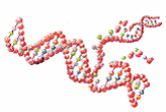
Chapter 14

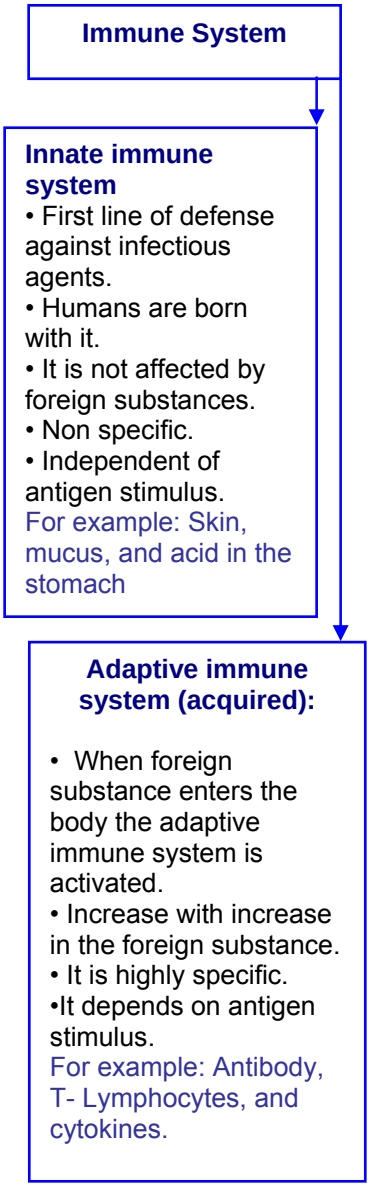


Genetics of the Immune System

Introduction

Higher organisms are unique in their ability to distinguish between "self" and "non-self" and to mount reactions against a very broad spectrum of foreign antigens. The wide variety of foreign substances including infectious agents present in the environment constitute an important components of non-self and if after entry into the body they multiply uncontrolled, this can lead to disease and eventually death. However, the human body is well equipped to deal with these organisms and other substances entering the body. Several defense mechanisms operate to provide resistance and protection against these organisms and are constituents of the immune system. The immune system can be divided into two parts i.e. Innate and adaptive immune systems. Figure 14.1 summarizes the characteristics of the two systems. In addition, immune response may be humoral (i.e. due to soluble substances e.g. anti-bodies) or cellular (i.e. due to different types of cells e.g. T lymphocytes). Both innate and adaptive immunities have cellular and humoral components [Figure 14.2].

Figure 14.1: The adaptive and innate immune systems.



Innate Immunity

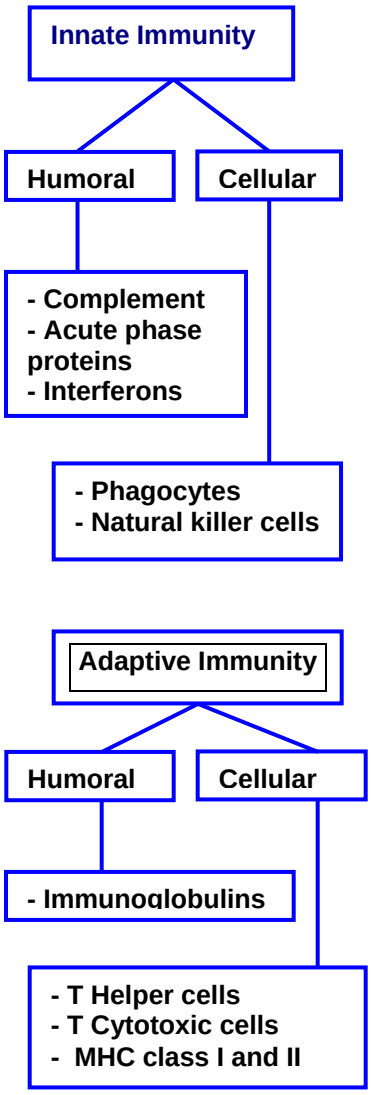
Innate immune response is the first line of defense against any foreign substance. It provides both physical and biochemical barriers in the form of the intact skin, mucous, acid in stomach, lysozyme in tears, ciliary movement in the respiratory tract, complement system which causes lyses to the foreign cellular organisms, opsonization and phago–cytosis of antigens. In additions, interferons secreted by several activated cells interfere with viral replication. Natural killer cells also destroy foreign

agents.

Adaptive Immunity

Adaptive immunity is also known as specific acquired immunity. The presence of a foreign substance activates this type of immunity, which is almost non-functional at birth. It has both humoral and cellular arms. The B- lymphocytes are responsible for the humoral arm of the adaptive immune system, while the T- lymphocytes are responsible for the cellular arm of the immune system. T-lymphocytes are also involved in activating the B- lymphocytes and other cells through the action of lymphokines secreted under different stimulatory responses. The developmental pathway of B- and T-lymphocytes is presented as Figure 14.3

Figure 14.2: Humoral and cellular arms of innate and adaptive immunity



Acquired Cellular Immunity

T-lymphocytes are responsible for cellular immunity including destruction of viral infected cells, transplantation immunity, graft rejection, delayed hypersensitivity and reactions to malignant cells. T-lymphocytes can be subdivided according to their function:

T- Cytotoxic or killer lymphocytes: These are sensitized to destroy cells bearing antigen induced by virus infection. The T-cytotoxic lymphocytes recognize the antigen presented to them by the antigen-presenting cell (APC) on MHC I molecules.

T- Helper lymphocytes: These are necessary for the induction of the antibody response by B-lymphocyte, and also help activation of the cytotoxic T lymphocytes to kills infected cells. The T-helper lymphocytes recognize the antigen presented to them by the B-lymphocytes or other antigen-presenting cell on MHC II molecules.

T- Suppressor lymphocytes: The lymphocyte,s which inhibit or suppress the immune response.

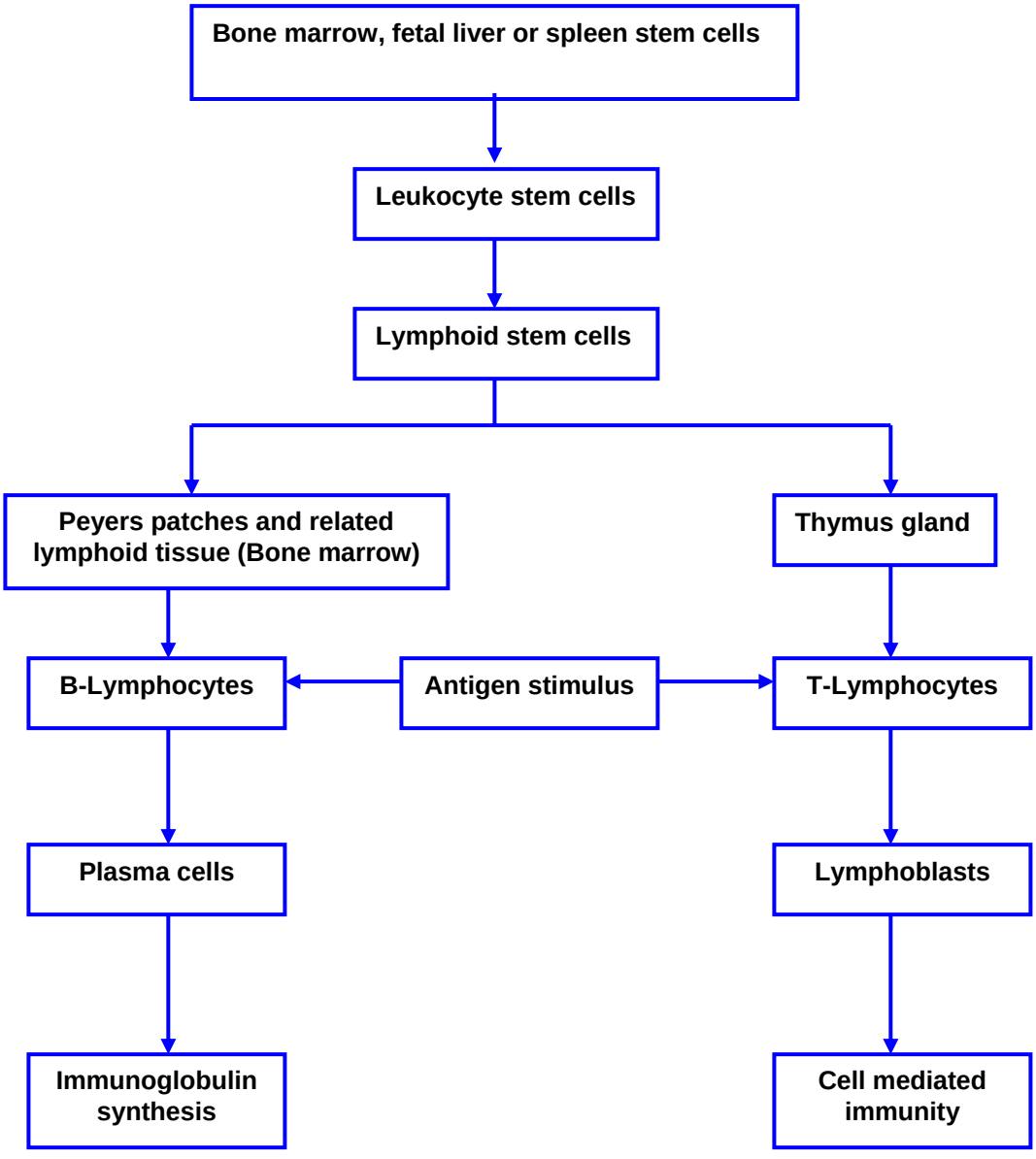
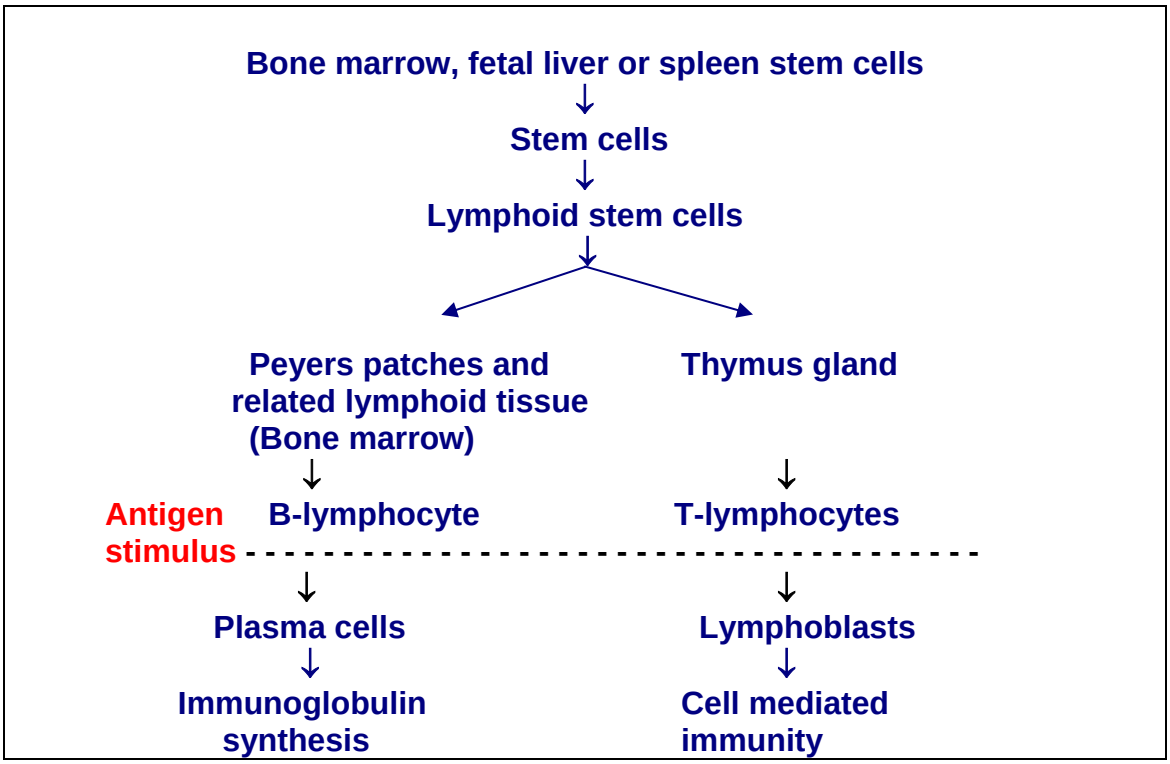

Bone marrow, fetal liver or spleen stem cells
Leukocyte stem cells
Lymphoid stem cells
Peyers patches and related lymphoid tissue (Bone marrow)
Thymus gland
B-Lymphocytes
Antigen stimulus
T-Lymphocytes
Plasma cells
Lymphoblasts
Immunoglobulin synthesis
Cell mediated immunity

Figure 14.3: The developmental pathway of B- and T- lymphocytes



Acquired Humoral Immunity

The main mediators of acquired humoral immunity are antibodies (immunoglobulins) produced by the B-lymphocytes. There are five main classes of antibodies, each with its own specific function (Table 14.1). Upon exposure, the antigen binds to surface receptors on the surface of the B-lymphocytes, which differentiate into mature antibody producing cells called 'plasma cells' in the bone marrow, in the red pulp spleen and the medulla of the lymph nodes. A specific antibody is produced against each antigenic determinant (epitope) by the differentiated B-lymphocytes. The first exposure to the antigen results in primary response and IgM antibodies are produced. Some B-lymphocytes change into memory cells and remain in the body. A later exposure to the same antigen results in a secondary response, which is a quick response and results in the production of other classes of Immunoglobulins, particularly IgG [Figure 14.4].

The secondary response is due to the presence of antigen specific immunological memory.

Antibodies (Immunoglobulins)

Immunoglobulins (Ig) constitute an important component of the body's defense mechanisms against infection. They play several important functions: i. bind antigen to form antigen-antibody complex and to cause the destruction of the antigen, ii. cause activation of classical pathway of complement fixation, leading the destruction of cellular antigen, and iii. accelerate phagocytosis, by binding to the Fc receptors on the membrane of the phagocytes.

There are five main classes of Igs. These are IgG, IgM, IgA, IgD and IgE. Table 14.1 lists the major functions of the Ig classes. IgG has four subclasses: igG1, IgG2, IgG3, and IgG4, while IgA has two subclasses: IgA1 and IgA2. Major characteristics of the five Ig classes are listed in Table 14.2.

Table 14.1: Major functions of immunoglobulins classes

Ig	Function
IgG	Major antibody of secondary response. Fixes complement. Antibacterial, antitoxin.
IgM	First antibody to be synthesized in response to antigen. Major antibody of primary response. Fixes complement.
IgA	Antibody in external secretions
IgD	On lymphocyte surface.
IgE	Antibody in allergic response & parasitic infections

Immunoglobulin Structure:

Immunoglobulins are glycoproteins made of two light (L) and two heavy (H) polypeptide chains of approximately 220 and 440 amino acids, respectively. The H and L chains are joined together by disulphide bonds to form a half subunit, and two half subunits are joined together by disulphide bonds to make an immunoglobulin subunit [Figure 14.5]. Each subunit has a Y shaped structure with two antigen-binding sites at the NH_2 terminal end. The light chains in all immunoglobulins are either κ or λ, but the heavy chains are different in the different classes of Ig's [Table 14.3] .

Table 14.2: Characteristics of Ig Classes

Ig	Serum conc. (mg/ ml)	M.Wt (Dalton)	*
IgG	8-16	150,000	+
IgM	0.5-2	900,000	-
IgA	1.4-4	160,000	-
IgD	0- .03	185,000	-
IgE	trace	200,000	-

* = Placental transfer.

The subunit Immunoglobulin structure is shown in Figure 14.5. Each Ig subunit is made of two light (L) chains and two heavy (H) chains. The L chain is linked to H chain by disulphide bonds and the two H chains are linked together with disulphide bonds. Each H and L chain has a variable region at the N-terminal and a constant region at the C-

terminal. The variable region has the antigen binding site, while the constant region has different effector functions i.e. i. it has carbohydrate attached to it, ii. It has complement- binding site, iii. It binds Fc receptor on macrophages and iv. It helps the IgG to cross the placenta. The hinge region, gives flexibility to the two antigen binding sites and gives the Ig subunit a **Y** shaped structure.

Figure 14.4. Primary and Secondary Immune Response

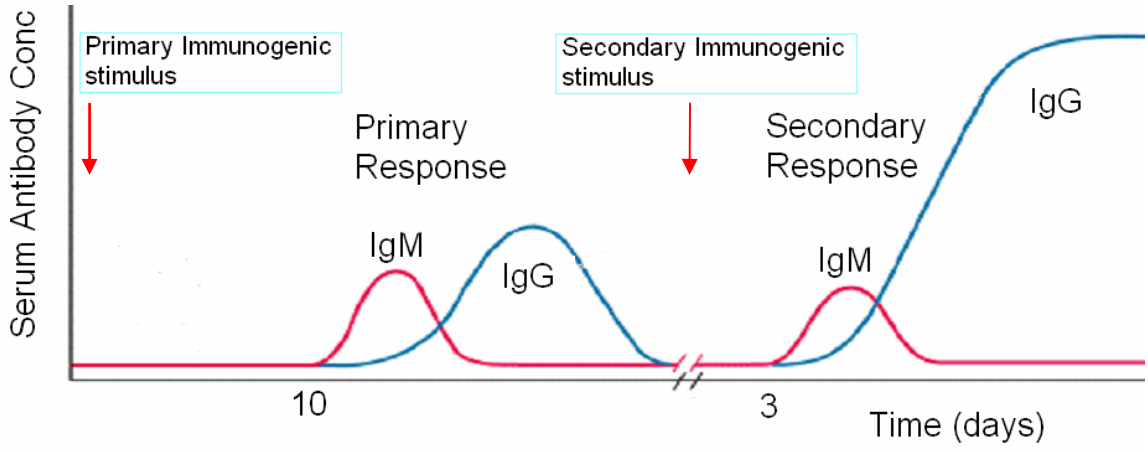
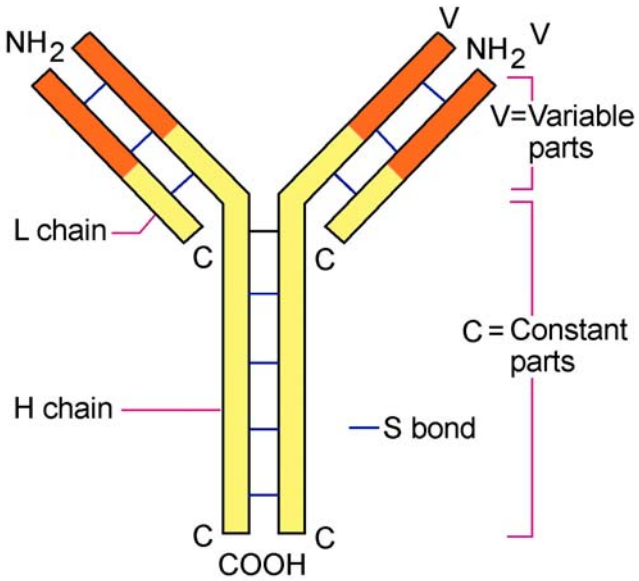


Figure 14.5: General structure of an antibody molecule.



Each Ig class has different H chains (Table 14.3) but there are only two types of L chains: κ chains or λ chains, in all classes. The antigen-binding site has three hypervariable regions (HVR), separated by four framework regions (FRs) (Figure 12.6). The hypervariable regions (HVR) have a very highly variable amino acid sequence and no two antibodies have the same HVR. The FR's are less variable. Each IgG, IgD and IgE exists as a single subunit. sIgA (the IgA found in external secretions) is made of two subunits joined together by an extra J peptide and has a secretary (S) peptide which gives it resistance against proteolytic breakdown during its secretion. While IgM is made of five subunits, joined at the C-terminal end by a J polypeptide.

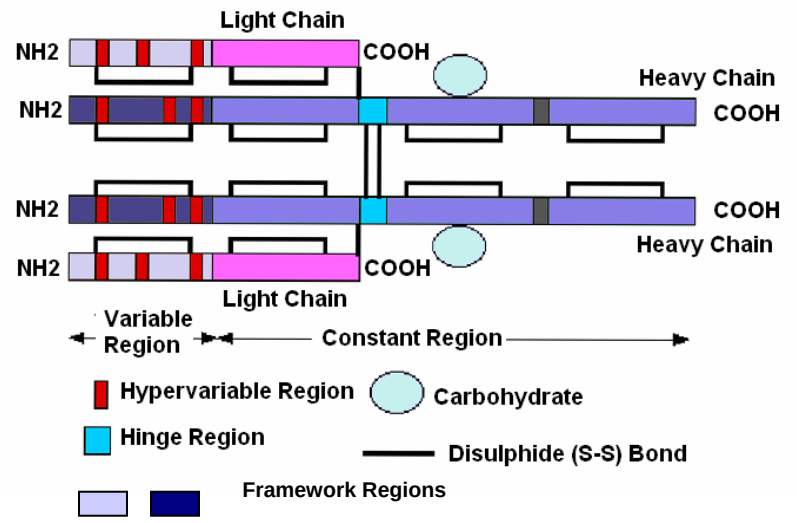
Table 14.3: H chains in Ig classes and sub-classes.

Ig class	H chain type	L chain type
IgG1	γ1	κ or λ
IgG2	γ2	κ or λ
IgG3	γ3	κ or λ
IgG4	γ4	κ or λ
IgM	μ	κ or λ
IgA1	α1	κ or λ
IgA2	α2	κ or λ
IgD	δ	κ or λ
IgE	ε	κ or λ

Immunoglobulin Genes

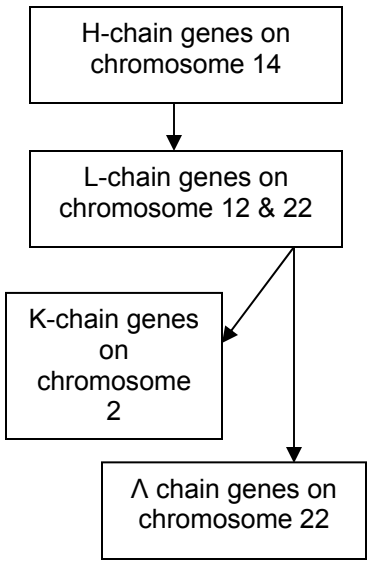
The genes for the κ and λ light chains and the heavy chains in man have been assigned to chromosomes 2, 22 and 14, respectively (Figure 14.7). For κ chain, there are 100-200 Vκ genes, up to 5 Jκ genes and one Cκ genes for the constant region (Figure 14.8). For the λ chains there are around 100 Vλ, 6 Jλ and each has its own Cλ gene.

Figure 14.6: The subunit structure of an Immunoglobulin Molecule



The H chain genes are located on the chromosome 14 and are around 200 V_H genes, 12 D_H genes and 6 J_H genes, followed by the genes for the constant regions of H chains. The C_H genes are arranged in the order: 5'- μ δ γ3 γ1 α1 γ2 γ4 ε α2 -3' as shown in Figure 14.9.

Figure 14.7: The L and H chain genes



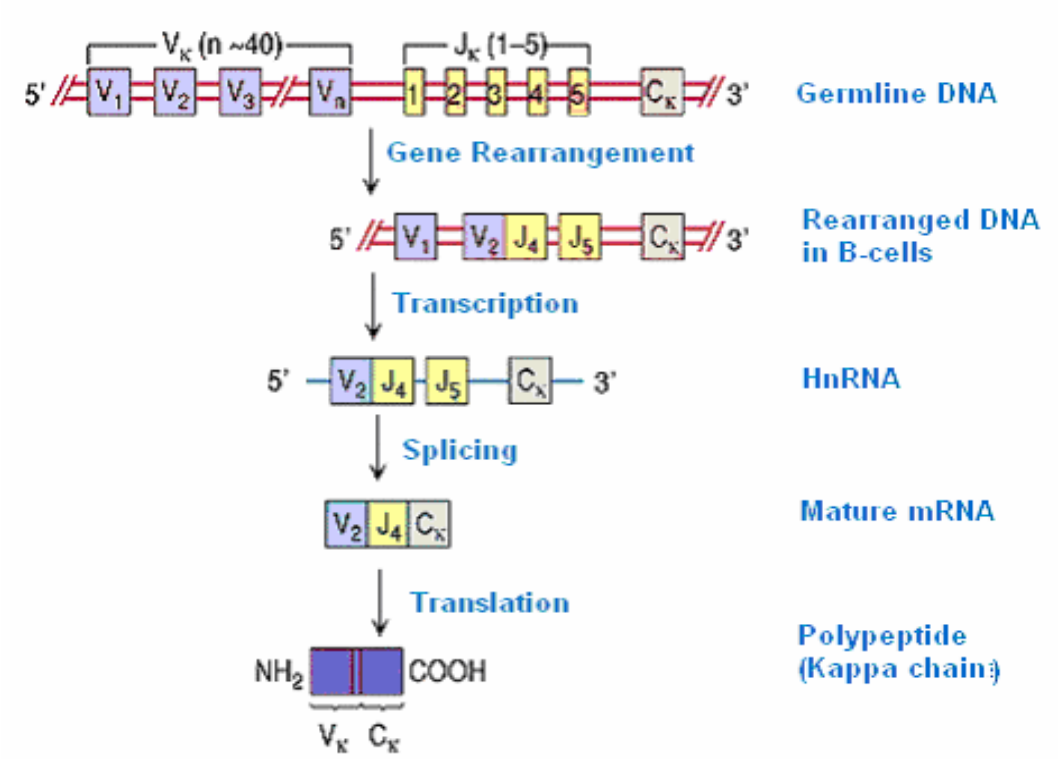
The undifferentiated B-lymphocytes have cell surface receptors (sIgM and sIgD molecules). When they are exposed to the antigenic determinant (epitope) of an antigen, the epitope selects the best the best fit receptor, and causes differentiation of the B lymphocytes. This involves gene rearrangement to produce H & L chains with a specificity against the specific epitope. The differentiated B cell, the proliferates and changes to plasma cells, which secrete specific antibodies against the antigenic epitope. It moves the one epitope is present on the antigen, the each epitope will produce its own differentiated B-lymphocyte, plasma cells and antibodies. This has been referred to as the clonal expansion theory and is presented schematically in Figure 14.10.

Immunoglobulin Synthesis

During synthesis of an antibody against a specific antigen the germ-line DNA is subjected to extensive rearrangement both for the L and the H chains. The differentiated B lymphocyte can make antibodies with only a single specificity, due to the rearranged genes.

For the L chains a "VJ rearrangement" occurs, in which one V gene is selected and moves next to a selected J gene (Figure 14.8). All genes lying in between are deleted. Transcription occurs and produces a primary RNA transcript or heterogenous RNA (HnRNA), which has the V, J and C gene sequences and has several additional sequences. These extra sequences are spliced out in the nucleus to produce mature RNA. The mRNA leaves the nucleus enters the cytoplasm and is translated on the ribosomes to produce the light chain polypeptide. The steps in the biosynthesis of Lκ chains are

Figure 14.5: Rearrangement of the Lκ chain genes during biosynthesis of κ chains.



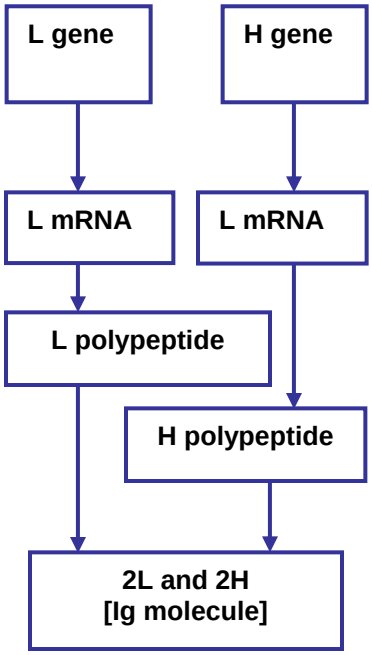
presented in Figure 14.8. Similar events result in the formation of Lλ chains. The H chain synthesis involves a VDJ rearrangement as shown in Figure 14.9. One selected V gene and one selected D gene move to a selected J gene. The VDJ rearrangement. is followed by splicing, to initially produce the mature mRNA for μ and δ chains of IgM and IgD respectively. This is the primary immune response.

The H and L chains come together and are linked by disulphide bonds during post-translation modifications and produce the Ig molecule. A schematic presentation and produce the Ig snthesis is presented in Figure 14.11.

Class Switching

There is a normal switch of antibody class produced by B cells on continued or further exposure to antigen, usually from IgM the initial class of antibody produced to IgA or IgG. This process, known as *class switching,* involves retention of the specificity of the antibody to the same antigen. This occurs during secondary response to the same antigen and involves switching (S) region present at the 5' end of the different H chain genes. The IgM is switched to IgG or IgA or IgE. The variable region remains the same only the C region is switched (Figure 14.12). Class switching occurs by a somatic recombination event which involves DNA segments designated S which lead to looping out and deletion of the intervening DNA. The result is to eliminate the DNA segment coding for the C region of the heavy chain of the IgM molecule and to bring the gene segment encoding the C region of the new class of heavy chain adjacent to the segment encoding the V region.

Figure 14.11: Schematic presentation of Ig (antibody) synthesis in the B-cells



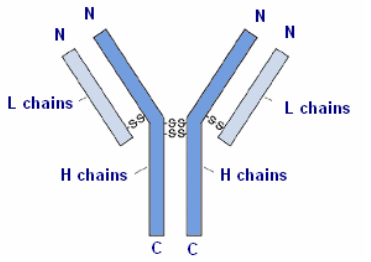
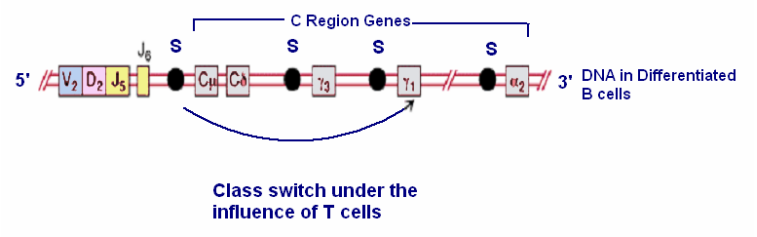


Figure 14.12: Class switching during immunoglobulin synthesis. [S= switch region-upstream of each heavy chain constant region gene except δ.



Immunoglobulin super family

The Ig Superfamily of genes consists of eight multigene families which include the κ and λ light chains genes, different classes of heavy chain genes, the alpha, beta, gamma and delta chain genes of the T cell receptor, the class I and II major histocompatibility complex human leucocyte (HLA) antigens and β_2-microglobulin. These also include the T lymphocyte antigens CD4 and CD8, the epithelial cell polyimmunoglobulin receptor and proteins with quite different function such as the intercellular adhesion molecule, ICAM-1.

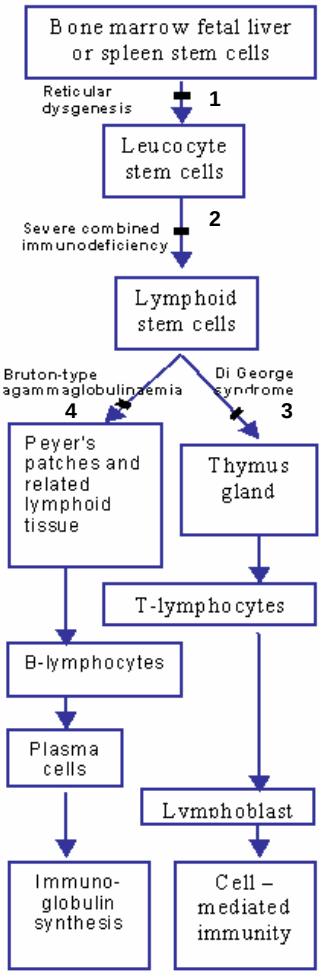
Immunodeficiency

Immunodeficiency can occur as a primary isolated abnormality or as a secondary or associated finding.

Primary Immunodeficiency Disorders

Primary Immunodeficiency Disorder occurs in some primary immunological deficiency diseases in humans. Defects have occurred at specific points in the biology of the immune system.

Figure 14. 8: Sites of hypothetical blocks in certain immunological deficiency diseases



1. Reticular dysgenesis
2. Severe combined immunodeficiency
3. Di George syndrome
4. Bruton-type agammaglobulinaemia

(a) Severe combined immunodeficiency

Severe combined Immunodeficiency (SCID), as the name indicates, is associated with an increased susceptibility to both viral and bacterial infections because of abnormal B- and T-cell function. Death usually occurs in infancy because of overwhelming infection, unless bone marrow transplantation is performed. Severe combined immune deficiency can be inherited either as an X-linked or autosomal recessive disorder. A deficiency of the enzymes adenosine deaminase or purine nucleoside phosphorylase can be demonstrated.

(b) Reticular dysgenesis (AR)

Children have abnormal cellular and humoral immunity and also have a deficiency of granulocytes. They usually die very early in the first year unless offered a bone marrow transplant.

(c) DiGeorge syndrome

DiGeorge syndrome presents with recurrent viral illnesses and are found to have markedly abnormal cellular immunity as characterized by severely reduced or absent T lymphocytes, due to their thymus gland being absent. The patients usually have congenital abnormalities which include congenital heart disease and absent parathyroid glands.`

(d) Bruton type agammaglobulinaemia

Male children with this X-linked immuno-deficiency usually develop multiple recurrent bacterial infections of the respiratory tract and skin after the first few months of life, being protected initially by transplacental maternal IgG. The diagnosis of this type of immunodeficiency is confirmed by demonstration of deficient immuno-globulins and absence of B lymphocytes.

Secondary or associated immunodeficiency

There are also a number of hereditarydisorders occur as one of a number of associated features as part of a syndrome.

Ataxiate langiectasia (AR)

Persons with this disorders have low serum IgA levels and a hypoplastic thymus.

Wiskott-Aldrich syndrome (XR)

Low serum IgM levels.

Disorders of phagocytic function

Chemotaxis, phagocytosis and subsequent cell mediated killing of microorganisms may be defective in some patients.

Chronic granulomatous disease (XR or AR)

Is associated with recurrent bacterial or fungal infections.. A high childhood mortality.

Leucocyte adhesion deficiency

A rare disorder of phagocytic function with life threatening bacterial infections of the skin and mucuous membranes with impaired pus for mation. Lack of migration of phagocytic cells due to abnormal adhesion-related functions such as chemotaxis and phagocytosis. This is due to absence of the β_2 integrin receptor subfamilty of leucocyte cell surface glycoproteins.

The complement system

Components of complement interact in sequence, resulting in increased inflammation and vascular permeability. Attact phagocytes and enhance phagocytosis, ultimately bringing about the destruction of cellular antigens.

Deficiency of the C1 inhibitor of the complement system, causes hereditary angioneurotic oedema.

Defects of the other components of complement are associated with an increased susceptibility to bacterial infections of a predisposition to a systemic lupus erythematosus-like disorder.

BLOOD GROUPS

Blood groups reflect the antigenic determinants on red cells.

Table 12. : Blood Groups

RBC			React with antiserum	
Phenotype	***Genotype***	***Ab***	***Anti-A***	***Anti-B***
O	OO	Anti-A, B	-	-
A	AA, AO	Anti-B	+	-
B	BB, BO	Anti-A	-	+
AB	AB	-	+	+

The ABO Blood Groups

The ABO blood groups were discovered by Landsteiner. There are four major ABO blood groups: A, B, AB and O.

Individuals of blood groups AB do not produce A or B antibodies they can receive a blood transfusion from individuals of all other ABO blood groups and are therefore referred to as *universal recipients*. Since individuals of group O dod not express either A or B antigens on their red cells they are referred to as *universal donors*.

Molecular basis of ABO blood groups

Individuals with blood groups A, B and AB possess enzymes with glycosyl transferase activity, which convert the basic blood group, which is known as the H antigens, into A or B antigens.

Rhesus Blood Group

The rhesus (Rh) blood group system involves three sets of closely linked antigens, Cc, Dd and Ee. Either Rh positive (possessing the D antigen) or Rh negative (lacking the D antigen).

Molecular basis

There to be two types of Rh red cell membrane polypeptide. One corresponds to the D antigen and the other to the C and E series of antigen.

The major histocompatibility human leucocyte antigen complex

The products of the genes of the major histocompatibility complex (MHC), named for its association with graft rejection, are involved in the immune response through the presentation of antigens to the T cell receptor. In humans, the MHC

Phenotype
O
A
B
AB

is known as the human leucocyte antigen or HLA system. This consists of a class I molecules (A, B, C, E, F and G) and the so-called class II molecules (D related to DR, DQ, DPA1, DPA2, DNA, DOB, DQB2 and DQA2).

The HLA system is highly polymorphic. A virtually infinite number of phenotypes resulting from different combinations of the various alleles at these loci are theoretically possible.

HLA polymorphisms and disease associations

A finding, which helps to throw light on the pathogenesis of certain diseases is the demonstration of the association of specific diseases with certain HLA types. The best documented is that between ankylosing spondylitis and HLA-B27. In the case of narcolepsy, a condition of unknown aetiology characterized by a periodic uncontrolled tendency to fall asleep, almost all affected individuals are HLA-DR2. The possession of a particular HLA antigen does not mean that an individual will necessary develop the associated disease, merely that he or she has a greater relative risk of being affected than the general population.

Explanation for the various HLA associated disease susceptibility include close linkage to a susceptibility gene near the HLA complex, cross reactivity of antibodies to environmental antigens or pathogens with specific HLA antigens and abnormal recognition of 'self' antigens through defects in T cell receptors or antigen processing. At present, however, the mechanisms involved in most HLA disease associations are still not completely understood.

Table 12. Examples of diseases associated with some HLA types

Disease	HLA
Narcolepsy	DR2
Ankylosing spondylitis	B27
Chronic hepatitis	B8
Hodgkin's disease	B18
Haemochromat-osis	A3
Insulin-dependent diabetes	DR3/4
Myasthenia gravis	B8, A2
Rheumatoid arthritis	DR4
Thyrotoxicosis	DR3
Pre-eclampsia	DR4

Disorders of the immune response genetic

Several disorders of the immune system that lead to immunodeficiency are known. Some are acquired while others are genetic in nature. Table …… lists examples of genetic and acquired immuno-deficiency disorders. Figure …… presents sites of hypothetical block in certain immunological deficiency diseases.

Generation of antibody diversity

The unique mechanism of gene rearrangement during B-lymphocyte differentiation into antibody secreting cells, produces enormous antibody diversity, so the body can produce millions of antibodies which can cope with the millions of antigenic determinants (epitopes) entering the body. Other mechanism also play a role in producing more extensive genetic diversity. These mechanisms are listed in Table 14.4.

Table 14.4: Mechanism involved in producing antibody diversity.

1. Multiple V genes.
2. Multiple J * D genes.
3. Combinatorial association.
4. Random association between H & L chains.
5. Somatic cell mutations

(i) Multiple V genes

The H & L chains have multiple V region genes which constitute the baseline from which the antibodies with different specificity are derived. The antigen is responsible for selecting the specific V gene during antibody synthesis.

(ii) Multiple J & D genes

Presence of multiple J genes for L chains and J & D genes provide additional diversity which is referred to as 'Junctional' and 'unsertional' diversity. The precise position at which the genes for the V & J or the V, D & J segments fuse together is not constant and this leads to changes in amino acid sequence at the sites where they join, leading to further diversity in the antigen-binding site. Changes or deletion in amino acids at the junctional sites during DNA rearrangement is referred to as 'junctional diversity'. In addition, small sets of nucleotides may be unserted at the V-D and D-J

junctions by a enzyme known as terminal deoxynucleotidyl transferase without requiring a template and this is referred to as a “Insertional diversity”

(iii) Combinatorial association

The possible association of any V gene with any D or J gene in chain genes and V with any J gene in L chain genes, which occurs during gene rearrangement results in the production of thousands of different H chains and a few hundred (α and λ chains).

(iv) Random association between H &L chains

These thousands of different H chains can associate with any other the hundreds of κ and λ chains, thus resulting in the production of millions of different antibodies. A hypothetical calculation resulting in the production of millions of antibodies based on the different number of V, D and J genes for H chains, and V & J genes for chains is presented in Table 14……

(v) Somatic cell mutation

Differentiated B-lymphocytes are prone to point mutations in the hyper variable regions of the V gene and this leads to further diversity. This is believed to be responsible for fine-tuning of an immune response. It has been shown that somatic cell mutation leads to an increase in affinity of the antibody for the antigen during secondary stimulation.

Table 14. : Examples of Disorder Immune Response

Immune deficiency disorders:

(a) Primary Disorders:

(i) Hereditary:

Associated with B-cell antibodies:
- X-linked infantile Agammaglobulinemia (Burtons agammaglobulinemia).
- Transient hypogammaglobulinemia.
- Selective immunoglobulin deficiency.

Associated with T-cells and cell-mediated immunity:
- Congenital thymic aplasia (DiGeorge syndrome)
- X-linked lymphoproliferative syndrome (Duncan's syndrome)

Severe combined immunodeficiency disease (SCID):
- Adenosine deaminase deficiency.
- Ataxia telangiectasia.
- Wiskott-Aldrich syndrome

Phagocytic dysfunction:
- Chronic granulomatous disease.
- Chediak-Higastic syndrome.

NK-cell deficiency
Leukocyte Adetusion deficiency
Comlement deficiency:
- Complement deficiency
- C1, C4, C2 deficiency.
- Hereditary angioedema
- Paroxyseal nocturnal hemoglobulinuria

(b) Secondary Immunodeficiency:

- Loss of T or B leucocytes or their functions secondary to other diseases.
- Immunodeficiency secondary to intake of chemotherapeutic agents.
- Immunosuppression induced during organ transplantation.

(ii) Acquired Immunodeficiency Syndrome (AIDS)
(iii) Immune diseases due to abnormal aproduction of immune components:
(a) Gammopathies:
  - Multiple myeloma.
  - Macroglobulinemia.
  - Heavy chain diseases

(b) B & T-cell malignancies.