCs) through the Argonaut (Ago) proteins, which guide the binding of miRNAs to complementary sites mainly located in the 30

Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00003-8
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untranslated regions (30 UTRs) of the target mRNAs (Ha & Kim, 2014; Zamore, Tuschl, Sharp, & Bartel, 2000). miRNAs are estimated to control 30%−80% of mammalian genes by repressing translation and reducing the stability of target mRNAs (Brennecke, Stark, Russell, & Cohen, 2005; Friedman, Farh, Burge, & Bartel, 2009; Lewis, Shih, Jones-Rhoades, Bartel, & Burge, 2003). The immunoregulatory role of miRNAs was first implicated by conditional ablation of key components of the miRNA biogenesis pathway, such as Drosha, Dgcr8, and Dicer, in various immune cells at distinct stages of immune responses (Chong, Rasmussen, Rudensky, & Littman, 2008; Liston, Lu, O'Carroll, Tarakhovsky, & Rudensky, 2008; Zhou et al., 2008; Baer et al., 2016; Cobb et al., 2006; Jeker, Zhou, Blelloch, & Bluestone, 2013; Wei et al., 2018). As a matter of course, a large number of studies subsequently investigated the immune function and downstream targets of individual miRNAs, normally using gain- and loss-of-function approaches, which have been thoroughly reviewed elsewhere (Josefowicz, Lu, & Rudensky, 2012; Wei & Schober, 2016). However, considering that substantial mammalian mRNAs are miRNA targets (Friedman et al., 2009) and that the miRNA machinery is extremely complicated, it is critical to understand how miRNA biogenesis and function are tightly regulated in the immune system, despite the currently limited number of studies. Modulation of miRNA-mediated gene regulation may take place in multiple steps, including miRNA transcription, miRNA biogenesis and turnover, and target selection (Ha & Kim, 2014; Kim, Han, & Siomi, 2009; Krol, Loedige, & Filipowicz, 2010).

## 13.2 MicroRNA biogenesis

Most mammalian microRNA genes are located throughout the entire genome and account for approximately 1% of the genome (Bartel, 2004). MicroRNA genes are housed within the protein-coding or noncoding genes and are often located in clusters that may undergo a polycistronic transcription (Lee, Jeon, Lee, Kim, & Kim, 2002; Rodriguez, Griffiths-Jones, Ashurst, & Bradley, 2004). MicroRNA genes are transcribed by RNA polymerase II into primary transcripts, termed pri-miRNA, and have a 5′ 7-methyl guanosine cap and a 3′ poly-adenylated hairpin structure (Denli et al., 2004). In the nucleus, the pri-microRNA is cleaved into a 60−70 nucleotide precursor miRNA (pre-miRNA) by a microprocessor which includes the RNase III enzyme Drosha and its cofactor DGCR8 (Gregory et al., 2004). Subsequently, pre-miRNAs are exported from the nucleus into the cytoplasm by a dsRNA-binding protein Exportin 5 in a Ran GTPindependent manner (Lund, Güttinger, Calado, Dahlberg, & Kutay, 2004). In the cytoplasm, another RNase III enzyme, Dicer, acts on the pre-miRNA to generate mature 22 nucleotide long double-stranded microRNA duplexes (Zhang, Kolb, Jaskiewicz, Westhof, & Filipowicz, 2004). Then, the functional strand of the duplex is assembled

into the RNA-induced silencing complex (RISC), which includes Dicer, TRBP, and the Argonaute proteins, while the other strand is released and degraded (Kawamata & Tomari, 2010). The Argonaute proteins provide a suitable circumstance for the microRNA to interact with its target mRNAs. The RISC specifically recognizes mRNAs by base pairing between the position 2−8 nucleotides of microRNAs, known as the seed region, and the complementary 3′-UTR of its target mRNAs (Ameres, Martinez, & Schroeder, 2007; Bartel, 2009). Partial binding between the microRNA and mRNA results in translation inhibition or destruction of the mRNA. An imperfect match between the microRNA and mRNA enables a microRNA to regulate a range of different genes and for a given gene to be regulated by several microRNAs (Meltzer, 2005).

## 13.3 Immuno-miRNAs: vital immune regulators

In recent years, a vast amount of miRNAs have been found to be involved in the regulation of immunological key processes encompassing development, lineage commitment, activation, function and ageing of innate and adaptive immune cells (Mehta & Baltimore, 2016; O'Connell, Chaudhuri, & Baltimore, 2010b; Paladini et al., 2016). Within these networks, striking miRNAs and miRNA-clusters have crystallized, that exert pivotal roles in the regulation of gene expression throughout the entire immune system. By targeting cellular signaling hubs (e.g., NF-κB signaling pathways), these so-called immuno-miRs have fundamental regulative impact in both innate and adaptive immune cells. Fig. 13.1 depicts the relationship between miRNAs and immunity.

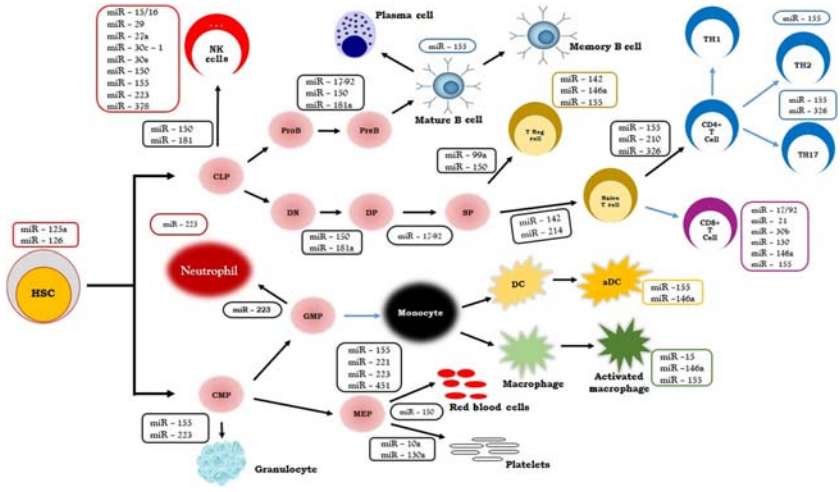


**FIGURE 13.1** The relationship between miRNAs and immunity.

### 13.3.1 miR-23∼27∼24 cluster

miRNAs of this cluster are organized within two paralogues: Mirc11 on chromosome 19 harboring miR-23a, miR24−2 and miR-27a, and Mirc22 on chromosome 9 encoding miR-23b, miR-24−1, miR-27b (Kong et al., 2010; Li et al., 2016; Zaidi et al., 2009). Growing evidence suggests a complex interplay between hematopoietic transcription factors and miR-23∼27∼24, influencing the regulation of immune cell lineage commitment. The cluster is transcriptionally induced by PU.1, a central myeloid transcription factor (Kong et al., 2010), and, in contrast, negatively regulated by lymphoid transcription factors (EBF1, E2A, and PAX5) (Kurkewich et al., 2017). In turn, cluster miRNAs directly target a number of fundamental lymphoid transcription factors (Runx1, Satb1, Bach1, and Ikzf1), thereby impairing lymphoid cell differentiation and promoting myeloid lineage commitment and cell proliferation (Ben-Ami, Pencovich, Lotem, Levanon, & Groner, 2009; Kong et al., 2010; Kurkewich et al., 2017; Mavrakis et al., 2011; Wang et al., 2015; Zaidi et al., 2009). Members of the miR-23∼27∼24 cluster exert complex, partially even opposing effects on T cell immunity. One central feature is repression of Th2 immunity by impairment of IL4 cytokine signaling mediated by all members of the cluster. miR-24 and miR-27 directly suppress IL4 (miR-24) and the central Th2 transcription factor GATA3, also by targeting BIM1 (Cho et al., 2016; Guerau-de-Arellano et al., 2011). Pua et al. discovered several more direct targets for miR-24 (Cnot6, Clcn3), miR-27 (Ccnk, Aff4), and miR24/miR-27 (Ikzf1, Gpr174, and Galnt3), mediating IL4-network-related suppression of Th2 immunity (Pua et al., 2016). Regarding other T helper cell subsets, miR-24 has been reported to promote Th1, Th17 and Treg differentiation by targeting TCF1 (Cho et al., 2016; Cho et al., 2017), whereas miR-23 and miR-27 have been shown to restrain Treg/Th17 cell differentiation and Th1 functions (Cho et al., 2016; Cruz et al., 2017; Rogler et al., 2009). Several studies provided evidence for impaired production and direct targeting of IFN$\gamma$ by miR-27 (Cho et al., 2016; Guo, Riley, Iwasaki, & Steitz, 2014). In contrast, in patients an increase in IFN$\gamma$+ T cells following overexpression of miR-27 was described (Guerau-de-Arellano et al., 2011). Very recently, this was attributed to a perturbed Treg compartment by Cruz et al. (Cruz et al., 2017). These partially opposing effects of members of the same miRNA cluster may serve as cluster-intrinsic negative feedback mechanisms, enabling a versatile molecular fine-tuning. In cytotoxic T cells, miR-23∼27∼24 is induced directly by TGF-$\beta$ and indirectly through TGF-$\beta$ mediated suppression of cMyc (Chandran et al., 2014; Lin et al., 2014). The cluster suppresses CD8+ T cell effector function through direct (miR-24, miR-27a) and indirect (miR-23a) targeting of IFN$\gamma$ (Chandran et al., 2014; Lin et al., 2014). In dendritic cells (DC), miR-27a has been shown to dampen Th1 and Th17 response (Min et al., 2012). miR-23b acts in the same direction by increasing

FoxP3 expression through inhibition of the Notch1 and NF-κB signaling pathways (IκBα phosphorylation) (Zheng et al., 2012).

### 13.3.2 miR-146a/-155 axis

miR-146a and miR-155 are abundant in most innate and adaptive immune cells, where they exert strong regulative impact (Ceppi et al., 2009; O'Connell, Taganov, Boldin, Cheng, & Baltimore, 2007; O'Connell, Zhao, & Rao, 2011). Although not encoded within one genetic cluster, both miRNAs are functionally strongly entangled and thus are viewed to act within a joint functional axis: They form a "yingyang" with miR-146a representing the antiinflammatory and miR-155 the proinflammatory part. The role of miR-146a as a brake on inflammation has been well defined: By targeting central signaling molecules (IRAK1/2, TRAF6, MyD88, TLRs, NOTCH1), miR-146a negatively regulates both TNFα as well as IL-6 immune pathways, thereby suppressing differentiation processes and inflammatory effector functions in innate immune cells (He et al., 2014; Hou et al., 2009; Self-Fordham, Naqvi, Uttamani, Kulkarni, & Nares, 2017; Taganov, Boldin, Chang, & Baltimore, 2006). In adaptive immunity, miR-146a has been shown to be a key negative regulator of Th1 cell differentiation (Möhnle et al., 2015). In line with these findings, massive upregulation of IFNγ in miR-146a defective CD4 + and CD8 + T cells has been observed (Huffaker et al., 2012). MiR-146a-deficient mice develop chronic hyperinflammatory and autoimmune disorders with accumulation of innate immune cells, activated CD4 + and CD8 + T cells, and autoantibodies. This phenotype is due to a lost targeting of signal transducer and activator of transcription 1 (STAT1), which reduced abilities of Treg cells to suppress IFN-mediated inflammatory Th1 responses (Lu et al., 2010). Furthermore, miR-146a has been identified as part of a negative feedback loop limiting NF-κB activity upon T cell activation via repressing the NF-κB activators TRAF6, IRAK1 (Boldin et al., 2011; Yang et al., 2012) and FADD. The latter interaction is also mitigating activation-induced cell death (AICD) in T cells (Curtale et al., 2010). Proinflammatory miR-155, in contrast, is elementary for mounting adequate immune responses (Rodriguez et al., 2007). Through direct targeting of suppressor of cytokine signaling-1 (SOCS-1), miR-155 positively regulates IFNγ production in CD4 + and CD8 + T cells, which was shown indispensable for sufficient T effector cell accumulation and action (Dudda et al., 2013; Huffaker et al., 2012). The interaction with SOCS-1 also has been implicated in conferring Treg fitness (Lu et al., 2009). Furthermore, miR-155 contributes to Th17 cell function by suppressing the inhibitory effects of Jarid2 (Escobar et al., 2014). These findings were further strengthened by analysis of miR-155-deficiency, which resulted in resistance to experimental autoimmune encephalitis, thereby providing evidence for significantly impaired Th1 and Th17 cell response (O'Connell et al., 2010a).

Based on the fact that miR-146a and miR-155 target elements of the same central immunological hub, namely, the NF-κB signaling cascade, Mann et al. provided evidence for a complex spatiotemporal regulatory network in macrophages, consisting of both microRNAs. Activation of NF-κB leads to enhanced expression of miR-155. By targeting of SHIP1 and SOCS1, miR-155 amplifies NF-κB signaling via a positive-feedback loop. With time, also miR-146a levels are upregulated by NF-κB, "switching off" the miR-155 signaling cascade by direct targeting of IRAK1 and TRAF6 (Mann et al., 2017).

### 13.3.3 miR-17∼92 cluster

The miR-17∼92 cluster, located on chromosome 13, encodes a polycistronic miRNA transcript that yields six individual miRNA components: miR-17, mir-18a, miR-19a/b, miR-20a, and miR-92 (Jiang et al., 2011). Individual miR-17∼92 components regulate a variety of cellular processes in a cell type and context-dependent manner (Olive, Li, & He, 2013). Relevant expression of miR-17∼92 and important functional implications have been described for lymphocytes, monocytes and macrophages (Poitz et al., 2013; Skinner, Keown, & Chong, 2014; Ventura et al., 2008). miR-17∼92 has revealed as a central proinflammatory cluster in T cell immunity. Induced upon NF-κB activation, cluster members directly target important regulatory molecules of JAK-STAT and NF-κB signaling pathways. As a result, Th1-related IFNγ response is increased, T effector cell proliferation and survival are promoted, cytokine production of Th2 cells (IL4, IL5, IL13) is amplified, and Treg differentiation inhibited (Gantier et al., 2012; Jiang et al., 2011; Simpson et al., 2014; Skinner et al., 2014; Xiao et al., 2008; Zhou, Hu, Gong, & Chen, 2010). Moreover, miR-17 has been shown to provide co-regulatory transcription factor Eos (Yang et al., 2016). The miR-17∼92 cluster is also essential for B cell development. Its absence causes increased levels of the proapoptotic protein Bim and inhibits B cell lymphopoiesis (Ventura et al., 2008). Furthermore, miR-17∼92 is required for T follicular helper cell (Tfh cell) differentiation, migration and function. By direct targeting of Pten and Phlpp2, miR-17∼92 effectively controls ICOS-PI3K signaling, thus enabling proper Tfh cell migration (Kang et al., 2013). Downregulation of key molecules (CCR6, IL1R1/2, IL22) via direct targeting of RORα through miR17∼92 further supports Tfh differentiation and function (Baumjohann et al., 2013; Kang et al., 2013). Additionally, macrophage differentiation is potently suppressed by miR-17∼92: Through targeting of HIF-1α and HIF-2α, miR-17∼92 decreases HIF-signaling and impairs macrophage differentiation, particularly in inflamed albeit hypoxic tissues (Poitz et al., 2013). In CD8 + T cells, another layer of complexity has been added to these regulatory multitargeting networks of miR-17∼92, as distinct biological functions require a strictly time-dependent cluster expression: In response to acute infections, elevation of miR-17∼92 is

required for sufficient cell expansion to counter the pathogens. In protracted states of infection, however, down-regulation of miR-17∼92 is necessary for adequate memory cell response and central memory development (Khan, Penny, Yuzefpolskiy, Sarkar, & Kalia, 2013; Wu, Gong, et al., 2012a).

### 13.3.4 miR-223

miRNA-223 is considered a classical innate miRNA (Fukao et al., 2007; Johnnidis et al., 2008). In earlier studies, miR-223 was viewed as myeloid-specific with relevant expression only in granulocyte precursor cells and mature granulocytes (Chen, 2004). Over time, also abundance and functional relevance in monocytes and macrophages has been discovered (O'Connell et al., 2011). Throughout granulocyte cell differentiation, miR-223 levels steadily increase (Johnnidis et al., 2008). Johnnidis et al. described neutrophil hyperactivity and hyperinflammatory disorders in miR-223 deficient mice, resulting from direct targeting of the transcription factor Mef2c, suggesting miR-223 to act as a negative regulator of granulocyte production and inflammatory response (Johnnidis et al., 2008). Other studies described miR-223 as a modulator within regulatory loops keeping granulocyte differentiation in check (Fazi et al., 2005; Pulikkan et al., 2010). In contrast to granulocytes, levels of miR-223 constantly decrease during monocyte/macrophage differentiation. In parallel, miR-223 target IKKα is up-regulated, leading to repression of noncanonical NF-κB signaling, thus preventing hyperactivation of macrophages (Li et al., 2010). Upon stimulation of macrophages, miR-223 is downregulated, which enhances proinflammatory cytokine expression (IL1β, IL6, IL18, TNFα) (Neudecker, Brodsky, et al., 2017a; Zhang, Fu, Bu, Yao, & Wang, 2017). Effects of miR-223 can be put down to a multitargeting network regulating NFκB-, TLR-, and MAPK-signaling, as well as NLRP inflammasome activity (Bauernfeind et al., 2012; Zhang et al., 2017). Thus miR223 may be regarded as key antiinflammatory miRNA. miR-223 has also been implicated in intercellular signaling by microvesicles. In response to immune challenge, polymorphonuclear cells (PMN) shuttle miR-223 to lung epithelial cells, thereby dampening inflammation during acute lung injury by direct targeting of poly (adenosine diphosphatase-ribose) polymerase-1 (PARP-1) (Neudecker, Haneklaus, et al., 2017b). Also, in patients undergoing cardiac bypass surgery, exosomal miR-223 has been described to suppress inflammation in monocytes by direct binding to NLRP 3′UTR and IL6 coding sequence (Poon et al., 2017).

### 13.3.5 miR-181

In both mature innate and adaptive immune cells, miR-181 is a potent antiinflammatory miRNA. In T cells, miR-181a strengthens T cell receptor-dependent activation by increasing both power and sensitivity of TCR

signaling (Li et al., 2007). These findings are the result of direct negative regulation of several Tyrosine- and ERK-phosphatases by miR-181 (Li et al., 2007). During thymic development, downregulation of miR-181 leads to reduced sensitivity of TCR, which results in maturation of auto reactive T cells, thus highlighting the importance of miR-181-mediated control of TCR threshold for T cell immunity (Ebert, Jiang, Xie, Li, & Davis, 2009). With respect to CD4 + cells, evidence was provided for miR-181 to inhibit Th1 cell differentiation and to promote Treg development, probably by direct targeting of Smad7, a negative regulator of TGF-β signaling (Ghorbani et al., 2017). Mir-181 also impacts immunosenescence. In aged CD4 + T cells, due to lower levels of miR181a, dual specificity phosphatase 6 (DUSP6) expression is increased. This leads to dampening of ERK-signaling, resulting in impaired TCR activation and reduced signaling strength (Li et al., 2012). In macrophages, Kruppel-like factor 6 (KLF6) and CCAAT/enhancer binding protein-α (C/EBPα) have been identified as direct targets of miR-181, thus promoting M2 over M1 polarization, including suppression of TNFα and IL1β (Bi et al., 2016; Ghorbani et al., 2017). Further, direct targeting has been confirmed for IL1α (Xie et al., 2013; Zhu et al., 2017). In DC, miR-181 was reported to act antiinflammatory through direct targeting of TNFα and proinflammatory transcription factor c-FOS (Wu, Wieland, et al., 2012b; Xie et al., 2013). Generally, antiinflammatory effects of miR-181 are further mediated by suppression of NF-κB signaling via direct targeting of importin α3 and the lysine deubiquitinase (CYLD) (Su et al., 2017; Sun et al., 2012).

## 13.4 miRNA-mediated regulation of T cell differentiation and function

It is increasingly recognized that miRNAs are important modulators of TH cell fate decisions and effector functions. Their fine-tuning activity is particularly well suited to their function early in the response when small changes in the expression of key genes can have a big effect on cell fate. In addition, miRNAs can regulate the plasticity and the effector functions of differentiated TH cell subsets.

### 13.4.1 TH1 cellular differentiation and function

TH1 cells, orchestrate immune responses against viruses, intracellular pathogens and tumors. The first evidence for miRNA regulation of TH cell differentiation came from studies with DICER-deficient CD4 + T cells, which exhibit increased differentiation into effector cells expressing the TH1 cell lineage-defining transcription factor T-bet and its transcriptional target, the TH1 cell hallmark cytokine IFNγ (Muljo et al., 2005) 27. T cells lacking DROSHA or DGCR8 display a very similar phenotype of aberrant TH1 cell differentiation (Chong et al., 2008; Steiner et al., 2011). A functional screen

in DGCR8-deficient CD4 + T cells revealed that among CD4 + T cell-expressed miRNAs, miR-29a and miR-29b are the most potent inhibitors of TH1 cell differentiation and IFNγ expression. miR-29 is highly expressed in naïve T cells, and it inhibits TH1 cell differentiation through multiple direct targets. T-bet is one of the direct miR-29 targets in CD4 + T cells, but miR-29 also targets the closely related transcription factor eomesodermin (EOMES), which is usually not expressed in CD4 + T cells (Steiner et al., 2011). EOMES regulates IFNγ production in natural killer (NK) cells and CD8 + T cells, and in the absence of miRNAs it is also inappropriately expressed in CD4 + T cells and contributes to the aberrant IFNγ31. miR-29 may also directly target the Ifng mRNA (Smith et al., 2012). Transgenic mice expressing a miRNA sponge [G] that reduces miR-29a-mediated control exhibited higher serum IFN-γ levels and more effective clearance of the intracellular bacterium Listeria monocytogenes (Smith et al., 2012). A recent study confirmed the importance of miR-29 in restraining IFN-γ expression by T cells using mir-29ab1 knock-out mice (Ma et al., 2011). Several other miRNAs regulate TH1 cell differentiation and function. miR-146a deficient CD4 + T cells produce more IFNγin vitro and in vivo (Huffaker et al., 2012; Lu et al., 2010). As discussed above, miR-146a inhibits T cell responses in general by targeting TRAF6 and IRAK146. However, it may also specifically regulate TH1 differentiation by targeting signal transducer and activator of transcription 1 (STAT1) (Lu et al., 2010). By contrast, miR-155 enhances both TH1 and TH17cell-dependent tissue inflammation. Among its many identified targets in T cells 49, miR-155 inhibits the negative regulators of cytokine signaling SOCS1 and SHIP1 (Huffaker et al., 2012; Lu et al., 2009). In addition, miR-155 targets Ifngr1 mRNA and may be responsible for the downregulation of IFNγ receptor in activated CD4 + T cells that differentiate into TH1 cells (Banerjee, Schambach, DeJong, Hammond, & Reiner, 2010). Epistasis experiments showed that miR-155 deficiency is dominant over miR-146 deficiency, as cells lacking both miRNAs are as defective in IFN-γ expression and antitumor immunity as those lacking miR-155 only (Huffaker et al., 2012). The miR-17∼92 cluster also impacts TH1 cell differentiation. CD4 + T cells freshly isolated from peripheral lymph nodes of 10- to 12-month-old mice that expressed a transgene encoding the human miR-17∼92 cluster showed a dramatic increase in the percentage of IFNγ-expressing cells (Xiao et al., 2008). Similarly, naïve miR-17∼92 transgenic CD4 + T cells produced higher amounts of IFNγ under TH1 polarizing conditions in vitro (Xiao et al., 2008). Conversely, T cell-specific deletion of the miR-17∼92 cluster in mice resulted in a gene dose-dependent reduction of IFNγ-secreting CD4 + T cells (Jiang et al., 2011). Reconstitution of miR-17∼92-deficient CD4 + T cells with individual miRNA mimics [G] identified miR-19b as sufficient to restore IFNγ production (Jiang et al., 2011). miRNAs can influence TH1 cell differentiation indirectly by targeting genes in immune cells other than CD4 + T cells. For

example, miR-21 regulates TH1 cell differentiation by modulating IL-12 production by DC. IL-12 is an important cytokine that induces T-bet and IFNγ expression and supports the growth and survival of TH1 cells (Lazarevic et al., 2011). miR-21 is highly expressed in DCs, and directly targets the mRNA that encodes IL-12p35 (Lu et al., 2009). miR-21 deficiency increases IL-12 expression in DCs, and enhances TH1 cell development and delayed type hypersensitivity responses in vivo (Lu et al., 2011). It is possible that other miRNAs that modulate DC activity and cytokine production also have important indirect effects on TH cell differentiation and immune function. Studies of TH1 cell differentiation and function have led the way to insights into the T-cell intrinsic and extrinsic roles for miRNAs in T cell differentiation, and set paradigms for the logic of miRNA-mediated regulation of cell differentiation in general. Some of the miRNAs that regulate TH1 cells also play identical or related roles in other IFN-γ producing lymphocytes, such as cytotoxic CD8 + T cells and NK cells (Beaulieu et al., 2013; Jeker, Zhou, Blelloch, & Bluestone, 2013). Lessons learned through the study of TH1 cells will likely continue to inform our understanding of miRNA regulation of the immune system.

### 13.4.2 TH2 cellular differentiation and function

TH2 cells orchestrate innate immune responses against helminths (Allen & Maizels, 2011), and dysregulated TH2 responses cause allergies and asthma (Locksley, 2010). Over the past decades, much progress has been made in our understanding of the molecular requirements for the generation of TH2 cells. In vitro differentiation systems and more recently elegant in vivo approaches have established the powerful influence of a positive feedback loop involving the hallmark TH2 cytokine IL-4 and the transcription factors STAT6 and GATA-binding protein 3 (GATA3) in amplifying TH2 responses, though the initial signals leading to TH2 cell differentiation have been harder to define (Paul & Zhu, 2010). In addition, to IL-4, TH2 cells express the genetically linked cytokines IL-5 and IL-13, which drive type 2 inflammation through their effects on myeloid and nonhematopoietic cells (Ansel, Djuretic, Tanasa, & Rao, 2006). Compared to TH1 cells, miRNA regulation of TH2 cell differentiation is poorly understood. Overexpression of miR-21 in T cells increases TH2 cell differentiation in vitro (Sawant, Wu, Kaplan, & Dent, 2013), whereas overexpression of miR-27 or miR-128 decreases the secretion of IL-4 and IL-5 by activatedCD4 + T cells (Guerau-de-Arellano et al., 2011). Further work is needed to characterize the importance of these miRNAs in TH2 cell responses. In addition, miR-155-deficient T cells display a mild bias towards TH2 differentiation in vitro. This is accompanied by dysregulation of many target genes, including MAF (also known as c-MAF), a transcription factor that regulates IL-4production (Rodriguez et al., 2007; Thai et al., 2007). Approximately 50% of ageing

miR-155 knockout mice develop spontaneous lung inflammation, which displays some but not all of the hallmarks of type 2 inflammation that have been associated with asthma (Rodriguez et al., 2007). This pathology may be caused by cell-intrinsic defects in TH2 cell differentiation, but it may also reflect other changes in T cell responses or requirements for miR-155 in other cells. The lung's accessibility for local drug delivery makes asthma an attractive target for miRNA-directed therapies. MicroRNA expression patterns associated with asthma have been detected in both lung epithelial cells and purified T cells from human asthmatic subjects (Seumois et al., 2012; Solberg et al., 2012). In addition, several proof-of-concept studies have shown that miRNA inhibitors can be protective in mouse models of allergic asthma. House dust mite allergen (HDM) challenge increases miR-126 expression in the airway wall, and intranasal administration of a miR-126-specific antagomir [G] (cholesterol-linked single stranded RNA complementary to miR-126) reduces airway hyper responsiveness and eosinophil recruitment to the lung (Mattes, Collison, Plank, Phipps, & Foster, 2009). Treatment with the miR-126-specific antagomir also reduced lungeosinophilia in a mouse model of chronic asthma, but it did not have sustained effects on inflammation or airway remodeling (Collison et al., 2011a). As miR-126 is strongly expressed in the airway wall (Collison, Mattes, Plank, & Foster, 2011b; Mattes et al., 2009), but is present in T cells and other hematopoietic cells at only very low abundance (Kuchen et al., 2010; Steiner et al., 2011), it seems likely that miR-126-specific antagomir acts on epithelial cells or other nonhematopoietic cells in the lung rather than directly on infiltrating TH2 cells in these models. A miR-145-specific antagomir also inhibited allergic airway disease in the HDM model, possibly through effects on smooth muscle cells, which express a large amount of miR-14578. Members of the let-7 family of miRNAs are highly expressed in most cells. Let-7 directly targets Il13 mRNA and reduces IL-13 production by T cells in vitro (Kumar et al., 2011; Polikepahad et al., 2010). However, intravenous administration of locked nucleic acid [G] (LNA) inhibitors of let-7 ameliorated lung inflammation and airway hyper responsiveness in a mouse model of asthma, rather than exacerbating the disease as may have been expected if the dominant effect of let-7 inhibitors was to de repress IL-13 expression in TH2 cells. Let-7 also targets IL-10 mRNA, and increased IL-10 levels in HIV patients correlate with decreased let-7expression in CD4 + T cells (Swaminathan et al., 2012). Taken together, these studies show that individual miRNAs can have a strong impact on type 2 immunity, but also underscore the complexity of miRNA biology in vivo. In particular, they indicate that targeted delivery of miRNAs or miRNA inhibitors specifically to T cells will be important for manipulating miRNA-mediated control of TH2 cell gene expression, differentiation and effector functions, be it for scientific or therapeutic purposes

### 13.4.3 TH17 cellular differentiation and function

TH17 cells are defined by expression of the transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and cytokines of the IL-17 family (Korn, Bettelli, Oukka, & Kuchroo, 2009) and have an important role in the defense against extracellular bacteria and fungi. In addition, they have been implicated in the etiology and pathology of several autoimmune diseases including psoriasis, multiple sclerosis, colitis, arthritis, and even asthma (Weaver, Elson, Fouser, & Kolls, 2013). miRNA profiling in T cells from patients with multiple sclerosis, as well as studies in mice and rats with experimental autoimmune encephalomyelitis (EAE) [G], a model system for multiple sclerosis, have revealed distinct contributions for miRNAs in the regulation of TH cell subsets involved in this disease (Thamilarasan, Koczan, Hecker, Paap, & Zettl, 2012). Expression of miR-326 in TH cells correlated with disease severity in mice with EAE and also in patients with multiple sclerosis (Du et al., 2009). miR-326 targets ETS1, a negative regulator of TH17 cell differentiation and increases TH17 differentiation in vitro (Du et al., 2009). Conversely, expression of miR-10a in CD4 + T cells can limit TH17 cell differentiation (Takahashi et al., 2012). This effect depends on the presence of retinoic acid, which induces both miR-10a and T-bet. Suppression of the miR-10a targets Bcl6 or Ncor by RNA interference limited TH17 cell differentiation in wild-type, but not T-bet-deficient T cells. As BCL-6 and NCOR modulate the expression and transcriptional activity of T-bet (Nurieva et al., 2009; Oestreich, Huang, & Weinmann, 2011; Yu et al., 2009) and T-bet in turn inhibits the expression of RORγt (Lazarevic et al., 2011), miR-10a and T-bet may cooperate to balance TH1 and TH17 differentiation in retinoic acid-rich microenvironments. In line with these observations, miR-10a overexpression in myelin oligodendrocyte glycoprotein (MOG)-specific CD4 + T cells delays neurological disease in EAE. miR-155 knockout mice are strongly resistant to EAE. Detailed analysis revealed both a T cell-intrinsic role for miR-155 in TH17 cell differentiation, as well as an indirect role through the regulation of production of TH17 cell-polarizing cytokines by DCs55 (Murugaiyan, Beynon, Mittal, Joller, & Weiner, 2011). Impaired TH17 cell responses of miR-155-deficient T cells were also observed in a mouse model of Helicobacter pylori infection and in a mouse model of TH17-driven chronic colitis (Oertli et al., 2011). Thus miR-155 promotes T cell-mediated tissue inflammation through the regulation of both TH1 and TH17 cell responses. Finally, miR-301a enhances TH17 cell differentiation, possibly through targeting PIAS3, which inhibits STAT3 signaling and TH17 cell differentiation (Mycko et al., 2012). Although the last few years have seen substantial progress in defining the transcriptional requirements for TH17 cells (Basu, Hatton, & Weaver, 2013), studies on miRNA function in TH17 cells remain sparse. Given the high plasticity associated with this TH cell subset, it is anticipated that miRNAs

will play an important role in defining and maintaining the gene expression program of differentiated TH17 cells.

### 13.4.4 Regulatory T cellular differentiation and function

Elucidating the roles of specific microRNAs in Tregs is necessary to understand the concrete regulatory mechanisms of Tregs by microRNAs and may provide another perspective to research immune-related diseases. Micro155 is highly expressed in Tregs and its expression is regulated directly by the Foxp3 transcription factor through binding to the promoter region of its host gene bic (Kohlhaas et al., 2009). In mice depleted of micro155, the number of Tregs was reduced in both the thymus and spleen, but their suppressive capacity was not evidently altered (Kohlhaas et al., 2009). Further work has revealed that micro155 is required for Treg development and is indispensable for proliferation or survival of Tregs in the thymus and periphery (Kohlhaas et al., 2009). However, no micro155-deficient mice developed a spontaneous inflammatory bowel disease (IBD) (Kohlhaas et al., 2009). Lu et al. confirmed that the proliferative capacity of micro155-deficient Tregs was reduced and Treg homeostasis was impaired in the absence of micro155 (Lu et al., 2009). They discovered that micro155 conferred Tregs with an advantage in competition for the limited growth factor IL-2 which has been proven to be necessary for Treg proliferation (Bayer, Yu, & Malek, 2007). Suppressor of cytokine signaling 1 (SOCS1), which suppresses IL-2 signaling, is a direct target of micro155 as shown by luciferase reporter experiments (Lu et al., 2009). In SOCS1 transgenic mice, the number of Tregs was reduced, which was analogous to that in the miR155- deficient mice (Lu et al., 2009). In conclusion, micro155 could affect IL-2 signaling by inhibiting SOCS1 expression to regulate Treg proliferation but has little effect on the suppressive function of Tregs.

Among the microRNAs differentially expressed between nTregs and conventional CD4 + CD25- T cells, micro146 was evidently upregulated in Tregs (Cobb et al., 2006). Micro146a, which is potentially regulated by Foxp3, was found to be upregulated in human nTregs (Sadlon, Wilkinson, & Pederson, 2010). Lu et al. also confirmed that micro146a expression in the mouse CD4 + - CD25 + Tregs is much higher than that in the CD4 + - CD25- CD62Lhi-naive T cells (Lu et al., 2010). To explore the role of micro146a in Tregs, micro146a-deficient mice were developed and exhibited serious lympho proliferative and myelo proliferative syndrome at 6 months of age (Lu et al., 2010). In the micro146a-deficient mice, the number of Foxp3 + Tregs was reduced in the periphery, but not in the thymus (Lu et al., 2010). Using micro146a-/-/Foxp3KO chimeras, researchers demonstrated that a lack of micro146a could increase Treg numbers in a cell-autonomous manner, but led to an impaired Treg function (Lu et al., 2010). In vitro, experiments indicated that micro146a deficiency in Tregs did not

change their overall suppressive function. Increased proinflammatory Th1 cytokine IFNγ was observed in micro146a-deficient Tregs. Further analysis showed that Stat1, which is a key transcription factor in the IFNγ receptor signaling pathway, is a direct target of micro146a as shown by luciferase reporter experiments. Stat1 expression was increased in micro146a-deficient Tregs (Lu et al., 2010). In conclusion, micro146a is indispensable for the Treg mediated suppression of the IFNγ-dependent Th1 response by a negative regulation of the Stat1 expression.

The micro17−92 polycistronic miRNA gene is transcribed as a primary transcript with the potential to generate six mature microRNAs including miR-17, miR18a, miR-19a, miR-20a, miR-19b, and miR-92. Micro17−92 is reported to inhibit the TGF-induced Treg generation in vitro (Jiang et al., 2011). To investigate the role of micro17−92 in vivo, mice were generated with micro17−92 depleted specifically in Tregs. However, there were no signs of disease in the mice, indicating that micro17−92 is dispensable for Tregs under healthy conditions (Jiang et al., 2011). To further analyze the role of micro17−92 in Tregs under disease stress, the TregmiR17−92-/- mouse model and controls were treated with myelin oligo dendrite glycoprotein (MOG) emulsified in complete Freund's adjuvant (CFA) to induce EAE. The TregmiR17−92-/- mice developed much more serious phenotypes than those in the controls. The proliferative activity of miR-17−92 deficient Tregs was not weakened in vivo. The overall numbers of Tregs in TregmiR17−92-/- mice under disease stress were not evidently altered but the number of antigen-specific Tregs was reduced, which may be due to increased cell death. There was also a reduced proportion of IL-10 secreting Tregs, suggesting that micro17−92 may regulate immunosuppressive IL-10 secretion in Tregs (Jiang et al., 2011). Therefore micro17−92 is not essential for Treg regulation under noninflammatory status but plays an important role in EAE disease by preserving antigen-specific Tregs and regulating IL-10 secretion in Tregs.

Li et al. discovered that micro568 expression was reduced during Treg activation (Li, Kong, & Li, 2014). Transfection of Tregs with micro568 mimicked inhibited Treg activation, reduced TGF-β and IL-10 production, and diminished their proliferative capacity (Li et al., 2014). Further research found that micro568 overexpression in Tregs could limit differentiation and immunosuppressive function of Tregs (Li et al., 2014). Nuclear factor of activated T cells 5 (NFAT5) is a direct target of micro568 and transfection with siRNA-NFAT5 inhibits the activation and differentiation of Tregs (Li et al., 2014). These results indicate that micro568 inhibits activation, differentiation, and function of Tregs by targeting NFAT5.

Okoye et al. reported that Foxp3 + Tregs could exert their inhibitory role on Th1 cells by secreting exosomes containing microRNAs (Okoye, Coomes, & Pelly, 2014). Rab27a and Rab27b are both required for exosome release. Tregs isolated from Rab27ashen/ashenRab27b-/- double knockout

mouse failed to secrete exosomes and lost their immunosuppressive ability (Okoye et al., 2014). The transfer of micro155, let-7b, and let-7d from Tregs to effector T cells was observed. Further research demonstrated that overexpression of let-7d in Th1 cells reduced Th1 cell proliferation and IFN-γ secretion. Tregs treated with a let-7d inhibitor which secrete let-7d depleted exosomes could result in an impaired immune suppression in vivo and vitro. Together, this work shows that let-7d plays an important role in the exosome-mediated immunosuppressive function of Tregs.

Foxp3 was confirmed to be a target of micro31 by luciferase reporter experiments and micro31 overexpression in Tregs reduced Foxp3 expression (Rouas, Fayyad-Kazan, & el Zein, 2009). Recently, Zhang et al. confirmed that T cell receptor (TCR) signaling could induce micro31 expression, while Foxp3 negatively regulated micro31 expression by binding to its promoter (Zhang, Ke, & Liu, 2015). Further analysis demonstrated that conditional deletion of micro31 in CD4 + T cells induced pTreg (periphery Treg) generation and ameliorated the severity of EAE in vivo. Gpra5a was confirmed to be a direct target of micro31 (Zhang et al., 2015). Gprc5a, which is also known as retinoic acid-inducible protein 3, contains two transcription factors binding sites: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) of RA (Ye, Tao, Wang, Cheng, & Lotan, 2009). Gprc5a expression was lower in EAE mice than that in controls, consistent with the higher micro31 expression in EAE mice than that in controls (Zhang et al., 2015). Under inflammatory conditions, Gprc5a-/- mice have fewer pTregs and exhibit increased EAE severity (Zhang et al., 2015). These results suggest that micro31 may affect EAE severity by regulating pTreg generation through targeting Gprc5a and may serve as a therapeutic target of EAE disease.

Micro27 is a member of the miR-23∼27∼24 family and is upregulated in T cells from multiple sclerosis patients (Guerau-de-Arellano et al., 2011). Further study demonstrated that mice with T cell-specific micro27 overexpression showed pathogenic Th1 responses and autoimmune pathogeny (Cruz et al., 2017). The abnormal immune reactions were driven by Treg dysregulation to some extent. Excessive micro27 expression in T cells could result in fewer Tregs. It was demonstrated that excessive micro27 impaired the development and homeostasis of Tregs by targeting SMAD2/3, RUNX1, and c-Rel, which are members of the NF-κB transcription factor and crucial for Treg biology. At the same time, overexpressed micro27 could inhibit Treg function by targeting IL-10 and GZMB. Another study demonstrated that micro27 was abundantly expressed in Tregs in mice, and overexpression weakened Treg generation (Cho et al., 2016). In conclusion, micro27 plays an important role in the development, homeostasis, and function of Tregs.

Becker et al. analyzed the microRNA expression profile of CD4 + T cells from the draining lymph node of a murine model of allogenic transplantation and found that micro466a-3p expression was increased after allogeneic

transplantation (Becker, Nagarkatti, & Nagarkatti, 2018). To further research the role of micro466a-3p in Treg, micro466a-3p was transfected into CD4 + T cells, which could reduce iTregs, while inhibiting micro446-3p expression in CD4 + T cells could increase Tregs and reduce effector cells. Luciferase reporter assays showed that TGF-β2 was a direct target of micro466a-3p. Inhibition of micro466a-3p in an allogeneic skin-graft model abates the T cell response to the graft through the increased expression of TGF-β2. These results demonstrate the role of micro466a-3p in Treg differentiation.

Micro4281 is specifically expressed in hominids and specifically binds the TATA box in the Foxp3 promoter. The expression of micro4281 was much higher in Tregs than that of naïve T cells sorted from human PBMCs (Zhang, Liu, & Chen, 2018). Experiments showed that micro4281 enhanced the promoter activity and transcription of Foxp3 and facilitated Treg development (Zhang et al., 2018). Overexpression of micro4281 significantly induces naïve T cells to Tregs. Micro4281 mimics ameliorate the graft versus host disease (GVHD) in a mouse model. IL2 signaling is essential for Treg development. IL2/STAT5 signaling induces micro4281 expression by binding to the promoter of SNCB, which then promotes Foxp3 expression (Zhang et al., 2018). In conclusion, micro4281 plays an important role in the development of Tregs by binding to the TATA box of the Foxp3 promoter.

Among the nTreg microRNA signature (micro21, micro31, micro125a, micro181c, and micro374), micro21 and micro31 were shown to regulate Foxp3 expression, while the other three microRNAs had no effect of Foxp3 expression and Treg phenotypes (Rouas et al., 2009). Compared with conventional T cells, micro21 was upregulated and micro31 was downregulated in Tregs. Micro21 positively regulates Foxp3 expression indirectly, and micro21 overexpression in non-Tregs can induce Foxp3 expression (Rouas et al., 2009). In the microRNA profile of circulating CD4 + Tregs in human adults, several microRNAs were confirmed to regulate Foxp3 and CTLA4 expressions (Fayyad-Kazan, Rouas, & Fayyad-Kazan, 2012). Micro24 and micro210 were under expressed in Tregs and could negatively regulate Foxp3 expression by directly binding to its 3′ UTR (Fayyad-Kazan et al., 2012). Overexpression of micro24 and micro210 in Tregs could lead to a reduced Foxp3 expression. However, other research demonstrated that micro24 expression was high in mouse Tregs, and blocking and overexpression experiments showed micro24 could induce Treg generation and differentiation by directly targeting IL-4 (Cho et al., 2016; Singh, Garden, Lang, & Cobb, 2015). The different influences of micro24 on Tregs shown in the abovementioned studies may be due to experimental differences in each study. Micro95 is upregulated in Tregs, positively regulates Foxp3 expression indirectly, and micro95 overexpression in CD4 + - CD25- T cells increases Foxp3 expression (Fayyad-Kazan et al., 2012). Micro145 is downregulated in Tregs, inhibits CTLA4 directly by binding to its 3′ UTR, and micro145 overexpression in Tregs reduces CTLA4 expression (Fayyad-Kazan et al., 2012). Micro23

and micro29a are both abundantly expressed in mouse Tregs. Micro23 overexpression inhibits Treg generation; in contrast, micro29a overexpression induces Tregs generation. Kaul et al. transfected peripheral blood mononuclear cells (PBMCs) with micro2909, which led to an evident increase of CD4 + - CD25 + Foxp3 + Tregs among the CD4 + T population (Kaul, Sasikala, & Raina, 2012). This indicates that micro2909 may play a regulatory role in Treg proliferation, but this requires further investigation. Together, these studies demonstrate that micro21, micro23, micro31, micro24, micro29a, micro95, micro145, micro210, and micro2909 are microRNAs with the potential to regulate the development, proliferation, and function of Tregs and further research may confirm their roles in immune regulation.

## 13.5 Conclusion

In conclusion, miRNAs are modulators of inflammatory and immune responses. Some miRNAs act as important inhibitors, while others tend to enhance the responses of the immune system by negatively regulating the response of the inhibitors. miRNAs either work on signal transduction proteins or support the inflammatory or antiinflammatory responses of immune systems. Hence, miRNAs can act as biomarkers or targets for treatment in a variety of infectious diseases. While the key role of miRNA-mediated gene regulation has been established in the immune system, there are still many unresolved questions regarding how immune cells modulate this process during immune responses. The transcription factors of most miRNAs are yet to be identified, which will greatly advance our understanding of changes in miRNA profiles in activated immune cells challenged by various stimuli. The precise and specific effects of RBPs on miRNA biogenesis and how these proteins are regulated needs to be investigated in the development and function of the immune system, as well as in pathological processes. Moreover, further studies are needed to reveal how miRNAs select their targets, depending on the cell type and immune microenvironment.

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Chapter 14

# Immunopharmacogenomics: a hope in the treatment of carcinoma

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## 14.1 Introduction

Cancer is a complex and heterogeneous process that is generally defined as accumulated cell mutations, manifested as uncontrollable growth as tumors. Currently, an extensive amount of research is going on to fully understand this disease for better treatment (Krzyszczyk et al., 2018). World health organization has reported the cancer as the second leading death cause with about 9.6 million globally estimated deaths in 2018 of which about 70% have been reported in low-middle income countries and the major risk factor being the use of tobacco which is responsible for nearly 22% of cancer deaths (Organization, 2018). In India, nearly 0.78 million people died and more than 1.1 million new cases have been reported in 2018 (Sahoo, Verma, & Parija, 2018). Currently, most of the cancers are treated using cytotoxic chemotherapies which do not target the mutations deriving malignant transformations (Wheeler, Maitland, Dolan, Cox, & Ratain, 2013). Four main types of standard treatments are available currently which is chemotherapy, radiation therapy, surgery, and immune therapy. Generally, combination of these approaches is commonly used for the treatment of cancer though some may require only one treatment approach. These traditional therapies for cancer like radiotherapy, chemotherapy, and others like clinical operations may have a periodic therapeutic effect but may cause side effects impacting the general well being and quality of life (Zhang et al., 2020). Even the more specific targeted therapies are also associated with severe adverse on-off target effects.

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00011-7**
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The field of immunopharmacogenomics aims to identify the basic molecular mechanisms of various underlying diseases by the sequencing of immune T and B cells and their underlying receptors and thus developing an accurate personalized treatment approach based on the identified genomic variations to help cure that disease. Analyzing the T cell characteristics in tumors and somatic changes can help in predicting response to anticancer treatment (Nakamura, 2015). The germline mutations in the tumor which affect the development of the disease and maybe the driver to define cancer subtypes and will also define the patients' response to the cancer treatment.

To achieve an effective immune response and avoid the possible toxicities using different immunotherapies, identification of the cancer-specific biomarkers to relate those patients most likely to benefit, or cancer antigens that can be targeted must be identified first (Figueroa et al., 2015; Schumacher, Kesmir, & Van Buuren, 2015). Identifying a potential biomarker is also necessary to determine specific combination therapies for particular malignancy and also to decrease the extra treatment cost in the patients unlikely to benefit from the treatment (Schumacher et al., 2015). With the occurrence and identification of DNA sequence variation commonly called single nucleotide polymorphism (SNPs) among the human genome and its association with cancer susceptibility, the interest in this field is increasing. SNP in the human genome is the usual type of genetic variation affecting regulation of cell cycle, DNA mismatch repair, metabolism, and immunity (Deng, Zhou, Fan, & Yuan, 2017; Lymberis, Parhar, Katsoulakis, & Formenti, 2004)

The important role of the immune system in the recognition and elimination of tumors was first revealed in 1943 by Gross et al. in mice who showed that the surgical removal of tumors in mice could protect it from subsequent re-exposure of tumor cells and also the exposure to the lethally irradiated tumor can also induce the protection. Subsequent other studies further supported this by reporting the immune protection of mice to the second challenge of the same tumor cells (Jiang et al., 2019). Various immunotherapies that aim at triggering the immune response toward the cancers are being actively examined currently. Immune checkpoint inhibitors for example, those affecting CTLA-4 and PD-1 have demonstrated better clinical efficacy and are under the rapid process of development (Pennock & Chow, 2015). The adoptive T cell CAR-T therapy which is the genetical engineering of T cells to express a synthetic receptor is also representing major advancements in immunotherapy (Feins, Kong, Williams, Milone, & Fraietta, 2019). Cancer vaccines are also designed to trigger an immune response against the tumor cells. These vaccines mainly contain cancer cells, cell parts, or pure antigens also represent an important form of immunotherapy (Even-Desrumeaux, Baty, & Chames, 2011). To provide an increased successful response these vaccines are usually combined with immune checkpoint

inhibitors so as to presensitize the immune system. Recognition of the cancer-specific neoantigens arising as a result of tumor derived mutations is now suggested to be a major element in the mechanism and activity of the immunotherapies (Schumacher & Schreiber, 2015). Personalized neoantigen based vaccine treatment given along with checkpoint inhibitors is a newer approach to the immunotherapy of cancer (Aldous & Dong, 2018).

Although immunotherapies are currently manifesting promising results in the management of cancer, the occurrence of fatal toxicities, high manufacturing cost, and limited effectiveness of some immunotherapies are the potential drawbacks that needs to be addressed. Pharmacogenomic studies may also provide negative results attributable to the genotyping errors, phenotypic errors, inadequate sample size, or lack of insertion of the genetic variation (Wheeler et al., 2013).

## 14.2 Cancer immunogenomics

Since host defensive-immune response has a crucial role in the pathogenesis of cancer, and gives a primary protection against it, immunogenomics is therefore currently a primary focus for cancer research. During cancer development immune system interacts dynamically with the tumor cells involving phases like immune surveillance where most of the tumor cells are eliminated by the immune defense. Next comes the equilibrium phase where the immune system is suppressed by the cancer cells by losing the antigen-presenting major histocompatibility complex (MHC) receptors. In the last phase of cancer escape, full-grown cancer cells escape immune response (Hossain, Majumder, & Miele, 2020).

Cancer cells undergo various mutations in the process of their division and growth. Some of these mutations are immunogenic-those which are immune recognized. These mutated peptides representing cancer cell antigens bind to MHC class-1 and are then expressed on the antigen-presenting cells (APCs) which are further recognized particularly by the cytotoxic CD8 + T cells. These peptides then bind with its surface receptor (TCR) and this is followed by the activation of the immune response and initiation of the target tumor-specific killing (Fig. 14.1). For effective T cell stimulation, APCs must also provide a costimulatory signal through cytokine molecules such as interleukin (IL-12), surface receptors like CD28 apart from the antigens present on MHC molecules. The epitope is the part of the antigen recognized by the TCR. The specific immune response to these mutated antigens can be a critical factor for the success of various immune therapies given in the cancer treatment of (Center, 2020; Seidel, Otsuka, & Kabashima, 2018).

After the recognition of the tumor cell antigen, the trans-membrane receptors present on the T and B cells that is T cell and B cell receptors (TCRs and BCRs) are activated by the tumor antigens and induce the

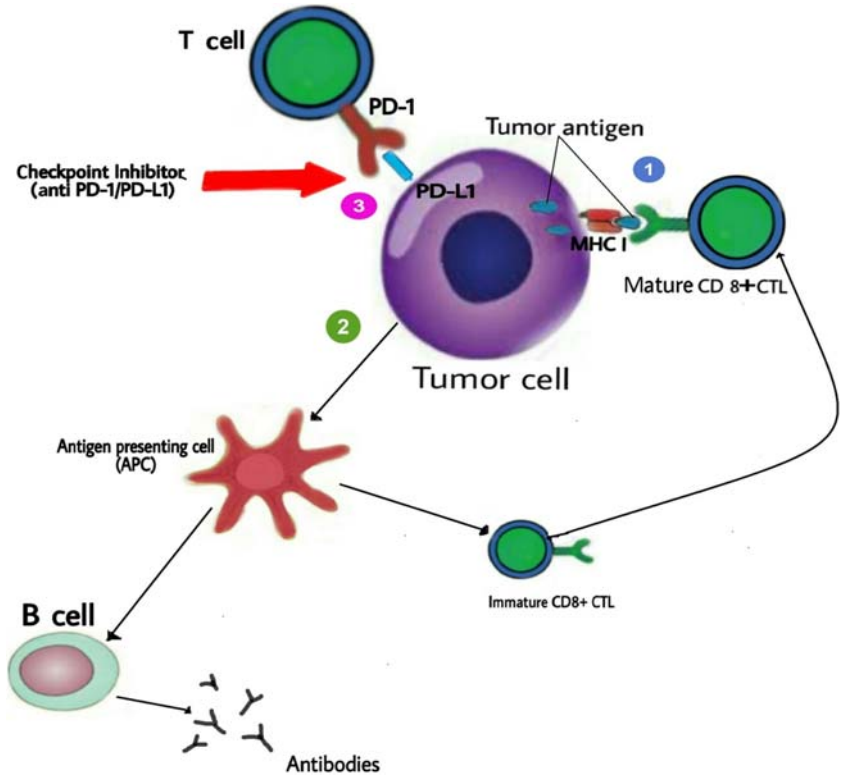


**FIGURE 14.1 Cancer immune response.** (1) mature cytotoxic CD8 + T lymphocyte bind to MHC class I presented antigen on tumor cell to induce the immune response against cancer. (2) Antigen-presenting cells will express antigen on the surface to recruit the immune responsive T and B cells to target the tumor. (3) T cells express immune checkpoints (e.g., PD-1 here) that bind to their tumor cell ligands (e.g., PD-L1 here) to suppress immune action. This interaction is blocked by using checkpoint inhibitors.

primary immunological responses. Both TCR and BCR have important "variable regions" responsible for the recognition of an antigen. This antigen-receptor binding will lead to immunological reactions such as the formation of plasma cell antibodies and activating the cellular T cell immunity. In the course of T and B cell differentiation, TCR and BCR encoding genes such as variable, diversity, and joining that is V D and J exon segments go through a networks of rearrangements to create fully-activated T and B cell receptors. Different TCR and BCR repertoires are generated by combining a diverse amount of above mentioned gene segments and by junctional variations with addition or deletion of nucleotides. This whole process results in the generation of highly variable "complementary determining region 3" (CDR3) which determines the affinity and specificity for

recognition of an antigen in TCR and BCR (Morris & Allen, 2012; Zewde et al., 2018). Sequencing of TCR and BCR to fully characterize the change in this receptor- repertoires is needed to enhance the immunotherapies against cancer.

During the active proliferation of T-lymphocytes, some of the cells begin to express other receptor that serve as checkpoints to the activated T cells (Fig. 14.1). Normally, the immune checkpoints keep activated T cells under control. These molecules such as programmed cell death protein-1 (PD-1), its ligand PD-L1, cytotoxic T lymphocyte antigen-4 (CTLA-4), usually help in the tumor cell proliferation by inactivating the host immune response (Mullard, 2013; Zewde et al., 2018). These checkpoint molecules work by providing a negative response to attenuate the overreactive activity of the T cells that can damage the normal cells. A cancer cell can utilize this immune checkpoint system to stop T cell action and allows the development and of tumor. For example, the interaction of the PD-1 with PDL-1 or PDL-2, would inactivate the function of T cells and thereby inhibiting its signaling (Topalian, Taube, Anders, & Pardoll, 2016). Targeting these checkpoint molecules to inactive them with the help of immune checkpoints inhibitors would necessarily reactivate the immune response against cancer cells. However, some tumor escape the T cell destruction by overexpressing the inhibitory checkpoint Ligands. Monoclonal antibodies (MAbs) can be used to block the Inhibitory checkpoints (Lee et al., 2016). In cancer immunogenomic studies, various methods like single-cell RNA-sequencing (scRNA-seq), TCR sequencing, mass cytometry, and others are used to evaluate the tumor cell microenvironment and characterize the immune cells (Center, 2020).

## 14.3 Cancer genomic biomarkers

Cancer biomarkers are the specifically produced biomolecules by the tumor or other body cells as a result of malignant transformations. Cancer biomarkers act as its diagnostic indicators and hence can be useful in the early detection and screening of cancer. These biomarkers may help in the detection of a subpopulation of patients more likely to be responsive to cancer therapies. Biomarkers can be certain specific genes or their products, specific cells, hormones, enzymes, detected in the blood tissues, urine, or other fluids (Kamel & Al-Amodi, 2017). The first biomarker of this kind was Bence-Jones protein found as a tumor derived light chain antibody of immunoglobulin G (IgG) (Long et al., 2019). After this numerous studies have evaluated other biomarkers like chorionic gonadotropin (hCG) in choriocarcinoma, enzymes like acid phosphate in prostate cancer, catecholamines in neuroblastoma, and pheochromocytoma.

Pharmacogenomic studies aim to improve the therapeutic response of the patients to a particular therapy and minimize the adverse effects. This is achieved by identifying the particular molecular biomarkers that can help in

predicting effective response and toxicity of the anticancer drugs (Rodríguez-Antona & Taron, 2015). The advanced progression in the gene-sequencing techniques has opened the way for developing genetic biomarker-based personalized therapies in cancer. Also, DNA microarray enables the extensive apprehension of transcriptional activities in cancer. Identification of Gene-specific polymorphism could help the rationalization of appropriate treatment for suitable patients as this polymorphism may incline susceptibility to some cancers. The most commonly found "Single Nucleotide Polymorphism" serves as a DNA biomarker-diallelic for most of the applications, producing three possible genotypes (Kamel & Al-Amodi, 2016; Kamel & Al-Amodi, 2017; Zewde et al., 2018). Tumor DNA biomarkers serve as a powerful tool for improving cancer treatment outcomes. Biomarkers can be prognostic-those predicting the progression of the disease and predictive-which detect the treatment response (Simon, Paik, & Hayes, 2009). Genetic alterations in cancer such as gene rearrangement, point mutation, gene amplification, subsequent variation with cell proliferation will be indicated by the expression of biomarkers of these variations. Novel drugs can target these genetic markers and thus can yield a unique anticancer activity.

The first mechanism of evaluation of biomarkers in any biological fluid is overexpression of the gene product for example, Human epididymal secretory protein-4 (HE4) in carcinoma of ovary which can be detected in serum. This biomarker or gene product is also overexpressed in breast, bronchial and endometrial and carcinoma (Galgano, Hampton, & Frierson, 2006). Secondly, evaluation of serum biomarkers which could be the shedding of membrane protein or the secretion of cellular proteins such as HER2-neu elevation in serum during breast cancer or elevation of alpha-fetoprotein (AFP) in circulation in hepatocellular carcinoma (HCC). The third mechanism is the angiogenesis or cell invasion such as increased levels of prostate-specific antigen (PSA) arising due to basement membrane deformation of the prostatic cell. Prostate cancer antigen (PCA3) is now considered as another new biomarker for prostate cancer detection (Kamel & Al-Amodi, 2016). Epigenetic changes, such as DNA methylation status, modification of chromatin, histones, may influence the patterns of gene expression without any modification of DNA sequence. Epigenetic changes are considered as the biomarkers of some cancers for example, in nonsmall-cell lung cancer (NSCLC) methylation signatures of selected genes, such as ALX1 are considered prognostic and correspond to the relapse-free patient survival (Kanwal & Gupta, 2012).

Pharmacokinetic biomarkers could provide tools to evaluate the drug efficacy and adverse reactions for example, in colon cancer- excessive irinotecan metabolite SN-38 can cause neutropenia, and its activity depends on UDP-glucuronosyltransferase 1A1 (UGT1A1). The allele UGT1A1*28 of UGT1A1 is taken as an indication for a low initial dose of Irinotecan to

reduce the risk of neutropenia (Tukey, Strassburg, & Mackenzie, 2002). Similarly, the genotype of CYP2D6 plays a role in tamoxifen treatment outcome in breast cancer (Schroth et al., 2009)

## 14.4 Cancer antigens and neoantigens

Cancer antigens are the biomarkers that are expressed by the tumor cells and are considered critical factors for tumorigenicity. Considerable antigenic differences occur between normal tissue and the tumors and these tumor antigens are currently considered ideal targets of anticancer therapy. Tumor antigens can be used as biomarkers and are often found in the circulation (Even-Desrumeaux et al., 2011). Neoantigens are the new group of somatic mutation derived antigens that are highly cancer cell specific and not found on the normal cells (Schumacher & Schreiber, 2015). Studies have reported the association of high neoantigen load at the tumor site for example, in colorectal or ovarian carcinoma, this causes more infiltration of the T cells and thus enhancing the survival rates (Giannakis et al., 2016; Howitt et al., 2015). Clinically important antigens are those specific to each tumor type, present only on the malignant cells, should define the survival of tumor cells, and those provoking both cell-mediated and humoral immunity (Baio, Kwak, & Weng, 2014). Tumor antigens can be considered of two types: mutation caused antigens called tumor-specific antigens (TSA), for example, melanoma-associated antigen 1 [MAGE-A1], and those which resulting from nonmutated protein over-expression called Tumor-associated antigens (TAA) such as glycoprotein 100 (gp100) and melanoma-associated antigen recognized by T cells [MART-1] (Kiyotani, Chan, & Nakamura, 2018). TSA are only malignant cells expressed antigens and can stimulate a specific antitumor immune response but unlike these antigens, TAA can be also expressed on the normal cells and are also called shared antigens as these are expressed by different tumor cell types. TSA may therefore be candidates for designing more specific personalized therapies. These antigens have improved clinical outcomes in renal carcinoma and melanoma (Su et al., 2003). For the identification of tumor antigens, various methods have been used such as expression cloning, discovering the peptides recognized by CTL and antigen elution from MHC molecule on cancerous cells. Reverse immunology and DNA microarray is currently a common strategy for identifying Tumor antigens (Rezaei & Keshavarz-Fathi, 2018).

Neoantigens are the type of antigens which the immune system recognizes as completely nonself. In nonviral etiological tumors, neoantigens are the derivatives of a variety of genetic mutations such as gene fusion, insertion or deletion, SNP, and other structural variants. In viral tumors such as Merkel cell polyomavirus (MCPyV) associated Merkel cell carcinoma (MCC) or human papillomavirus (HPV) associated cervical and oropharyngeal carcinoma or head-neck carcinomas related to Epstein-Bar virus (EBV)

(Pan et al., 2018), epitopes associated with open reading frames in the viral genome are also coming up as the neoantigen source (Jiang et al., 2019). The significance of neoantigens in cancer immune escape, immunoediting, and immune checkpoint inhibitor sensitivity has been suggested in recent studies (Yi et al., 2018). The recognition of the neoantigens presented on MHC molecule by immune checkpoint inhibitors is indicated as the first step in the elimination of an established tumor. Promising results have been shown by the neoantigen based vaccines and adoptive T cell therapies. Advances in the application of next-generation sequencing (NGS) have allowed an understanding of T cell recognition of neoantigens more profoundly (Jiang et al., 2019).

## 14.5 Cancer immunotherapy

Cancer immunotherapy is mainly directing immune system stimulation to fight cancer. Binding of modified antibodies to the tumor antigen causes recruitment of immune cells against the malignant transformations. Immune checkpoint antibodies are currently the most recommendable therapy in cancer immunotherapy. Other strategies are the cancer vaccines, adoptive T cell CAR-T inhibition therapy which are also becoming clinically validated.

### 14.5.1 Immune checkpoint inhibition

Immune checkpoint inhibition is the major therapy against cancer also sometimes given as first-line therapy (Topalian, Drake, & Pardoll, 2015). The checkpoint inhibitors work by subduing the action of immune checkpoints majorly PD-1 and CTLA-4 which are mainly responsible for T cell induced immune response suppression. Checkpoint inhibitors are coming as the major immune therapy choices for many types of carcinomas such as renal cell carcinoma (RCC), nonsmall cell lung carcinoma (NSCLC). Currently, FDA approves two classes of checkpoint inhibitors for clinical use which include PD-1/PD-L1and CTLA-4 inhibitors (Michot et al., 2016). The first anti-CTLA-4 antibody *Ipilimumab* (Yervoy) was approved in 2011 followed by the other antibodies like *pembrolizumab* (Keytruda) and *nivolumab* (Opdivo) targeting PD-1 and *atezolizumab* (Tecentriq) and *durvalumab* (imfinzi) targeting PD-L1 (Robert, 2020). Recently published data suggests that most of the patients respond minimally to these checkpoint inhibitors and some may even elicit adverse immune reactions to these drugs as well. Again this suggests the significance of identifying specific biomarkers to select the patients who will respond to the immunotherapy with lower risks of adverse immune reactions (Postow, Callahan, & Wolchok, 2015) and design patient-specific personalized immunotherapy based on these biomarkers. Other approaches like a combination of inhibitory CTLA-4 or PD-1 along with targeted approaches or photodynamic therapy have also been tested to improve the

clinical outcomes of current immunotherapies and a combined approach is considered as an effective treatment approach for multiple tumors (He et al., 2016; Wargo, Cooper, & Flaherty, 2014). However, the mechanism of these checkpoint inhibitors is still largely not known. The list of various immune checkpoint inhibitors is given in Table 14.1

#### *14.5.1.1 PD-1/PDL-1 checkpoint inhibition*

PD-1 is a checkpoint receptor related to the family of CD28/B7 present on the T cells and PD-L1 is the ligand of this immunoinhibitory PD-1 present on the tumor cells and also on APC. PD-1 is also expressed on B cells, natural killer T cells (NKT) CD4 + and CD8 + T cells, dendritic cells, bone marrow cells, and lymph nodes (Ishida, Agata, Shibahara, & Honjo, 1992; Keir, Butte, Freeman, & Sharpe, 2008; Natarajan et al., 2015) also on the surface of highly immunosupressive CD4 + Foxp3 + T-regulatory cells (Tregs) (Francisco, Sage, & Sharpe, 2010). The degree of PD-1 expression in B cells is though tightly regulated during their differentiation (Ohaegbulam, Assal, Lazar-Molnar, Yao, & Zang, 2015) and their levels are increased steadily during their development (Thibult et al., 2013). However, the surface stimulation of the mature B cells with Toll-like receptor 9 (TLR9) agonist increases the extent of PD-1 expression and the inhibition of the PD-1 has increased the antigen-specific antibody response of the B cell (Ohaegbulam et al., 2015). High expression of PD-L1 could serve as a biomarker of the tumor and can predict the clinical outcome of immunotherapy but every tumor does not express this antigen and hence its inhibition could not possibly benefit in each case. Some studies have also reported that some negative PD-L1 tumors also respond to these immunotherapies (Aguiar et al., 2016). The binding of PD-1 and PD-L1 deactivates the cytotoxic activity of T cells and leads to neutralization, exhaustion, and interleukin-10 (IL-10) production on tumor cells which. Various proinflammatory cytokines, like tumor necrosis factor-alpha (TNF-$\alpha$), interferon-gamma (IFN-$\gamma$), interleukin-2 (IL-2). PD-L1 on the tumor cells also interacts with other molecules like APC and T cell expressed B7−1 (CD80) and results in negative regulation of T cell activation (Alsaab et al., 2017). Inhibition of PD-1/PD-L1 activity is therefore applied to design antitumor immunotherapy (Xu-Monette, Zhang, Li, & Young, 2017). For example, blockade using MAbs checkpoint inhibitors have shown positive results in many advanced malignancies such as RCC, NSCLC, also advanced melanoma, and other tumors phase-1 studies of anti-PD-1 drugs such as Nivolumab, Pembrolizumab, have shown favorable results with fewer side effects. Further advanced studies of these inhibitors have shown very encouraging results in phase-III trials in advanced melanoma than RCC and NSCLC after which Nivolumab was approved by FDA as first-line approach for advanced melanoma and as second line

**TABLE 14.1 Currently approved immune checkpoint inhibitors (cancer.gov).**

| Immune checkpoint inhibitor | Trade name | Target | Indications |
|---|---|---|---|
| *Nivolumab* | Opdivo | PD-1 | 1. Metastatic melanoma<br>2. Small-cell lung cancer (SCLC)<br>3. Renal cell carcinoma (RCC)<br>4. Nonsmall-cell lung cancer (NSCLC)<br>5. Head and neck squamous cell carcinoma (HNSCC)<br>6. Metastatic colorectal cancer<br>7. Hepatocellular carcinoma (HCC)<br>8. Metastatic urothelial carcinoma |
| *Pembrolizumab* | Keytruda | – | 1. Metastatic melanoma<br>2. HNSCC<br>3. HCC<br>4. RCC<br>5. Metastatic squamous/ Nonsquamous NSCLC<br>6. Gastroesophageal adenocarcinoma<br>7. Metastatic urothelial carcinoma<br>8. Metastatic cervical cancer<br>9. Metastatic Merkel cell carcinoma (MCC) |
| *Cemiplimab* | Libtayo | – | 1. Metastatic squamous cell carcinoma |
| *Durvalumab* | Imfinzi | PD-L1 | 3. Metastatic urothelial carcinoma<br>4. NSCLC |
| *Avelumab* | Bavencio | – | 1. MCC<br>2. RCC<br>3. Metastatic urothelial carcinoma |
| *Atezolizumab* | Tecentriq | – | 1. Metastatic urothelial carcinoma<br>2. NSCLC |
| *Ipilimumab* | Yervoy | Anti-CTLA-4 | 1. Metastatic melanoma<br>2. RCC<br>3. Metastatic colorectal cancer |

for RCC and squamous NSCLC (Alsaab et al., 2017). Similarly, Pembrolizumab was approved as first line drug for *meta*static NSCLC and metastatic melanoma (Postow et al., 2015) and in 2018 Cemiplimab was

approved for metastatic cutaneous squamous cell carcinoma (SCC). Atezolizumab has been also been approved as first line treatment in metastatic urothelial carcinoma, and metastatic NSCLC (Alsaab et al., 2017). Other inhibitors like avelumab and durvalumab have also been approved (Vaddepally, Kharel, Pandey, Garje, & Chandra, 2020).

#### 14.5.1.2 CTLA-4 checkpoint inhibition

cytotoxic T-lymphocyte−associated antigen 4 (CTLA-4), homologous to CD28, is also a T cell expressed inhibitory molecule (Li, Qiu, Lu, Jiang, & Wang, 2018) and is a member of B7/CD28 that also impedes the function of T cell. It is expressed by Tregs constitutively and can be regulated by CD4 + subset of T cells also and exhausted T cells. It is mainly found in the intracellular vesicles expressed only transiently upon activation (Leung, Bradshaw, Cleaveland, & Linsley, 1995). CTLA-4 stops the autoreactive immune T cells at the early stage of their lymph nodal activation, and can also act at the tumor site since the Tregs and CTLA-4 (exhausted) expressing T cells can also assemble in the tumor tissue (Alsaab et al., 2017; Seidel et al., 2018). Immunosuppression is indirectly mediated by the CTLA-4 by indirect diminishing of the signaling through CD28 co-stimulatory receptor. Binding of both these molecules occur to CD80 and CD86 but CTLA-4 has a greater affinity for the receptor (Rudd, Taylor, & Schneider, 2009). Binding of CD28 with CD80 or CD86 causes increased metabolism and release of growth cytokines like interleukin-2 (IL-2) this causes proliferation of the T cells. Unlike CD28, binding of CTLA-4 with CD80 or CD86 does not produce any stimulatory signal and as such, it stops the co-stimulatory signal provided by the CD28 binding and the relative amount of CTLA-4 and CD28 binding will direct the activation of the T cell (Buchbinder & Desai, 2016). CTLA-4 through trans-endocytosis can also eliminates CD80 and CD86 from the cell surface of APCs and decreases their CD28 accessibility, an important mechanism for immunosuppression mediated by Tregs (Wing et al., 2008). The exact process of inhibition of antitumor response induced by CTLA-4 inhibitors is not exactly known. Ipilimumab is the first approved anti-CTLA-4 immune checkpoint antibody for cancer and studies have indicated the beneficial effects of this antibody in NSCLC and advanced melanoma. CTLA-4 inhibitors have also been found beneficial in RCC, SCC. Another CTLA-4 inhibitor Tremelimumab has been tested in combination with tumor ablation for HCC and has been found beneficial (Duffy et al., 2017). Tremelimumab is also shown durable response rates but with low frequency in metastatic melanoma and has been combined with durvalumab for NSCLC (Antonia et al., 2016; Ribas et al., 2013). It has also been found that overexpression of CTLA-4 in nasopharyngeal carcinoma leads to the poor patient's prognosis hence anti-CTLA-4 therapy can be ideal in this case (Huang et al., 2016).

#### 14.5.1.2.1 CAR- T cell therapy

Chimeric antigen receptor (CAR) T cell therapy is a recently developed kind of genetically engineered immunotherapy that necessitate the separation of immunocompetent cells from the patient's blood and then reinfusing them back into the patient after a successful genetically engineered in-vitro functional identification and expansion (June, O'Connor, Kawalekar, Ghassemi, & Milone, 2018). These genetically engineered T cells are altered to express a CAR which is a tumor-specific antigen molecule on their surface comprising of intracellular, transmembrane, and extracellular domains. The extracellular domain is a specific antigen recognition domain fused to the transmembrane and then signaling-intracellular domain. T cell modification may occur through methods like virus-based gene transfer, others like DNA based analogs, CRISPR/Cas9 technology, or direct transfer of transcribed mRNA via electroporation (Miliotou & Papadopoulou, 2018). CAR-T cell therapy is independent of MHC molecular recognition for effective tumor killing and hence can overcome the immune escape mechanism of tumor cells through changes in HLA expression (Figueroa et al., 2015). However, the extracellular surface target on tumor cells is still required in CAR-T. First-generation CAR-T therapy showed discouraging results in clinical trials for various cancers. After that, the second-generation CAR-T cells encoding costimulatory domains emerged as ideal treatment options in cancer therapies which targeted CD19. Studies have reported aplasia and long-term remission of B cell with CAR CD19 treatment (Davila, Kloss, Gunset, & Sadelain, 2013) which can be controlled by i.v immunoglobulin replacement therapy.

Although the therapy has made significant development in the area of cancer immunotherapy, still some chronic and acute toxicities are there associated with it, including most common Cytokine release syndrome (CRS), off-tumor/target effects that is targeting the normal cells, neurological toxicities such as paralysis, seizure, autism movement, and speed disorders (Zhang et al., 2020). Besides CAR-T cells lack infiltration into the tumor space and are currently effective mostly toward the hematological tumors and not much in solid tumors (Srivastava & Riddell, 2018). First-generation CAIX-CAR-T cell infusions given to the patients of renal carcinoma targeting carbonic anhydrase (CAIX) antigen, showed no clinical response due to less expression of targeted antigen on the biliary epithelium and resulted in off-tumor effects like hepatitis and anti- CAR-T immunity (Lamers et al., 2006). However, the use of this T cell therapy for solid malignancies is still under current scientific evaluation and can potentially become a successful treatment option for these tumors in the future. A study recently reported the use of third-generation CAR-T cell product HER-2-CD28/4−1BB in refractory, metastatic colon cancer but the development of acute respiratory distress has been a setback (Morgan et al., 2010). The safety of HER-2, however, is

currently been evaluated in osteosarcoma with no consequential counter effects reported so far (Ahmed et al., 2012; Figueroa et al., 2015). Regardless of various challenges and limitations, CAR-T therapy is under clinical development for treatment in head and neck carcinoma, sarcomas, mesothelioma, and other solid tumors against various tumor antigens such as HER-2, GD2, VEGFR-2, EGFRVIII, CEA, etc. (Figueroa et al., 2015). To improve the effectiveness and to control the related restrictions of CAR-T cell therapy, other drug therapies are combined with it such as radiotherapy or chemotherapy or checkpoint inhibition therapy. Currently, clinical studies of CAR-T cells have main targets like GPC3, CD20, BCMA, CD19, CD22, etc.

#### 14.5.1.2.2 Cancer vaccines

Cancer vaccines mainly lead to the development of active immunity. Although the study of these vaccines is going on for several decades but with slow advances compared to other immune therapies. These vaccines containing cancer cells, cell parts, or pure antigens are meant to increase an immune specific response against existing tumors and do not mainly prevent the disease. However, some preventive vaccines have also been developed which are importantly designed to reduce the risks of cancers caused by viral infections for example, HBV vaccine with the hepatitis B surface antigen is the first successful vaccine against HCC. Similarly, a monovalent HPV-16 L1 virus-like particle (VLP) vaccine and a bivalent HPV-16/18 VLP vaccine is given for the prevention of cervical carcinoma (Itoh, Yamada, Mine, & Noguchi, 2009). Recently FDA has approved a therapeutic prostate cancer vaccine to treat prostate cancer (Even-Desrumeaux et al., 2011). To achieve the maximum benefit from immunotherapies cancer vaccines are combined with the immune checkpoint blockers to presensitize the host immune response to the tumor. Tumor neoantigens are considered as the major target of the cancer vaccines and in recent trials of tumor vaccines, the strong neoantigen-specific T cell response has been reported. Currently, the development personalized vaccines based on neoantigens is emerging rapidly for patients with multiple tumors. The peptide, DNA, RNA, and Dendritic cell (DC) based approaches are currently used (Hu, Ott, & Wu, 2018). Peptide vaccines are used in the treatment of colorectal carcinoma. Also, peptide vaccines in combination with chemotherapy have shown to prolong survival in NSCLC. Tumor-associated antigens (TAA) are being used as targets for developing such vaccines (Wagner, Mullins, & Linnebacher, 2018). Sipuleucel-T (Provenge)-a DC vaccine for advanced prostate cancer have shown better results so far and are under clinical trials currently for testing its safety and efficacy in metastatic melanoma. However, the DC vaccines are facing the same shortcomings as CAR-T therapy (Oshita et al., 2012). Many other vaccine antigens are under evaluation in clinical trials like

CMV-related antigen, EGFR, CEA, MAGE antigens, PAP and PSA, and other personalized neoantigens (Institute, 2020).

## 14.6 Personalized cancer therapies

According to United States National Cancer Institute (NCI), personalized medicine is "*a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease*" (Kash, Mckahan, Mack, & Nanda, 2017; Le Tourneau et al., 2012). Cancer precision medicine initiative was launched in January 2015 in the USA by the Obama administration to improve the treatment of cancer (Harris, 2018). Cancer personalized therapies are designed to achieve a goal of "right drug to the right patient in the appropriate dose." For this individual genomics, pharmacogenomics, proteomics, and immunogenomics need to be integrated. Since there is strong genetic variability in cancer immune response and the safety and efficacy of immunotherapies is influenced by individuals genetic variability, identification of a predictive, prognostic, and diagnostic biomarker is necessary to design the individualized therapies, to identify which patient will benefit or which patient will suffer toxicities. Personalized therapies are important to manage the short and long-term toxicities associated with chemotherapeutic, surgical, or other conventional cancer therapies (Jackson & Chester, 2015). Various factors derive the development of personalized therapy for cancer such as toxicity and limited efficacy of current therapies, increase in the cancer burden, and most importantly advanced developments in molecular diagnostics, and a combination of diagnostics with therapeutics. Previously for the diagnosis of tumor blood sample analysis by nucleic acid methods was used to detect the circulating cancer cells. For the detection of adenocarcinomas such as prostate cancer, RT-PCR has shown to be useful in detecting circulating cancer cells but incase of epithelial carcinomas like NSCLC RT-PCR was not reliable. The development in gene expression profiling have enabled to classify tumors which is important for development of personalized therapies for example, the identification of bladder carcinoma using DNA microarray technique (Jain, 2005). Companion diagnostics (DC) is highly powerful to determine the patient's response to the particular treatment by measuring patients gene levels, specific proteins or mutations for example, the determination of HER2 protein and gene overexpression in gastric, breast, or gastroeasophageal junction adenocarcinomas using Dako Denmark's HERCEPTEST and HER2 FISH PharmDx Kit (Krzyszczyk et al., 2018).

The identification of a specific predictive, prognostic and diagnostic, and pharmacogenomic biomarker as well as a new biomarker that can predict the response (sensitivity or resistance) is important to design a personalized therapy. A known deriver genomic alteration also acts genomic predictive biomarker and specific treatment therapy can be designed to target such

mutations. For example, *EML4-ALK* fusion in NSCLC or *BRAF p. V600E* mutation in melanoma (Dumbrava & Meric-Bernstam, 2018). FDA has approved anaplastic lymphoma kinases (ALK) blockers such as crizotinib and ceritinib in ALK mutation-positive patients after ALK mutations have been found to derive tumor formation in about 5% NSCLC cancers. Similarly, Olaparib which is a poly ADP polymerase inhibitor is used in the treatment of BRCA mutant ovarian cancer (Krzyszczyk et al., 2018).

## 14.7 Conclusion

Immunopharmacogenomics is the consolidation of immunogenomics and pharmacogenomics to identify the genetic status of cancer and immune regulatory molecules and design accurate and specific immunotherapy in selective groups that may benefit. Evaluating the role of patients' germline genetics and comprehensive analysis of highly polymorphic genes controlling the immune response of an individual toward immunotherapy is necessary to formulate accurate specific therapies for cancer. Improvement in the neoantigen based therapies, combination therapies, or engineered adoptive T cell therapies could be effective in managing solid tumors.

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Chapter 15

# Immunopharmaco-genomics: future of clinical medicine

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## 15.1 Introduction

The ongoing progress of high-performance DNA sequencing has resulted in hasty growth in genetics and genomics science, and resolved a broad spectrum of physical challenges. Population and medical genetics, particularly cancer genomics, entire-genome or even whole-exome sequencing, and genome-wide association studies (GWAS) utilizing single-nucleotide polymorphisms (SNPs) have resulted in the identification of disease allied genetic loci and variants (Rabbani, Tekin, & Mahdieh, 2014). Over the last era, the International Cancer Genome Consortium (ICGC) and Cancer Genome Atlas (TCGA) defined genomic triggers of the infections by conducting a systematic genetic analysis of distinct types of human leukemia (Cancer Genome Atlas Research Network, 2008; Hudson et al., 2010).

Immunogenomics and immunopharmacogenomics have achieved a lot of attention due to the advancement in immunotherapies, specific immune

**Immunogenetics: A Molecular and Clinical Overview. DOI: https://doi.org/10.1016/B978-0-323-90053-9.00007-5**
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checkpoint inhibitors, subsequent technological advances to sequence cancer genomes, and distinguishing T cell or B cell receptors (Erdag et al., 2012). Immunogenomics is a wide-ranging scientific principle concentrating on immune-specific gene variation. An extensive list of significant genomic interactions with immune-mediated ailments and IM-ADRs has been promoted by Immunogenomics (Karnes et al., 2017). These usually manifest as correlations with Human leukocyte antigen (HLA) alleles as HLA alleles acquire the amino acid sequence. Hence, the specific binding and signal transduction ability of major histocompatibility complex (MHC) molecules.

Immunogenomics also deals with the interplay of diseases and IM-ADRs with other immune-related genetic variation such as killer immunoglobulin-like receptors (KIRs), T cell receptors (TCRs), and B cell receptors (BCRs), as well as expression levels for immune-related molecules such as cytokines and chemokines (Nakamura, 2015). The association of diseases and IM-ADRs with many other immune-related genetic variants like the KIRs, TCRs, and BCRs and the extent of expression for immune-related molecules like cytokines and chemokines are discussed by Immunogenomics (Nakamura, 2015).

Immunopharmacogenomics encompasses immunogenomics and pharmacogenomics approaches. The impact of genomic and other "omic" differences on drug response and ADRs are premised on pharmacogenomics. Immunopharmacogenomics emphasized the implications of immune-specific "omics" differentiation on drug disposition and next-generation immunotherapy design. Immunopharmacogenomics can anticipate and deter IM-ADRs, such as possibly fatal severe skin adverse reactions (SCARs). Immunopharmacogenomics can promote the selection and tracking of autoimmune and cancer immunotherapy, monitor and avoid antigenic food reactions, and better understand the autoimmune disease and transplantation rejection, which may result in new therapies (Nakamura, 2015).

## 15.2 Adverse drug reactions

Adverse drug reactions (ADRs) are a noteworthy reason for higher healthcare costs and induce or refer to at least 5%−7% of hospitalizations and approximately 3% of all mortality (Odar-Cederlöf et al., 2008; von Euler, Eliasson, Ohlén, & Bergman, 2006; Wester, Jönsson, Spigset, Druid, & Hägg, 2008). ADRs are assumed to be the 4−6th major cause of death, to be the consequence of 3%−6% of hospitalizations, and to cost the United States up to $136 billion annually (Bond & Raehl, 2006). ADRs are induced by predictable effects based on pharmacological activity (type A) or off-target frequently immune-mediated effects independent of pharmacological activity (type B). Approximately 20% of all ADRs are Type B or IM-ADRs, but they are significantly more expensive than pharmacologically predictable ADRs (Pavlos et al., 2015). IM-ADRs were categorized as primary immune cell involvement, namely B cell mediated (antibody-mediated, Gell-Coombs type I-III) and T cell mediated (delayed hypersensitivity, Gell-Coombs type IV)

reactions. Type A ADRs have generally been thought predictable and type B ADRs unpredictable, but the latest evidence has paved the way to predict and prevent IM-ADRs (Karnes et al., 2019).

Practically all medications are hazardous in a subgroup of patients, even though used on the certified tag and a substantial proportion is mediated by genetic predisposition (Carr & Pirmohamed, 2018). In certain circumstances, dose-dependent ADRs are primarily initiated by mutations in the genes associated with drug metabolism or drug target. An illustration of this is dose-dependent bone marrow suppression, which arises in patients with flawed thiopurin detoxification associated with genetic variants of the main enzyme thiopurin methyl transferase (Liu et al., 2015).

Other forms of severe ADRs tend to be fewer dose-dependent. These ADRs, called idiosyncratic, or type B reactions, may influence multiple organ tissues or induce widespread hypersensitivity reactions (HSR) (Daly et al., 2009). The origin is typically elusive in this group of ADRs, and no apparent possible genes exist. The microarray-based genotyping of several genetic variants and next-generation sequencing (NGS), helped in carrying out GWAS of such reactions and other forms of association analyses. According to the statistical requirements for multitest correction and validation of results, significant amounts of patients are needed in both methods (Böhm, Proksch, Schwarz, & Cascorbi, 2018).

## 15.2.1 Immune-mediated adverse drug reactions

### *15.2.1.1 Classification of immune-mediated adverse drug reactions*

Drug reactions that take place within one hour of dosing are alluded to as immediate ADRs. This can include both dose-independent IgE-mediated mast cell reactions characterized as anaphylaxis marked by cardiovascular failure, airway constriction, angioedema, and urticaria rash, and dose-related non-IgE-mediated mast cell reactions. Non-IgE-mediated reactions have symptoms analogous to IgE-mediated reactions but are variable in their occurrence and reproducibility. The functions of non-IgE-mediated mast cell stimulation is allied to drugs namely opioids, fluoroquinolones and neuromuscular blockers. These are secondary to stimulation of a particular G-protein coupled receptor (MRGPRX2) and occur only in mast cells and dorsal root ganglia (McNeil et al., 2015).

Mild delayed cutaneous reactions (urticarial and maculopapular exanthems) are the utmost common indices of allergic drug reactions (Khan, Knowles, & Shear, 2019). The most severe IM-ADRs are known as SCARs, which usually comprise multiple organs and represent three major T cell-mediated syndromes (Pirmohamed, Aithal, Behr, Daly, & Roden, 2011). These SCARs comprise drug reactions with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis (AGEP) and

Stevens-Johnson syndrome (SJS)/toxic epidermal necrosis (TEN) (Lehloenya & Kgokolo, 2014; Pavlos, Mallal, Ostrov, Pompeu, & Phillips, 2014). More than 100 preparations were correlated with SCARs, but allopurinol, aromatic anticonvulsants, antimicrobials, antiretrovirals, and oxicam nonsteroidal anti-inflammatories are the most prominent objectionable drugs for SCARs (Roujeau & Stern, 1994).

#### 15.2.1.2 Stevens-Johnson syndrome/toxic epidermal necrosis

SJS/TEN is the extreme IM-ADR having death rate of about 50%. SJS/TEN frequency is elevated in patients having unique class I HLA alleles and may also be enhanced with preexisting comorbidities and drug allergies such as HIV, cancer, and systemic lupus erythematosus (SLE) (Zimmermann et al., 2017). Mucocutaneous presence and the maximum thickness of epidermal necrosis are common pathological characteristics of SJS/TEN (Su, Hung, Fan, Dao, & Chung, 2016; Yacoub et al., 2016). SJS and TEN identify the continuum of diseases mainly identified by the total body surface area (TBSA) whereby SJS is associated with less than 10% TBSA, 10%−30% SJS/TEN and more than 30% TEN. The intensity of SJS/TEN can be determined from clinical features using the validated test SCORTEN (Bastuji-Garin et al., 2000; White et al., 2018). Systemic corticosteroids appear to be the recommended treatment, but there is a possibility for more chemotherapeutic agents that are antagonizing the impact of granulysin or TCR participation. Evidence-based approaches are required to drive clinical intervention in SJS/TEN beyond effective intensive care (White et al., 2018). The very first accessible randomized clinical study of 96 persons diagnosed plausible or definitive SJS/TREN had a promising treatment-contrary TNF-alpha antagonist etanercept with corticosteroids (Wang et al., 2018). The study indicates a possibility for TNF-alpha antagonists in diagnosing SJS/TEN (González-Herrada et al., 2017; Kirchhof, Miliszewski, Sikora, Papp, & Dutz, 2014). It also illustrates the need for a retrospective chart with other medicines, such as cyclosporine, that have potency in smaller observational trials.

#### 15.2.1.3 Drug reaction with eosinophilia and systemic symptoms

In contrary to SJS/TEN, there is no skin detachment and mucocutaneous interference in DRESS. The DRESS death rate is typically 10%, and subsequent complications may imitate viral illness, like lymphadenopathy, pneumonia, encephalitis, myocarditis, and nephritis (Chen, Chiu, & Chu, 2010). It is linked with hematological anomalies (eosinophilia, atypical lymphocytosis), internal organ association, and an unspecific rash of variable intensity (Shiohara, Kano, Takahashi, Ishida, & Mizukawa, 2012). DRESS occurs typically 2−8 weeks after introducing treatments, and long-term inflammatory

sequelae are severe, like type 1 diabetes, thyroiditis, and SLE (Minegaki et al., 2013).

#### 15.2.1.4 Other immune-mediated adverse drug reactions

Other T cell arbitrated deferred IM-ADRs involve abacavir hypersensitivity syndrome (HSN). HSN is accompanied by nausea, malaise, respiratory illnesses and simplistic skin infection through the initial few weeks of abacavir therapy in about 5%−8% patients (Ostapowicz et al., 2002). IMADRs may also display as organ-specific appearances like pancreatitis and drug-induced liver injury (DILI). DILI is uncommon; however, life-threatening hepatic malfunction, liable for 7%−15% of acute liver failure cases in Europe and the United States (Russo, Galanko, Shrestha, Fried, & Watkins, 2004). Agranulocytosis entails an extreme, life-threatening decline in white blood cell count and can be caused by HLA-dependent antithyroid drugs (Chen et al., 2015). Erythema multiform majus (EMM) is a severe bullous disease with elevated lesions affecting less than 10% of TBSA (Sokumbi & Wetter, 2012). This condition may resemble SJS/TEN but is distinct in that EMM is correlated with the causes of infections, like herpes simplex virus (HSV) and *Mycoplasma pneumonia*, instead of drug exposure. A significant portion of cases diagnosed as SJS/TEN has observable *M. pneumonia* antibodies (Finkelstein et al., 2011; Olson, Abbott, Lin, Prok, & Dominguez, 2017).

Advancing IM-ADRs showed combat metastatic melanoma with recently approved immune control point inhibitors nivolumab and pembrolizumab (Michot et al., 2016). While moderate immune reactions noted in up to 50% of cases (probably after 3−6 months of treatment), a rising number of severe IM-ADRs, like fatal myositis, were confirmed with these inhibitors (Johnson et al., 2016). The magnitude of IM-ADRs caused by checkpoint inhibitors correlates with enhanced tumor response and patient survival, thus emphasizing the need to control these toxicities to optimize drug gain (Sanlorenzo et al., 2015).

## 15.3 Genomics approaches to illuminate the complexity of drug response

Immune-related genetic variants were implicated as risk factors in comparatively limited huge genome-wide experiments carried out already on extreme ADRs. These factors are drug-specific and differ among diverse ethnic groups (Esmerian et al., 2011). Pharmacogenomical research emphasized genetic markers, which are highly predictive of drug effects. Genetic variants of CYP2C9 and VKORC1 are said to determine warfarin dosage or reaction (Scott, Edelmann, Kornreich, & Desnick, 2008; Takeuchi et al., 2009). The HLA B*5701 variant is concerned with hypersensitivity to abacavir (nucleoside reverse transcriptase inhibitor) (Mallal et al., 2002; Rauch et al., 2006).

Flucloxacillin-induced liver damage is associated with HLA B*5701, HLA DRB1 * 0107 DRB1 * 0103, and TNF a238G/A (Daly et al., 2009). The IL28A/IL28B locus SNPs links with antihepatitis C therapeutic effect to interferons and ribavirin (Ahlenstiel, Booth, & George, 2010; Tanaka et al., 2009). Such findings indicate pharmacogenomics' ability for evaluating hereditary risk aspects. The recognition of vulnerable SNPs or loci, however, do not eventually lead to pathways (Giacomini et al., 2017; Manolio, 2010). Table 15.2 shows the effects of Single SNPs on cancer treatment with mAbs.

As per the GWAS catalogue managed by the National Human Genome Research Institute, a maximum of 48 GWAS pharmacogenomics of different chemotherapeutic drugs were quantified in concentration, therapeutic efficacy, detrimental impact or cytotoxicity (Hindorff et al., 2009). In contrary to these effective cases, the findings of several pharmacogenomics analysis, particularly belonging to antidepressants and antipsychotic agents, includes many loci, several of which indicates more complicated heritable susceptibility and complex phenotypes influenced by several discrete biological practices (van Westrhenen, Aitchison, Ingelman-Sundberg, & Jukić, 2020). Furthermore, various genetic loci are situated in intergenic regions having no apparent applicant genes are closely involved. These are similar to the earlier findings. About 40% of the trait-linked PNS are present in intergenic zones, 40% are intronic and just 12% are present in or around protein-coding gene regions (Manolio, 2010).

Consequently, understanding pharmacogenomic outcomes is seldom an easy task. Interdisciplinary genomics strategies using functional genomics and network biology were subsequently implemented to classify genes, pathways and gene networks, comprehending GWAS observations for different ailments and characters (van Westrhenen et al., 2020). These approaches often leverage a significant amount of useful knowledge obtained in the GWAS. Usually, without a prior theory to emphasis, the study on a segment of the genomic regions, the extensive testing modification used in the genome-wide statistical analysis enables only the most decisive consequences to be observed. It penalizes the weakest correlations that are clinically significant.

Screening for HLA has arisen as a therapeutic technique for IM-ADRs and immune-mediated diseases. With over 8000 class over 3000 class IIβ-chain forms, HLA alleles and I are highly polymorphic, with peptide-binding grooves mapping the most heterogeneity (Robinson et al., 2013). The HLA system mediates immune response, and peptide association and antigen appearance to T lymphocytes assessed by the amino acid sequence of each HLA molecule (Nepom & Erlich, 1991). To prevent allograft rejection, heterogeneity in HLA is critical and plays a vital role in assessing infection susceptibility and autoimmune disease. A variety of HLA alleles has correlated with immune-mediated phenotypes, and more phenotype correlations were

found in the GWAS catalogue than in any genomic area (MacArthur et al., 2017).

Even when small case numbers are available, such HLA correlations with immunologic condition and IM-ADRs are strong and sustained (Daly et al., 2009). On all nucleated cells, Class I HLA molecules (HLA-A, -B, and -C) are extant and activates cytotoxic lymphocytes $CD8^+$. Only antigen-presenting cells, such as B cells, dendritic cells, and macrophages, and activated $CD4^+$ helper T lymphocytes are present in class II HLA molecules (HLA-DP, -D.Q., and -D.R.). Since four-digit HLA alleles distinguish the amino acid sequence of class I and II MHC molecules, robust associations with immunological disease and IM-ADRs are frequent, even though small case numbers are available (Minegaki et al., 2013; Shiohara et al., 2012). HLA allele associations present valuable insights regarding immunopathology, besides having the potential for clinical prediction and diagnosis of IM-ADRs.

Abacavir HSN, a significant correlation with HLA-B*57:01 is the oldest examples of HLA-associated IM-ADRs (Mallal et al., 2002). For the HSN reaction to occur, the appearance of one HLA-B*57:01 allele is essential, though it is not able to assess the HSN. Consequently, HLA-B*15:02 are closely correlated with carbamazepine-induced SJS (Chung et al., 2014). Despite small case numbers, these findings demonstrate the widely observed strength of HLA associations with IM-ADRs, for which as few as 6 cases would have been adequate to characterize this correlation. Likewise, in a GWAS that included only 51 patients, HLA-B*57:01 were essential predictor of DILI attributable to flucloxacillin (Daly et al., 2009). A range of IM-ADRs, namely SJS/TEN, maculopapular erythema, and DRESS, are correlated with carbamazepine. The interaction of HLA-B*15:02 is relevant to carbamazepine-induced SJS/TEN, although there were relations of other HLA alleles with B75 serotypes, most notably HLA-B*15:21, but also HLA-B*15:08 and HLA-B*15:11 (Phillips, Chung, Mockenhaupt, Roujeau, & Mallal, 2011). The allele's prevalence is relatively high in Asian populations, but uncommon in European populations (Genin et al., 2014). The massive influence of race/ethnicity on HLA associations and the particular obstacles that nationality poses while implementing HLA alleles as determinants of IM-ADRs were demonstrated in this case (Phillips et al., 2018). Other IM-ADRs aligned with HLA alleles comprise dapsone hypersensitivity and HLA-B*13:01, amoxicillin-clavulanate-induced DILI and numerous HLA alleles (risk and defensive) and antithyroid-induced agranulocytosis and the HLA-B*38:02-HLA-DRB1*08:03 haplotype (Carroll, Smith, & Shaak, 2017; Chen et al., 2015, 2018; Cheung et al., 2016; He et al., 2017; Lucena et al., 2011). Table 15.1 illustrates HLA allele associations with IM-ADRs and their characteristics (Table 15.2).

**TABLE 15.1** Human leukocyte antigens associations for immune-mediated adverse drug reactions (Karnes et al., 2019).

| Drug (usage) | IM-ADR | HLA risk alleles | Prevalence of ADR | Carriage rate (%) of HLA allelea | OR | PPV | NPV | NNT | References |
|---|---|---|---|---|---|---|---|---|---|
| Abacavir | HSR/DIHS | B*57:01 | 8% of population 3% true, 2%–7% false positive HSR | 1.4–11.2 (European) <1 (Sub-Saharan African) 0–2 (Southeast Asian) 0–2 (African American) | 960 | 55% | 100% | 153 | Mallal et al. (2002) |
| Carbamazepine | SJS/TEN | B*15:02 | < 1–6/1,000 | <1.2 (European) ≤ 34 (Southeast Asian) | >1,000 | 100% (Southeast Asian) | 2%–8% | 1,000 | Chang et al. (2001), Zhang et al. (2003), Kulkantrakorn et al. (2012) |
| | | B*15:11 | – | Korean, Japanese | – | – | – | – | Kaniwa et al. (2010), Kim et al. (2011) |
| | | B*15:18, B*59:01 and C*07:04 | – | Japanese | – | – | – | – | Ikeda et al. (2015) |
| | | B*15:21 | – | Japanese, northern European, Korean | – | – | – | – | Jaruthamsophon et al., 2017 |
| | DRESS/DIHS | A*31:01 | 0.05% | ≤ 6 (European) <1 (Sub-Saharan African) | 57.6 | 99.9% | 0.89% | 3,334 | Ozeki et al. (2011), Niihara et al. (2012) |
| | HSR/DIHS/DRESS | 8.1 AH (HLA A*01:01, Cw*07:01, B*08:01, DRB1*03:01, DQA1*05:01, DQB1*02:01) | – | Caucasians | – | – | – | – | Alfirevic et al. (2006) |
| | | A*31:01 | – | Europeans | | 0.89% | 99.98% | | Genin et al. (2014) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A*31:01 | – | Chinese | – | 0.59% | 99.97% | – | Genin et al. (2014) |
| | | A*31:01 | – | Northern Europeans, Japanese, and Korean | – | – | – | – | Ozeki et al. (2011), Niihara et al. (2012) |
| | | A*11 and B*51 (weak) | – | Japanese | – | – | – | – | Niihara et al. (2012) |
| | MPE | A*31:01 | – | – | – | 34.9% | 96.7% | – | Yip et at. (2017) |
| | Any ADR | A*31:01[26] | – | – | – | – | – | – | Yip et al. (2015) |
| Allopurinol | SJS/TEN/DIHS/DRESS/ MPE | B*58:01 | 1–4/1000 | 0–6.7 (European) 5.5–14 (Sub-Saharan African) 2–22 (Southeast Asian) >5.3 (African American) | 580 | 100% (Han Chinese) | 3% (Han Chinese) | 250 | Génin et al. (2011), Somkrua et al. (2011), Sukasem et al. (2016) |
| Oxcarbazepine | SJS/TEN | B*15:02 and B*15:18 | – | <1.2 (European) ≤34 (Southeast Asian) | 27.9 | 99.9% (Han Chinese) | 0.73% (Han Chinese) | Unknown | Hung et al. (2010), Chen et al. (2017) |
| Lamotrigine | SJS/TEN | B*15:02 (positive) | – | Han Chinese | – | – | – | – | Hung et al. (2010) |
| | | B*15:02 (no association) | – | Han Chinese | – | – | – | – | Shi et al. (2012) |
| Phenytoin | SJS/TEN | B*15:02(weak), Cw*08:01 and DRB1*16:02 | – | Han Chinese | – | – | – | – | Chung et al. (2014) |
| | DRESS/MPE | B*13:01 (weak), B*5101 (weak) | – | Han Chinese | – | – | – | – | Chung et al. (2014) |
| Nevirapine | DRESS | C*04:01 | – | ≤33 (European) ≤42 (Sub-Saharan African) ≤39 (Southeast Asia) | 3–7 | 95%–97% | 5%–27% | – | Carr et al. (2017) |
| | HSR/DIHS/DRESS | DRB1*01:01 & DRB1*01:02 (hepatitis and low $CD4^+$) | – | Australian, European and South African | – | 18% | 96% | – | Yuan et al. (2011) |

(*Continued*)

**TABLE 15.1** (Continued)

| Drug (usage) | IM-ADR | HLA risk alleles | Prevalence of ADR | Carriage rate (%) of HLA allelea | OR | PPV | NPV | NNT | References |
|---|---|---|---|---|---|---|---|---|---|
| | | Cw*8 or Cw*8⁻B*14 haplotype | – | Italian and Japanese | – | – | – | – | Gatanaga et al. (2007) |
| | | Cw*4 | – | Blacks, Asians, Whites, Han Chinese | – | – | – | – | Yuan et al. (2011) |
| | | B*35, B*35:01, B*35:05 | – | Asian | – | 16% | 97% | – | Yuan et al. (2011), Karnes et al. (2014), Chantarangsu et al. (2009) |
| | Delayed rash | DRB1*01 | – | French | – | – | – | – | Vitezica et al. (2008) |
| | | Cw*04 | – | African, Asian, European, and Thai | – | – | – | – | Likanonsakul et al. (2009) |
| | | B*35:05 | – | Thai | – | – | – | – | Chantarangsu et al. (2009) |
| Dapsone | DRESS/DIHS/HSR | B*13:01 | 1–4/100 | ≤3.8 (European) 2–52 (Southeast Asian) | 20 | 99.8% | 7.8% | 84 | Zhang et al. (2013) |
| Efavirenz | Delayed rash | DRB1*01[49] | – | French | – | – | – | – | Vitezica et al. (2008) |
| Sulfamethoxazole | SJS/TEN | B*38 | – | European | – | – | – | – | Lonjou et al. (2008) |
| Amoxicillin-clavulanate | DILI | DRB1*15:01, A*02:01, DQB1*06:02, and rs3135388, a tag SNP of DRB1*15:01-DQB1*06:02, DRB1*07 and HLA-A1 (protective) | – | European | – | – | – | – | O'Donohue et al. (2000), Lucena et al. (2011) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lumiracoxib | DILI | DRB1*15:01-DQB1*06:02-DRB5*01:01-DQA1*01:02 haplotype | – | International, multicenter | – | – | – | – | Singer et al., 2010 |
| Ximelagatran | DILI | DRB1*07 and DQA1*02 | – | Swedish | – | – | – | – | Kindmark et al. (2008) |
| Diclofenac | DILI | HLA-A11 | – | European | – | – | – | – | Berson et al. (1994) |
| Flucloxacilin | DILI | B*57:01 DRB1*07:01-DQB1*03:01 | 8.5/100,000 | 1.4–11.2 (European) < 1 (Sub-Saharan African) 0–2 (Southeast Asian) 0–2 (African American) | 81 | 99.99% | 0.12% | 13,819 | Daly et al. (2009) |
| Lapatinib | DILI | DRB1*07:01-DQA2*02:01-DQB1*02:02/02:02 | – | International, multicenter | – | – | – | – | Spraggs et al. (2011) |
| Methimazole/ Carbimazole/ | Agranulocytosis | HLA-B*38:02 (*5 SNPs) HLA-B*27:05(3/5 SNPs) HLA-DRB1*08:03 | unknown | <1 (European) < 1 (Sub-Saharan African) ≤ 20 (Southeast Asian) | 266–753 | 7%–30% | 99.9% | 211–238 | Chen et al. (2015), Cheung et al. (2016), He et al. (2017) |
| Clozapine | Agranulocytosis/ Neutropenia | HLA-B*59:01 HLA-DQB1 (126Q) HLA-DQB1*05:02; HLA-B (158 T) (HLA-B*39:01, HLA-B*39:06, HLA-B*38:01)HLA-DQB1 | – | Japanese and European | – | 35.1% | – | – | Legge et al. (2019) |
| Azathioprine | Pancreatitis | HLA-DQA1*02:01; HLA-DRB1*07:01 | – | European | – | 9% | – | – | Heap et al. (2014) |
| Statins | Myopathy | HLA-DRB1*11:01 | – | European and African | – | – | – | – | Mammen et al. (2012) |
| Asparaginase | Anaphylaxis | DRB1*07:01 | – | European | – | – | – | – | Fernandez et al. (2014) |

*DIHS*, drug-induced hypersensitivity syndrome; *DILI*, drug-induced liver injury; *DRESS*, drug reaction with eosinophilia and systemic symptoms; *HLA*, human leukocyte antigen; *HSR*, hypersensitivity reaction; *IM-ADR*, immune-mediated adverse drug reaction; *MPE*, maculopapular exanthema; *NPV*, negative predictive value; *PPV*, positive predictive value; *SJS*, Stevens—Johnson syndrome; *TEN*, toxic epidermal necrolysis.

**TABLE 15.2** Single nucleotide polymorphisms influence on cancer treatment with mAbs (Shek et al., 2019).

| Gene | Single nucleotide polymorphisms | Drugs tested | Mechanism of action | Cancer type | Outcome |
|---|---|---|---|---|---|
| VEGF | rs699947 (AA) | Bevacizumab | Decrease blood vessel proliferation by binding to VEGF (prevent interaction of VEGF with its receptors Flt-1, KDR) | Metastatic EGFR-2 negative breast cancer | Better response from therapy and overall survival |
| VEGF | rs833061 (TT) | Bevacizumab | Decrease blood vessel proliferation by binding to VEGF (prevent interaction of VEGF with its receptors Flt-1, KDR) | Metastatic colorectal cancer | Decreased progression-free survival |
| KRAS | rs121913529 (KRAS G12A/V) | Bevacizumab | Decrease blood vessel proliferation by binding to VEGF (prevent interaction of VEGF with its receptors Flt-1, KDR) | Metastatic colorectal cancer | Decreased progression-free survival and overall survival |
| BRAF | rs113488022 (BRAFV600E) | Panitumumab and Cetuximab | EGFR binding antibody and Inhibit cell growth, induct apoptosis, reduce production of VEGF, by binding to epidermal growth factor receptors | Colorectal cancer | Initial metastasis of lymph node, decreased response |
| PD-L1 | rs4143815 (CC) | Nivolumab | Binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response | Non-small cell lung cancer | Higher progression-free survival |
| CTLA-4 | rs4553808 (G) rs11571317 (T) rs231775 (A) | Ipilimumab | CTLA-4 (Cytotoxic T-lymphocyte antigen-4) blocking antibody | Metastatic melanoma | Better response correlated with immune-related adverse reactions in grades 3–4 |

| | | | | | |
|---|---|---|---|---|---|
| PD-L1 | rs2282055 (GG) | Nivolumab | Binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response | Non-small cell lung cancer | Better progression-free survival |
| FcgR3A | rs396991 (G) | Trastuzumab, Cetuximab, and Rituximab | HER2 (c-erb82) binding antibody, Inhibit cell growth, induct apoptosis, reduce production of VEGF, by binding to epidermal growth factor receptors and Cell lysis, by binding to CD20 antigen on B lymphocytes | HER-2 positive breast cancer Colorectal cancer, B cell lymphoma | Higher response rate, increase response rate and progression-free survival |
| CTLA-4 | rs733618 (G) | Ipilimumab and Tremelimumab | CTLA-4 (Cytotoxic T-lymphocyte antigen-4) blocking antibody | Metastatic melanoma | Higher response rate |
| CTLA-4 | rs4553808 (G) | Ipilimumab | CTLA-4 (Cytotoxic T-lymphocyte antigen-4) blocking antibody | Metastatic melanoma | Higher risk of adverse effects associated with endocrine immunity |
| CTLA-4 | rs733618 (G) rs3087243 (G) | Ipilimumab | CTLA-4 (Cytotoxic T-lymphocyte antigen-4) blocking antibody | Metastatic melanoma | 3−4 years of better long-term survival relative to heterozygous survival. |
| PIK3CA | rs17849079 (T) rs7640662 (C) | Cetuximab, Panitumumab | Inhibit cell growth, induct apoptosis, reduce production of VEGF, by binding to epidermal growth factor receptors and EGFR binding antibody | Metastatic colorectal cancer | Lower objective response rate, lower overall survival and progression-free survival |

## 15.4 Challenges for genetic association studies of IM-ADRs

For the study of IM-ADR risk factors and evidence-based treatment of IM-ADRs, the prevalence of SCARs poses significant challenges. The incidence of SCARs makes it extremely difficult for observational studies and emphasizes the need for preclinical systems to guide criteria for research, implementation, and evidence-based treatment (White et al., 2018). Diagnosis and management keep relying extensively on case reports interpretations and causality judgments assisted by hematology and laboratory findings, despite significant advances in the comprehension of mechanisms for SCARs (Peter et al., 2017). Although huge spectrum of clinical presentations for IM-ADRs is available, but there is no universal system for grouping reactions into phenotypes. More study provides an opportunity to enhance phenotype classifications of IM-ADRs by using biological data and emerging experiments to retaxonomize such reactions. Induced pluripotent stem cells (IPSCs) originating from patients were suggested as a means of evaluating patient-specific drug hypersensitivity. This technique will be similar to the basophil activation test used to recognize sensitized patients with several medications, including antibiotics, anesthetics and chemotherapy, for IgE-mediated ADRs (González-de-Olano et al., 2016). Such advances will fix issues associated with the phenotyping of IM-ADRs.

Evolving data indicates that HLA variation and other genomic variation dynamics play a vital role in IM-ADR growth. This is indeed an obstacle for research on IM-ADR and an avenue to recognize immunopathogenesis and explore biomarkers that are not HLA. For instances, HLA-B*15:02 is linked with phenytoin-linked SCARs but is also enhanced by loss of function alleles by CYP2C9 (Caudle et al., 2014; Chung et al., 2014). Such alleles decrease the function of the drug-metabolizing enzyme CYP2C9 that reacts explicitly to the metabolism of phenytoin. Comparably, nevirapine-induced SCARs were correlated with both CYP2B6 weak metabolizer genotypes and multiple HLA alleles (Yuan et al., 2011). These studies highlight the impact of drug clearance and pharmacokinetics on the production of IM-ADRs. Besides, the association of HLA-C*04:01 with variability in the endoplasmic reticulum aminopeptidase two gene (ERAP2) is affected by SJS/TEN secondary to nevirapine (Carr et al., 2017). Given these findings, it would be important to consider non-HLA heterogeneity in future investigations of genomic influences on IM-ADRs.

Also, the HLA region's highly polymorphic nature poses technical problems in HLA typing, and the direct sequencing of HLA loci with four-digit resolutions requires considerable effort and competence (Fan et al., 2017). Subsequently, due to the accessibility of GWAS data and the large cohorts which can be examined without further cost, HLA alleles are often ascribed from SNP-level data. Though its accuracy rate is high (up to 98%), this data's decreased reliability in class II alleles and varied race/ethnic groups

makes it unlikely to be clinically implemented (Karnes et al., 2017). The reduced reliability in non-Caucasian populations illustrates the restricted availability of suitable HLA allele reference panels and diverse populations' need to acquire immunogenomic data.

## 15.5 Approaches to determine IM-ADR mechanisms

Critical insights into immunopathogenesis were offered by HLA associations with IM-ADRs and enabled frameworks for T cell mediated IM-ADRs. In particular, carriage of an HLA is essential but not adequate to trigger an IM-ADR. The low PPVs indicate that there are alternate underlying causes that can be recognized. It is crucial to specify such approaches to explain IM-ADR mechanisms and drive pharmacogenomics screening to prevent IM-ADRs. In proving the extent of HLA-B*15:02-carbamazepine and HLA-B*57:01-abacavir drug binding, surface plasmon resonance experiments using site-directed mutagenesis were productive (Wei, Chung, Huang, Chen, & Hung, 2012; Yang et al., 2007). In certain instances, the combination of multiple HLA alleles will provide insights into how essential amino acid residues in HLA binding defects (Pavlos et al., 2017). Lately, *in silico* strategies were used to model drug binding affinities and simulate and anticipate new therapeutic agents' immunogenicity. Specific evolving initiatives involve TCR and BCR sequencing, HLA expression research, and experiments exploring the microbiome's effect in SCAR immunopathogenesis (White et al., 2018).

TCR sequencing of the next generation has developed as an adequate criterion for measuring immunological pathways and biomarkers for drug-induced SCARs (Robins et al., 2013). Clonotypic analysis can be used to quantify disease-associated T cells, to test expression profiles of pathogenic T cells, and to assess associations between TCR clonotypes and clinical manifestations (Hunder et al., 2008). While clonotypic analysis is a possible technique for early treatment of immunological processes, such methods could be problematic due to inadequate knowledge of such strategies' accuracy and the unusual occurrence of antigen-driven T cells in peripheral blood after and after acute drug reactions.

## 15.6 Role of immunopharmacogenomics in preventing IM-ADRs

Immunopharmacogenomic biomarker clinical applications provide a preconceived removal of possibly allergic patients, revaluation of patients initially known to be allergic, and recognition of patients with possible benefit from desensitization practices (Muraro et al., 2017). To treat already unknown, immune-mediated diseases, HLA alleles have appeared as highly beneficial immunopharmacogenomic biomarkers. Limited clinical efficacy of in vivo

biomarkers like skin monitoring which is unavailable for most drug allergens and for which results may have uncertain diagnostic value, may be substituted by alleles predicting drug IM-ADRs (Empedrad, Darter, Earl, & Gruchalla, 2003). HLA alleles are genotyped to drive drug therapy and avoid IM-ADRs, leading medical technology to existence. Notable outcomes of an SJS/TEN interrogator-driven events, however, illustrated the need to expand results associated with the implementation of immunopharmacogenomic testing across various populations and correlative medicines (White et al., 2018).

Abacavir and HLA-B*57:01 are hallmark examples of genetic testing to avoid HLA-associated drug reactions. Discovered in 2002 the HLA-B*57:01 biomarkers is suggested for the prevention of abacavir HSN in HLA-B*57:01 allele carriers (Churchill et al., 2016; Mallal et al., 2008; Martin et al., 2012). The efficacy of pharmacogenomic testing for abacavir demonstrates the effectiveness of appropriate HLA genotyping and IM-ADR phenotyping, and high NPV and PPV. HLA-B*57:01 avoiding Abacavir-induced HSN presents a basis through which retrospective immunopharmacogenomic screening may aim to decrease deadly immunological reactions.

A key factor for the efficacy of abacavir-containing HLA screening is that it only happens in people with the allele. The 100% (negative predictive value) NPV, together with the 55% (Positive Predictive Value) PPV, implies that perhaps the allele is indispensable however, inadequate to stimulate the IM-ADR. High NPV is of greatest concern to the physician since this eliminates the probability of a reaction based on the lack of an HLA allele. A low NPV (less than 100%) restricts the biomarker's clinical utility. Furthermore, 55% of HLA-B*57:01 patients subjected to abacavir will experience a hypersensitivity reaction (HSR), offering this experiment an exceptionally high PPV for an IM-ADR-associated HLA allele. Specific ADRs associated with HLA have much lower PPVs (approximately 8%) or NPVs ($<$100%), rendering screening less realistic. For efficient clinical application, recognizing many other factors predisposing patients to the IM-ADRs is vital (Karnes et al., 2019).

In flucloxacillin-induced DILI case, 14,000 patients would need to be screen to avoid a single incident; in these instances, due to a low NPV, PPV, and number required for treatment (NNT), application of HLA biomarkers is unrealistic (Teixeira et al., 2020). The effectiveness of HLA-B*57:01 testing for the prevention of abacavir HSN is an exception. As only 13 patients are required to be evaluated for the prevention of one medically diagnosed HSN reaction and only 30 patients are needed to be screened for the prevention of immunologically mediated abacavir HSN. The PPV is inadequate for several widely used medicines, like antibiotics, to use HLA as a diagnostic tool. In the future, when several drugs or antibiotics are dosing simultaneously, HLA may have applications in conjunction with operational and other tests to assist in diagnosis.

Besides, HLA allele frequencies vary significantly among populations (González-Galarza et al., 2015). For example, in several African communities, carriage of HLA-B*57:01 is virtually irrelevant but is as high as 11.2% in Southern Ireland and 17.8% in Sri Lanka. In some communities, the overall carriage rate of IM-ADR-associated HLA alleles and the reduced prevalence of IM-ADRs in noncarriers indicate that HLA screening across all cities would need to be enforced. The American College of Rheumatology suggests the screening of allopurinol HLA-B*58:01 for people with serious kidney impairment and for high-risk groups. Since it is deemed a genetic marker in many communities worldwide for allopurinol-induced SCARs (Yip, Alfirevic, & Pirmohamed, 2015). The United States Food and Drug Administration (FDA) advises that HLA-B*15:02 be screened earlier the commencement of carbamazepine in high-risk populations, especially Southeast Asian origins (Mehta et al., 2009). The specific clinical use of HLA testing by race/ethnicity allows patients to recognize their ethnicity correctly, which may be challenging in nations where overlapping is frequent, like the United States

The approval of standard medical recommendations is a crucial element in the translation of HLA tests into medical care. Adoption of guidelines to endorse HLA testing is necessary for useful application but depends on reliable medical evidence. There are several explanations of why abacavir HLA screening has been introduced. The main reason is that a clinical trial has been realistic to validate the efficacy of abacavir HLA-B*57:01 screening. This randomized controlled trial (RCT) was feasible given the high prevalence of abacavir HSN, high NPV and HLA-B*57:01 screening and high HLA-B*57:01 carriage rate in HIV care populations. Logistic, ethical, and statistical barriers for RCTs are challenging in pharmacokinetics and pharmacodynamics, considering the criteria of a sample size to illustrate therapeutic significance across genotype groups and replication requirements. The RCT for personalized medicine can be seen as a paradox because it employs a randomized treatment structure that is supposed to be personalized to an individual (Karnes et al., 2014).

Generally, RCTs were used to endorse novel medications or innovative criteria for drug use; however, data suggesting most contraindications to drug use and widely performed genetic screening is not drawn from RCTs (Johnson, 2013). HLA-B*15:02 tests to preclude carbamazepine-related IM-ADRs were cost-effective in the analysis of the Singapore drug regulatory authority, the Health Sciences Authority (HSA), while HLA-B*58:01 tests to prevent allopurinol-related IM-ADRs were not cost-effective (Dong, Tan-Koi, Teng, Finkelstein, & Sung, 2015; Toh et al., 2014). HLA-B*58:01 evaluation for allopurinol Medicare applicants for allopurinol could be recommended in patients with other preexisting health complications such as kidney failure. The RCT does not endorse either the HLA screening test, but the HLA-B*15:02 genotyping test for new carbamazepine consumers in

Singapore lowered the amount of associated SJS/TEN cases from 18 per year to 1 in the four years since the application (Yacoub et al., 2016). HLA typing may already be accessible from bone marrow or organ implant data in other places like the United States, reducing the economic burden linked with genotyping. Once new drugs are prescribed for drugs with HLA-associated IM-ADRs, current HLA forms offer an option to use electronic health records for clinical information systems.

## 15.7 Complexities of the human immune system

The immune system is highly problematic, as T cells and B cells composition is distinctive for every entity (Cano & Lopera, 2013). The variation in HLA types, which show antigens in the T cell and B cell repertoires, is extremely distinct for individual and each T cell has a specific TCRs for recognizing the cell surface-specific antigens of the HLA molecules (Fang et al., 2015). The body produces T cells, which can sense its cells, and it often positively selects T cells to attack pathogens or chemicals from an undesirable collection of thymus (Nicholson, 2016).

T cells are distinguished by TCRs into two classes: the α/β T cell and the γ/δ TCRs. The α/βT form of the TCRs is exhibited by most lymphocytes (95%). A huge percentage of V and J exons are present in the α/β receptor genes (Pennock et al., 2013). The TCRs α comprises approximately 60 V exons. The genes for TCRs and BCRs through recombination process develop receptors during the differentiation of lymphocytes, and a V exon, a D exon (only for the β chain), and a J exon integrate throughout this process (Janeway, Travers, & Walport, 2001). In fact, at V(D)J junctions, nucleotides are deleted or substituted unexpectedly. The traits of each TCRs are described by the V(D)J combination and insertion/deletion of each chain, and α/β chain combination, with their competence to track a particular antigen on the HLA molecule (Roth, 2014). The α-chain of the TCRs produces 3000 various V-J combinations, and the β-chain produces 2000 different V(D)J combinations. Furthermore, nucleotides are added and removed during recombination among the V-J junction (α-chain) and among the V-D and D-J junctions (β-chain), creating different forms of TCRs having same V(D)J combinations (Redmond, Poran, & Elemento, 2016).

Recently, two approaches have been applied to determine our immune system's complex structure and explore the underlying mechanisms implicated with different diseases. The first approach was to confirm the location of different TCRs families by analyzing variable (V) sequences of other TCRs (Langerak et al., 2001). CDR3 size spectra typing was another technique used for determining the repertoire's clonality utilizing fluorescent primers to assess the CDR3 region length variation within each V family of TCRs (Memon, Sportès, Flomerfelt, Gress, & Hakim, 2012). However, they provide some information, but comprehensive evidence on the CDR3 region

sequences will not be available. Such approaches are not valid if unknown exons for V and/or J segments are found in some persons and communities. A systematic, reliable and impartial study of TCRs and BCR transcripts is required to address these inherent limits. While this former generation of high-performance DNA sequencers reported restrictions due to shorter read-length (50−100 bp long) or poor sequencing quality, the progress of longer read-lengths empowered us to analyze thousands of TCRs and BCRs in one solitary study (Bashford-Rogers et al., 2013; Lange et al., 2014).

## 15.8 Applications of T cell receptors/B cell receptors sequencing

### 15.8.1 T cell receptor and B cell receptor sequencing with next-generation sequencers

For TCR and BCR cDNA sequences using next-generation sequencing (NGS) technique identified as 5′ rapid amplification of the cDNA end (5′RACE) PCR is developed, wherein one specific forward primer as adapter sequence is configured at the 5′ end, and second is used as a reverse primer in the C region of the TCR, or each BCR isotype (Lange et al., 2014). The process allowed robust and less-biased PCR amplification of TCR and BCR cDNA. With this cDNA library approach, the implementation of NGS technologies helps us to accumulate an enormous quantity of data on TCRs and BCRs. This method was employed to medical specimens to recognize specific T cell populations that invaded tumor tissues or cancerous ascites (Inoue et al., 2016; Jang et al., 2015; Kato et al., 2017). The BCR repertoires were used to analyze and regulate B cell related disorders like food allergy, autoimmune and infectious diseases (Kiyotani et al., 2018).

### 15.8.2 Identifying neoantigen-specific T cell receptors for cancer immunotherapy

Monoclonal antibodies which blockade immune checkpoint molecules like CTLA-4, PD-1, and PD-L1 were successfully showed substantial therapeutic benefit and have pioneered tumor immunotherapy (Drake, Lipson, & Brahmer, 2014). Tumor tissues frequently develop various ways to get away the cancerous cells' immune-mediated degradation (Drake, Jaffee, & Pardoll, 2006). One such tool includes cell-surface expression of immune checkpoint molecules, such as CTLA-4, PD-1, and PD-L1 (Pardoll, 2012). Reliable responses of antibodies against such molecules were regularly observed (Schadendorf et al., 2015). Many somatic mutations are associated to such antiimmune checkpoint antibodies (Le et al., 2017; Schumacher & Schreiber, 2015). Higher numbers of somatic mutations generate a higher amount of healthy, cancer-specific somatic mutation antigens expressed in HLA

molecules in cancer cells. Such antigens activates T cells, which develops immune checkpoint inhibitor's therapeutic efficacy. The usage of neoantigen-specific immune response has been very significant (Leisegang et al., 2016). The adoptive transfer of patient-derived penetrating tumor lymphocytes (TILs) provided promising clinical results in a limited subgroup of patients with solid tumors and took two weeks from T cell priming with applicant neoantigen peptides to neoantigen-specific TCR exploration (Kato et al., 2018; Maoz, Rennert, & Gruber, 2017). This vigorous method is vital in the introduction of neoantigen-specific TCR-engineered cells in clinical practice

### 15.8.3 Describing T cell changes during immunotherapy

Considering dynamic and varied nature of the immune microenvironment of the tumor, it is imperative for patients treated with cancer immunotherapy to investigate the molecular nature of immune responses. The preexisting interaction in the microenvironment of the tumor among immune-active and immune-suppressive molecules facilitates immune outcomes. Several immunotherapies like ipilimumab-targeting CTLA-4, pembrolizumab and nivolumab-targeting PD-1 were certified by the United States Food and Drug Administration for melanoma. Nevertheless, small number of patients responds to such immunotherapies (Tumeh et al., 2014). In tumor TCRβ repertoire assessment of melanoma patients, nivolumab response was correlated with oligoclonal TIL expansion and increased expression of PDL1, granzyme A (GZMA) and HLA-A (Inoue et al., 2016). Thus comprehensive knowledge on T lymphocytes in tumors, blood and cancer-associated results can be obtained by TCR sequencing and the precise processes of immune responses in certain therapies can be further characterized.

### 15.8.4 Characterizing T cell changes during nonimmune targeted cancer therapy

The treatment of cancer patients treated with nonimmune-targeted treatments, utilizing NGS to assess the immune components has provided handy knowledge for ailment tracking and clinical outcome prediction. The correlation between TIL and patient diagnosis was primary stated in epithelial ovarian cancer (Zhang et al., 2003). Improved survival observed in patients showing a high fraction of CD3 + TILs, suggesting that the immune response may play a crucial part in deciding clinical outcomes. In these instances, the corresponding study of the invaded lymphocytic fraction explored the significance of CD8 + T cell populations in decisive improved consequences (Sato et al., 2005). The absence of CD8 + T effector cells in malignant pleural effusion was assumed as a consequence of CD8 + T cell recruitment defect due to the immunosuppressive impact of the disease

(Scherpereel et al., 2013), leading to the spread of cancer cells. The use of TCR sequencing will assist further in differentiating populations of T cells present in a particular environment. In contrast with typical flow cytometry or immunological studies, the wide categorization of the T cell repertoire by NGS offers details regarding specific subpopulations existing in tumor tissue or ascitic/pleural effusions. Enrich T cell clones were identified in a malignant effusion sample of ovarian cancer patients not familiar with those in tumors. Besides, the predominant TCR clonotypes in CD4 +, CD8 +, and CD4 + CD25 + T cell populations are generally incompatible, suggesting that the immune microenvironments are altogether different in tumors and ascites (Jang et al., 2015).

In certain malignant tumors, the existence of TILs is correlated with beneficial clinical results. A small cohort study reported a spike in infiltrative B and T cells in bladder tissues after BCG intravesical intervention in bladder cancer, and these cases often have a reduced risk of relapse of the disease (Chang, Lee, Huh, & Lee, 2001). Oligoclonal expansion of TILs was allied with prolonged recurrence-free survival (RFS) with higher neoantigen loads in patients receiving conclusive surgery while studying immune control in muscle-invasive bladder cancer (Choudhury et al., 2016). Consequently, certain molecular patterns provides valuable prognostic markers for predicting disease recurrence after such treatments.

In different tumor types, regulatory T cells (Tregs) in tumors reported negative clinical consequences (Shang, Liu, Jiang, & Liu, 2015). However, one study showed better clinical outcomes in follicular lymphoma (F.L.) tissues (Carreras et al., 2006). A later investigation found that poor survival was related to a specific intrafollicular Treg pattern instead of Tregs' number (Farinha et al., 2010). Moreover, whether Tregs suppress the immune system in an antigen-specific manner remains uncertain. Intriguingly, Tregs characterized by F.L. biopsy pretreatment specimens were extremely clonal (Liu et al., 2015). Further, perifollicular CD8 + T cells in tumors which that certain unspecified mechanism may exclude antigen-specific CD8 + T cells proficient of addressing F.L. cells from malignant follicles (Liu et al., 2015).

Although nivolumab and pembrolizumab were authorized as second-line therapy of recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN). SCCHN is primarily being diagnosed with chemoradiation therapy and surgery in the advanced stage. To explore the functions of the host immune system in SCCHN, tumor tissues from subjects with locoregionally advanced SCCHN prior to chemoradiotherapy were examined (Saloura et al., 2017). Interestingly, in human papillomavirus (HPV)-negative tumors, clonal expansion of T cells was slightly more significant than HPV-positive tumors. Such results indicate discrepancies between HPV-negative and HPV-positive tumors in the immune microenvironment. Besides, pretreatment levels of the resistant marker, like GZMB, could function as determinants of the risk of complications in advanced disease patients.

For renal cell carcinoma (RCC), and other forms of cancer, cryoablation is used, and it triggers immune cells by inflammatory signals. Indeed, cryoablation studies in RCC and prostate cancer showed potential to invade a cytotoxic T cell tumor-specific response (Thakur et al., 2011). Cryoablation can contribute to local and systemic T cell response in the TCRβ repertoire of tumor tissues and samples taken from patients with chronic kidney cancer before and three months after Cryoablation. In addition, an upsurge in CD11c + cells (macrophages and D.C.s) in the postcryoablation tissues was correlated significantly with antigen presentation, assessed by HLA-A expression (Zewde et al., 2018). Overall, we have been able to describe better the immune-mediated response to cryoablation via these molecular changes. In the clinical sense, for cancer-antigen-specific TCR-engineered T cell therapy, extended TCR sequences emerging after cryoablation might be implemented.

## 15.8.5 T cell receptors/B cell receptor sequencing in other diseases

### *15.8.5.1 Pathogenesis of autoimmune diseases*

In the pathophysiology of autoimmune diseases, tissue infiltrating lymphocytes in a compromised abscess have a critical part. For instance, the primary infiltrating lymphocytes in the thyroid of patients with autoimmune thyroid disease (AITD) were classified as CD20 + B cells and CD8 + T cells. In the production of renal dysfunction and enhancement of salivary gland CD3 + CD4-CD8 (double negative) T cells, infiltration of CD4 + and CD8 + T cells is suggested to react to tissue damage in patients with systemic lupus erythematosus (Alunno et al., 2013; Winchester et al., 2012). Definition of human immune cells including Type 1T support cells (Th1), Type 2T help cells (Th2), and B cells in the blood and/or infected lesions in patients with autoimmune diseases, can also include useful knowledge to better explain autoimmune disease molecular pathogenesis. In this respect, the quantification of unique subpopulations of T cells and B cells through the temporal and spatial use of high-throughput sequencing of TCRs and BCRs could contribute to a deeper understanding of these conditions. Furthermore, it can be applicable to improvements in patient diagnosis, prognosis, and evaluation for suitable treatments (Maecker et al., 2012). Oligoclonally expanded CD4 + T cells were insistently found in preoperative and postoperative illness sites in inflammatory bowel disease (IBD). IBD is a persistent intestinal inflammatory disease triggered by innate and adaptive immune system dysfunction (Baumgart & Sandborn, 2012). Inflamed tissue extracts from CD patients revealed the production of oligoclonal T cells whose TCR sequences were not present in subsequent normal tissues. The culprits of intestinal inflammation in CD may be unique disease-causative T cells.

A deeper knowledge of immune dysfunctions, which lead to CD/IBD pathogenesis, will be presented by further examining sorted T-cell subpopulations.

#### 15.8.5.2 Pathogenesis of food allergy

Nearly 15 million people in the United States are susceptible to food allergies and there are specific regional variations of known food allergy antigens (Ho, Lee, Wong, Ip, & Lau, 2012; Lee, Gerez, Shek, & Lee, 2012). Allergy are represented in 2 phases: the sensitization phase and the effector phase. Dendritic cells carrying food-specific antigens in their HLA molecules activate CD4 + Th2 cells by TCRs which identify such antigens. These stimulated CD4 + cells encourage B cells to develop IgE-specific allergens at the sensitization stage (Palomares, 2013). Such T cells often helps in improving an allergen-specific IgE level at the later stages.

Various experiments have demonstrated distinct potential functions of Th1, Th9, Th17, and Th22 effector T cells in food allergies (Akdis et al., 2011; Akdis, Palomares, van de Veen, van Splunter, & Akdis, 2012). In food allergy situations, the quantitative examination of the TCR and BCR repertoires can help to discern interindividual differences and intra-individual improvements in the era. We performed a preliminary review of the BCR repertoire of patients with peanut allergy pre- and postoral immunotherapy (OIT) to ascertain improvements in the BCR repertoire during therapy (Kiyotani et al., 2018). OIT has oligoclonally enriched some immunoglobulin heavy chain alpha (IGHA) and IGH gamma (IGHG) clones, and the total BCR repertoire diversity has been substantially reduced. A crucial next step is the detection of particular BCR sequences concerned in the production of peanut allergy, to explore the comprehensive mechanism of food allergy molecular pathology

#### 15.8.5.3 Pathogenesis of graft rejection or graft-vs-host disease after hematopoietic cell transplantation

Hematopoietic cell transplantation (HCT) is becoming an essential therapeutic choice for patients with hematological disorders. Obstacles to this procedure involve graft rejection or graft-vs-host disease (GVHD) that hinders the effectiveness of HCT. In both graft rejection and GVHDD, T cells play a crucial function (Ferrara, Levine, Reddy, & Holler, 2009). For patients with hematologic disorders, HCT has become the appropriate therapeutic options. In both graft rejection and GVHD, T cells are considered to have a pivotal role (Ferrara et al., 2009). The host cells' immune response to the donor cells is induced by the expression of the antigen(s) posed by the donor cells' HLA molecules. Numerous reports showed that cytotoxic T cells obtained from recipients can prompt acute graft rejection by detecting donor cell antigens like HLA-A, HLA-B, or HLA-C (Anasetti et al., 1989; Fleischhauer, Kernan,

O'Reilly, Dupont, & Yang, 1990). The variance of these HLA alleles raises the probability of acute graft rejection.

GVHD emerges after infiltrating of donor T cells into the target tissue, and an immunological reaction against the graft cells could not be deployed by the host cells (Shlomchik, 2007). The progression and intensity of GVHD is dependent on different factors, like age and source of graft. GVHD's prevalence is usually greater in people whom donors are unknown or those whose types of HLA are not well paired. Further, cytotoxic T cells resulting from activated donor play a vital role in acute GVHD production (Schmaltz et al., 2003). In comparison, acute and persistent GVHDD is correlated with a lower frequency of regulatory T cells (Dong et al., 2013; Hau et al., 2007). A systematic high-throughput analysis of the TCR repertoire reported that extensive sequencing description of the TCR repertoire is useful in acute GVHD patients and in non-GVHD patients utilizing next-generation sequencing framework and can lead to a detailed understanding of the pathways of pathogenesis of GVHD (Yew et al., 2015).

## 15.9 Future perspectives of immunopharmacogenomics

The development of immunopharmacogenomics will result in new immunotherapy through recognizing TCRs that detect cancer-specific antigens and distinguish T cells capable of killing cancer cells to classify the TCRs. This area of research could be used for immunotherapy (e.g., immune checkpoint antibodies), and for chemotherapy and radiation to predict how a patient would react to anticancer care. It may also be used to indicate whether adverse events will occur in a patient (Zewde et al., 2018). Different aspects of the immune microenvironment can determine the reaction of a patient to immunotherapy. Several studies showed somatic mutations are associated with the outcome of immune control point antibodies, including those aiming programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) (Durgeau, Virk, Corgnac, & Mami-Chouaib, 2018; Nixon et al., 2019). A further vigorous reaction to treatment with immune checkpoint antibodies is expected in patients with DNA repair gene mutation, a greater number of somatic mutations, further penetration of CD8 + T lymphocytes into cancer tissues and clinically expanded T cells (Arora et al., 2019; Trujillo, Sweis, Bao, & Luke, 2018).

Several decades prior, there have been no approaches to describe the thousands of TCRs in T cells or B cell receptors in B cells. However, millions of TCRs may now be described by a single test (Sanchez-Trincado et al., 2017). The quality of read sequences was not high adequate, and the duration of each reading wasn't even long enough to cover essential areas of the TCRs or B cell receptor that really can distinguish antigen recognition locations, whereas next-generation DNA sequences provide a considerable amount of sequence knowledge. However, improvement in DNA sequencing

technologies has permitted us to acquire the mixture of variable/diversity/joining (V[D]J) and inserted/deleted nucleotides through the recombination phase of the TCRs β-chain (the D portion is not present in the TCRs of the alpha-chain) (Mourad, 2020). Further, examining the levels of expression of immune-based molecules, particularly γ-interferon, perforin, and granzyme, as they are relevant to the cytotoxic activity of T cells is required. Lastly, it is important to establish appropriate methods for elucidating passive transcript details to decode antigen specificity.

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VOLUME I

# Immunogenetics: A Molecular and Clinical Overview

*A molecular approach to immunogenetics*

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*Immunogenetics: A Molecular and Clinical Overview* provides an updated overview of scientific knowledge, achievements and findings in the field of immunogenetics. The field of immunogenetics is dynamic and the emerging trends, especially related to clinical solutions, necessitated the writing of this book. *Immunogenetics: A Molecular and Clinical Overview* gives insight into the molecular and clinical aspects of immunogenetics that will help researchers, scientists, teachers as well as clinicians. The most critical problem that readers usually face is the lack of compact knowledge on this subject. This book addresses this problem as it provides knowledge from basics to developments and clinical applications to future prospects.

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ISBN 978-0-323-90053-9
9 780323 900539

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