جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

مبادئ الوراثة المناعية

المرحلة الرابعة

الفصل الاول

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Introduction to the Immunogenetics

The field of Immunogenetics is at the core of research aiming at identifying and understanding associations between genetic factors and immunological phenotypes or immunity-related diseases.

Immunogenetics is made from the words **immunity** and **genetics** and is concerned with the genetics of the immune system. **Immunity** derives from the Latin word **immunis** which means ”exempt” and ”genetics” derives from the Greek word **genesis** which means **origin**.

Immunogenetics has now become one of the most challenging disciplines in modem Imrnunogenelin biology. In recent times with the advant of modern techniques like, genetic engineering and monnclonal antibodies, it has come to the forefront of modem sciences.

Immunology has its origins in the study of how the body protects itself against infectious diseases caused by microorganisms, such as bacteria, viruses, protozoa, and fungi, and also parasitic organisms, such as helminth worms.

In its most complex forms, the immune system consists of two branches:

- The Innate immune system that provides a rapid, general, response when alerted by certain typical signals of infection (essentially forming a first-line of defence)
- The adaptive immune system that is able to develop highly specific responses (and a persistent ‘immune memory’) to target the infection with extraordinary accuracy.

Both systems work in close cooperation and, to an important extent, the adaptive immune system relies upon the innate immune system to alert it to potential targets, and shape its response to them.

1- Innate immunity

Innate immunity consists of protective mechanisms we are born with, and are the first line of defence against anything recognized as non-self. The produced immune response is not specific to the antigen and no memory of the antigen persists. However, innate immunity is the crucial first step in most adaptive immune responses.

The following are the protective mechanisms of innate immunity(see Table 1):

- Physical and Chemical Mechanisms
- Phagocytosis
- Molecular Response
- Inflammatory Response

Mast cells and basophils are innate cell types that, when activated, secrete histamine, which can be an important inflammatory mediator produced in response to initial tissue damage as a result of infection. Mast cells are tissue resident (e.g. in mucosal tissues) whilst basophils are found in the blood. In particular, they play a key role in the so-called allergic response. Innate immunity comprises both cellular and humoral ('in solution') elements. The cellular elements are represented notably by **phagocytes** (specifically **neutrophils** and **macrophages**) that can respond to signs of infection (i.e. inflammation) in the tissues and home-in on infective bacteria before

neutralising and engulfing them ('phagocytosis'). Recognition of microorganisms by the innate system occurs via characteristic pathogen-associated molecular patterns (PAMPs) on microbial surfaces, and an important family of innate receptors called pattern-recognition receptors (PRRs) are responsible for this (notably including Toll-like receptors [TLRs]). **The natural killer (NK) cell** is another important innate cell that is able to detect and target intracellular infection of body cells by viruses. A further specialised innate cell is the **eosinophil** that plays a particular role in targeting larger infective organisms, such as parasitic worms.

-The complement system represents the humoral arm of innate immunity, and consists of a number of proteins (found in solution in the blood) that can interact directly, or indirectly, with infective bacteria (through different activation pathways). Inflammation, as a result of infection, allows plasma, containing complement proteins, to enter infected tissues. Once activated, the member proteins assemble to form complexes on the surface of microbes that punch holes in the membrane. The complement activation pathways are termed: the classical pathway, the alternative pathway, and the mannose-binding lectin pathway.

Cytokines form an important family of proteins that function as immune mediators and have important roles during immune responses , they can serve to both stimulate or inhibit the differentiation, proliferation or activity of immune cells. A subset of cytokines, chemokines, play an important role in guiding immune cells to sites of infection by forming a chemical 'trail'.

Table 1 - Innate Immunity

Physical and Chemical Mechanisms	Phagocytosis	Molecular Response	Inflammatory Response
Physical barriers: • intact skin • mucous membrane barrier (sneezing, coughing) • cilia **Chemical barriers:** • tears • acid (pH) • saliva • bile	**Macrophages:** • engulf and kill invading organisms **Dendritic cells:** • engulf pathogen • display antigen on cell surface • travel to lymph node to present antigen to T cells • critical link between the innate and adaptive immune responses.	**Cytokines:** Cytokines are small proteins made by a cell that affect the behavior of other cells. Examples: • Cytokines cause vasodilation (heat and redness) • Some types of *interferon* are antiviral cytokines which help healthy cells resist viral infection **Chemokines:** Chemokines are proteins secreted by macrophages that attract cells out of the blood stream and into the infected tissues. **Complement:** The complement system is a group of approximately 20 proteins that coat bacterial surfaces and promote bacterial destruction by macrophages.	The accumulation of fluid and cells at the site of infection causes the redness, swelling, heat, and pain known as inflammation. Inflammation is beneficial because it: • recruits cells out of the blood stream, • increases the flow of lymph to take away microbes and antigen-bearing cells to the lymphoid tissue which will lead to adaptive immunity, and • brings the T cells and B cells back to the site of infection.

2- Adaptive immunity

Adaptive immunity is the second line of defence against anything recognized as non-self and it provides protection against re-exposure to the same pathogen.

- **Characteristics of adaptive immunity:**

1- Specificity: the immune response is specific to the antigen that produced it (e.g. antibody for measles antigen has no effect on rubella antigen).

2- Tolerance: the immune response is able to differentiate between self and non-self so that body tissues are not destroyed

3- Memory: with subsequent exposure to an antigen there is a rapid and strong immune response. This is called an anamnestic response.

-The adaptive immune response consists of two branches: : (See Figure 1)

- Cellular adaptive response (effected by cytotoxic T cells)
- Humoral adaptive response (effected by B cells).

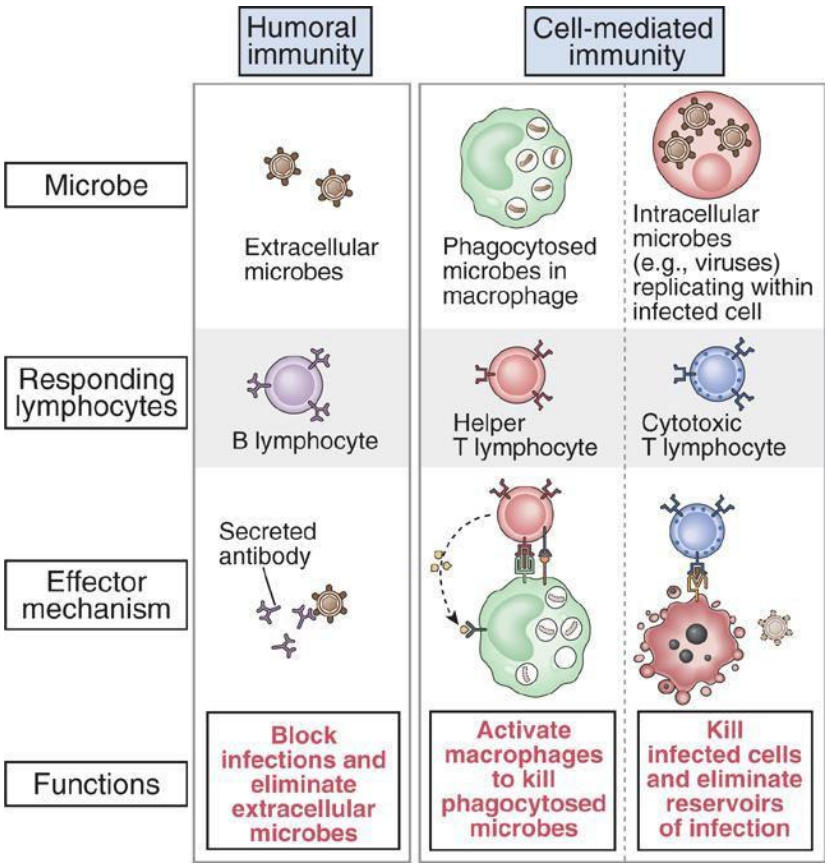


Figure 1 adaptive immune response.

Cellular adaptive response

A key to the adaptive immune response is the lymphocyte. There are several subtypes, however, these fall under two broad designations: **T lymphocytes** and **B lymphocytes** (commonly known as T cells and B cells).

Although both originate in the bone marrow, T cells mature in the thymus, whilst B cells mature in the bone marrow. During an organism's early development a large number of B- and T cells are produced, each of which has the ability to recognise a specific, and essentially unique, molecular target.

Adaptive immunity utilises many kinds of receptor to coordinate its activities. T cells carry **T-cell receptors (TCR)**, whilst B cells carry **B-cell receptors (BCR)**. In addition, another set of receptors, encoded by the major histocompatibility complex (MHC), play an important role in adaptive immunity. **MHC class I receptors** are displayed on a majority of body cells, whilst **MHC class II receptors** are restricted to antigen-presenting cells (APCs). Both of these receptor types interact with TCRs.

T cells do not recognize microorganisms in the extracellular fluids. Instead, T cell receptors bind to fragments of antigens (*epitopes*) that are presented on the surface of antigen presenting cells (APC).

There are three main types of APC:

- Macrophages
- Dendritic cells
- Naïve B cells

When T cells recognize an antigen presented by the APC, they can differentiate into several different types of T cells: (See Figure 2)

- ✓ **Cytotoxic T cells: (also called CD8+ T cells)**.

  Kill cells infected with intracellular pathogens such as viruses

  Helper T cells: (also called CD4+ T cells)

- Activate antigen and stimulate B cells to differentiate and produce antibodies .

- Activate macrophages to become more efficient at killing the pathogen

- Control intracellular bacterial infections (e.g. tuberculosis) that grow in intracellular membrane-bound vesicles of macrophages. The macrophages can't kill the bacteria but instead display the bacterial antigen on the surface so that it can be recognized by T cells

✓ **Regulatory T cells:**

Suppress lymphocytes and control the immune response

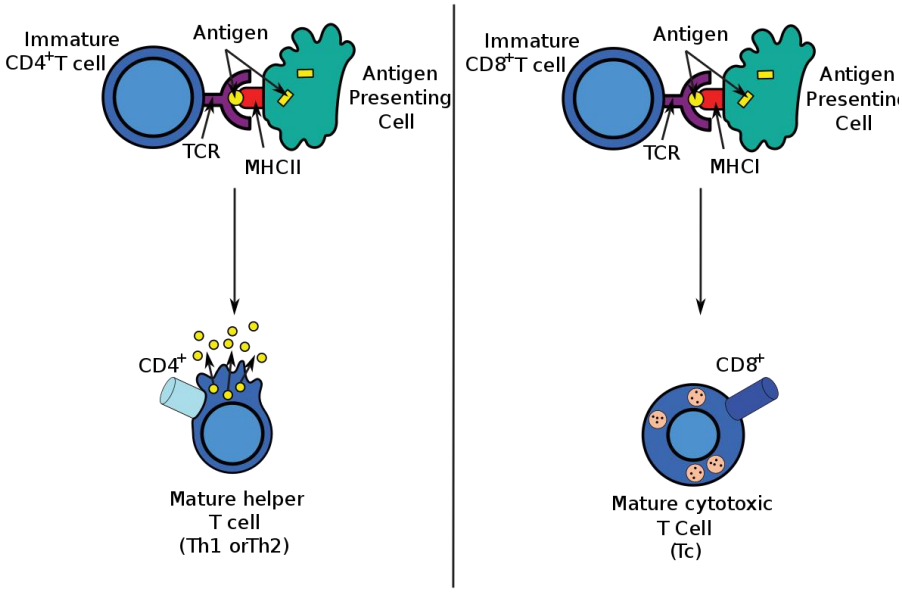


Figure 2 Antigen presentation stimulates T cells to become either "cytotoxic" CD8+ cells or "helper" CD4+ cells.

Humoral adaptive response

Humoral immunity is mediated by B cells. B cells react against foreign substances in the extracellular spaces of the body by producing and secreting

antibodies (Abs). These Abs are present in the biological fluids of the body (the humours); hence the term humoral immunity.

B cells display immunoglobulin molecules (antibodies) on their surface membranes, which act as receptors for the antigens. B cell antibody receptors can either bind to helper T cells that have interacted with an APC or bind to extracellular microorganisms such as bacteria.

Once an antigen binds to an antibody with the best "fit", the B cell differentiates into plasma cells or B memory cells.

- Plasma cells:

These cells operate as factories to manufacture the chosen antibody and then secrete those antibodies.

- B memory cells:

These cells mediate immunological memory. They respond rapidly on re-exposure to the antigen that originally induced them.

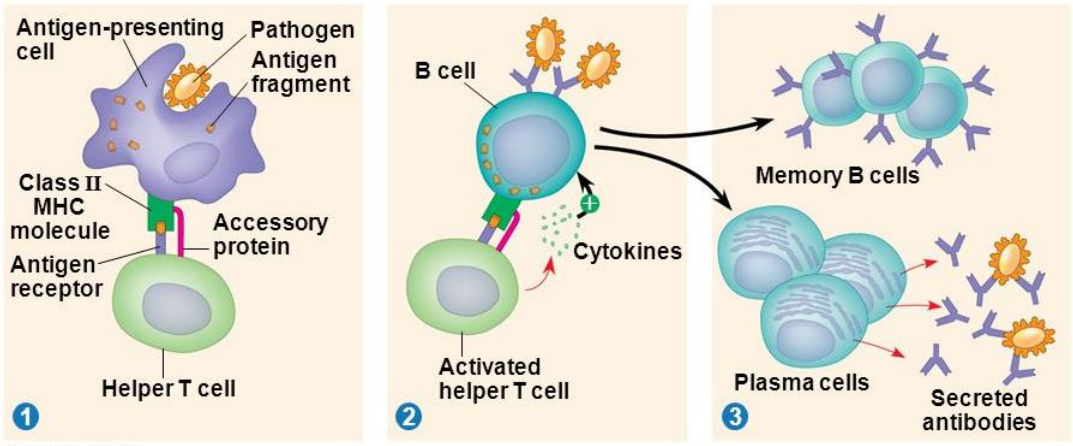


Figure 3 antigen presenting cells

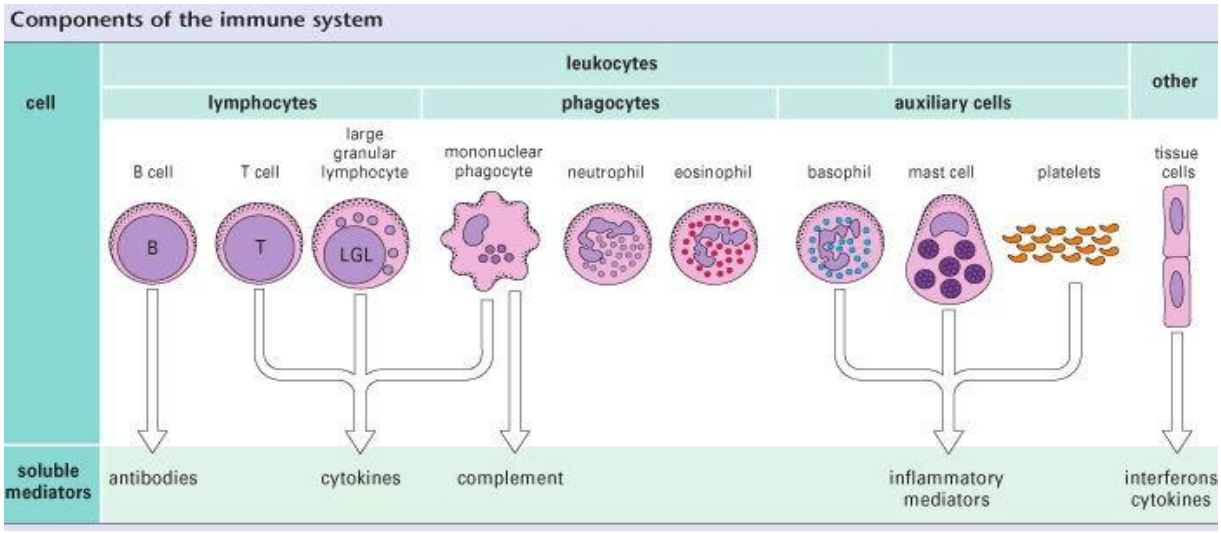
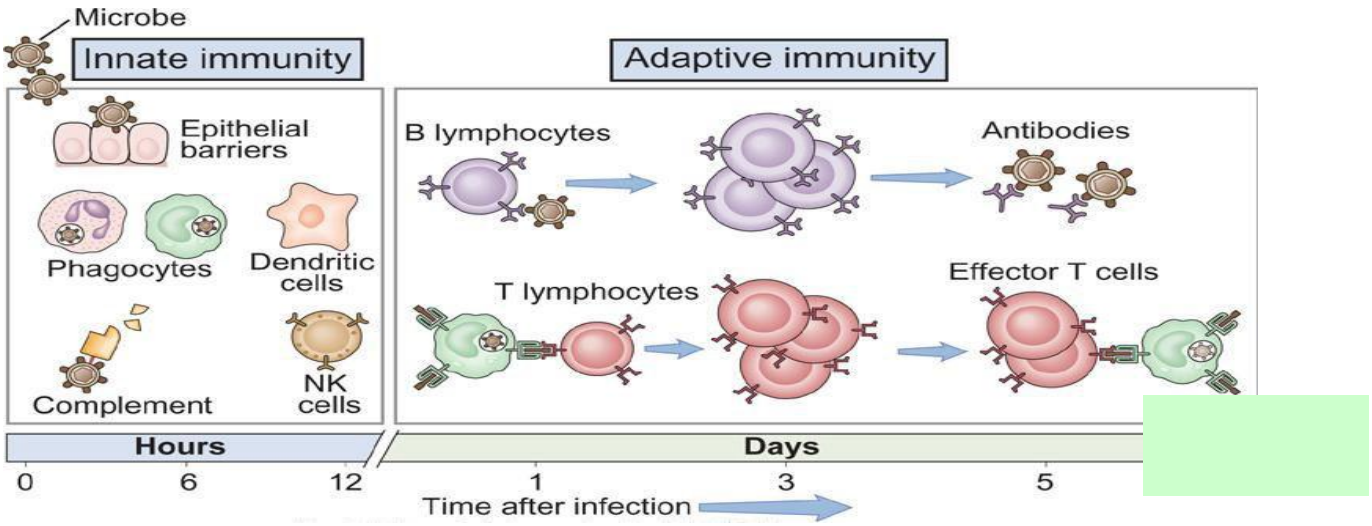


Figure 4 components of immune system .



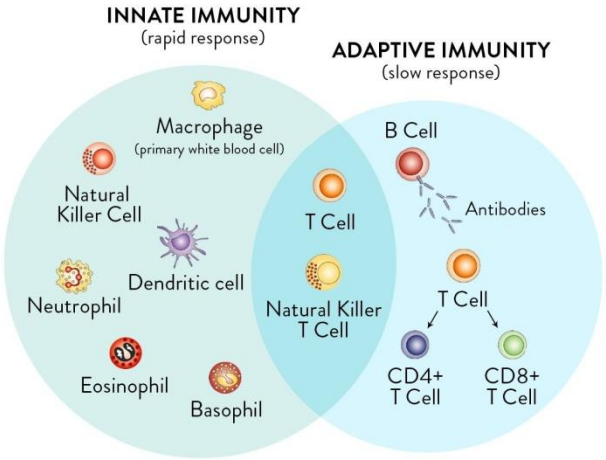


Figure 5 Innate and Adaptive immune system

The major histocomtpatibility complex (MHC)

The mammalian's immune system is capable of distinguishing self from non-self due to a group of protein markers (also called antigen here) known as the **major histocomtpatibility complex (MHC)**. In humans, they are called **human leukocyte antigen (HLA)**. The MHC in mice is called the Histocompatibility system 2 or just the H-2. Two organisms are said to be histocompatible if they can accept solid tissue transplants from each other and incompatible if they cannot.

"Histocompatibility" is a word that says pretty much what it means: "histo" means "tissue" and "compatibility" means "getting along." The *major histocompatibility complex* (MHC) genes were first identified in experiments investigating why the tissues and organs from one individual of a species were destroyed when introduced into another member of the same species.

The genes controlling the histocompatibility of tissue transplantation were localized to a large genetic region containing multiple loci; hence, the term "complex." The molecules encoded by these genes were found to have striking effects on histocompatibility, and to distinguish them from other molecules (encoded elsewhere in the genome) that had relatively minor effects on histocompatibility, these molecules were called the "major" histocompatibility molecules. Thus, the genes encoding these molecules were dubbed the "major histocompatibility complex." Because of the multiple loci present in the MHC, any one individual was found to express a Variety of different MHC molecules on his/her cells.

-The first descriptions of the MHC were made by British immunologist Peter Gorer in 1936. MHC genes were first identified in inbred mice

strains. Clarence Little transplanted tumors across differing strains and found rejection of transplanted tumors according to strains of host versus donor. George Snell selectively bred two mouse strains, attained a new strain nearly identical to one of the progenitor strains, but differing crucially in histocompatibility—that is, tissue compatibility upon transplantation—and thereupon identified an MHC locus. For this work, Snell was awarded the 1980 Nobel Prize in Physiology or Medicine, together with Baruj Benacerraf and Jean Dausset.

The Functions of MHC

MHC is the tissue-antigen that allows the immune system (more specifically T cells) to bind to, recognize, and tolerate itself (autorecognition). MHC is also the chaperone for intracellular peptides that are complexed with MHCs and presented to T cell receptors (TCRs) as potential foreign antigens. MHC interacts with TCR and its co-receptors to optimize binding conditions for the TCR-antigen interaction, in terms of antigen binding affinity and specificity, and signal transduction effectiveness.

Essentially, the MHC-peptide complex is a complex of auto-antigen/allo-antigen. Upon binding, T cells should in principle tolerate the auto-antigen, but activate when exposed to the allo-antigen. Disease states occur when this principle is disrupted.

MHC role in antigen presentation

The events that occur inside a host cell after a protein antigen has entered it are summarized in (Figure 1) .In summary:

• The protein is broken down (catabolized or "processed") to peptides—linear fragments—of varying length.

• Some of these peptides bind to an MHC molecule inside the cell. This binding is selective; that is, not all the peptides formed bind to MHC molecules.

• The MHC molecule with bound peptide moves to the cell surface.

• The combination of peptide bound to an MHC molecule is recognized at the cell surface by a T cell that expresses the "appropriate" or "correct" TCR—one of the billions of different TCRs the host can generate.

Thus, MHC molecules have two key functions:

(1) to **selectively bind** to peptides produced when proteins are processed inside cells of the host

(2) to **present** peptides on the surface of a host cell to a T cell with the appropriate TCR.

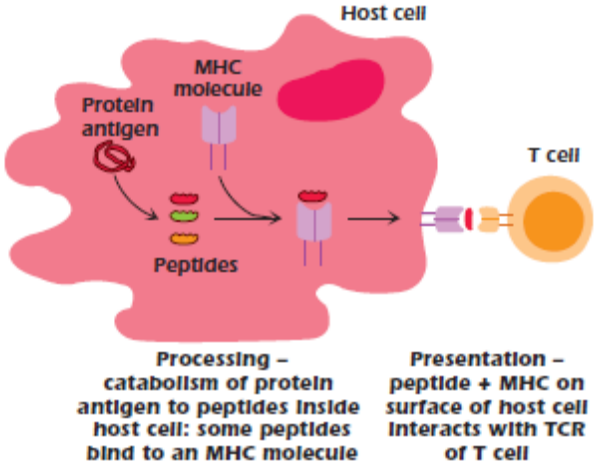


Figure 1 : The role of MHC in antigen presentation to T cells.

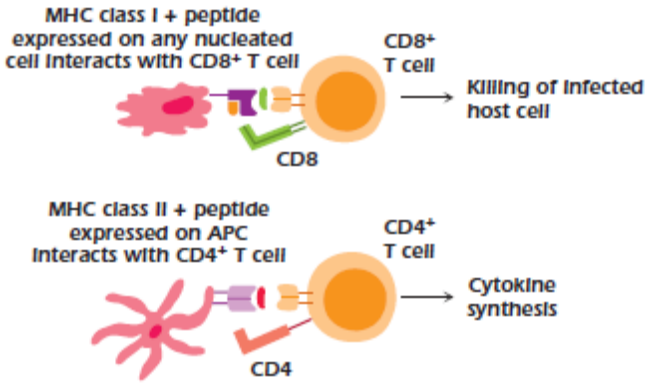


Figure 2 Cells expressing MHC class I interact with CD8+ T cells, which kill infected host cells; cells expressing MHC class II interact with CD4+ T cells, which synthesize cytokines.

MHC Class I

MHC class I molecules interact with CD8, whose expression defines the subset of T cells called **CD8+ T cells**. Thus, to expand on the definition of MHC restriction of T-cell responses we introduced earlier, we say that *the responses of CD8+ T cells are restricted by MHC class I molecules*. MHC class I molecules are expressed on all nucleated cells (thus, not on red blood cells), any of which may be infected by a pathogen such as a virus, bacterium, or parasite. The main function of CD8+ T cells is to kill athogeninfected host cells, as well as tumors and transplanted tissue. Thus, MHC class I molecules and CD8+ T cells play critical roles in the responses to pathogens that infect host cells. In addition to their interaction with CD8 expressed on CD8+ T cells, MHC class I molecules also interact with molecules expressed on natural killer (NK) cells. This interaction prevents NK cells from killing normal host cells.

Structure of MHC class I: (Figure 3)

- Class-I MHC gene encodes a glycoprotein molecule which expressed on the surface of all nucleated cells and platelets.
- MHC-I molecule contains a 45KDa α-chain associated non-covalentely with a 12KDa β2 microglobulin molecule.
- Association of α-chain and β2 microglobulin is required for expression of class-I MHC molecule on cell membrane.

α-chain of MHC-I:

- The α-chain is a transmembrane glycoprotein encoded by polymorphic gene within A, B and C region of Human HLA complex
- The α-chain is anchored in the plasma membrane by its hydrophobic trans-membrane segment and hydrophilic cytoplasmic tail.
- α-chain is made up of 3 domains (α1,α2 and α3). Each domain containing approximately 90 aminoacids, a transmsmbrane domain of about 25 hydrophobic aminoacids followed by short stretch of charged (hydrophilic) aminoacids of cytoplasmic tails of 3o aminoacids.
- α1 and α2 domains interacts to form a deep groove on the top which is a **peptide binding clift**. It can binds antigen of 8-10 animoacids long.
- α3 and β2 are organized into β-pleated sheets, each formed by antiparallel β-strand of aminoacids, this structure is known as immunoglobulin fold. Because of this structure α-chain and β2 microglobulin are classified as member of immunoglobulin super-family receptor.

β2 microglobulin of MHC-I:

- β2 microglobulin is a protein encoded by a highly conserved gene located on different chromosome
- β2 microglobulin is similar in size and organization to α3 domain.
- B2 microglobulin does not contain transmembrane region and is non-covalently linked with α-chain.

Functions of MHC class I:

- The Major function of MHC-I is to bind peptide antigens and present to CD8+ T cells (T helper cells)
- CD8 T cells are specific for MHC-I antigen
- MHC-I bind endogenous antigen and present to T helper cells.
- MHC-I molecules are found on the surface of all nucleated cells.

MHC Class II

MHC class II molecules interact with CD4, whose expression defines the subset of T cells called **CD4+ T cells**. *The responses of CD4+ T cells are restricted by MHC class II molecules.*

MHC class II molecules have a more limited distribution than MHC class I molecules: They are expressed ***constitutively*** (that is, under baseline conditions) only on ***antigen-presenting cells*** (APCs) but can be induced on other cell types. APCs are cells that take up antigen and present it to T cells. In humans, the principal APCs that express MHC class II are dendritic cells, macrophages, and B lymphocytes; thymic epithelial cells also express MHC class II molecules. In the absence of inducing factors, most cells (for

example, liver and kidney tissue cells) express MHC class I but not MHC class II molecules; by contrast, APCs constitutively expresses *both* MHC class I and class II molecules.

In response to activation, CD4+ T cells synthesize a vast array of cytokines, and hence cooperate with multiple types of cells, including helping B cells synthesize antibody. Thus, MHC class II molecules and CD4+ T cells play critical roles in the responses to agents—pathogens and antigens—that are taken into APCs.

Structure of MHC class II: (Figure 3)

- Class-II MHC is the glycoprotein molecule expressed primarily on antigen presenting cells such as macrophages, dendritic cells and B-cells.
- MHC-II molecules contains two different polypeptide chains, 1 33 KDa α-chain and 28KDa β-chain which are associated by non-covalent interactions.

α-chain and β-chain of MHC-II:

- α-chain and β-chain of MHC-II is a membrane bound glycoprotein that contains external domains, a transmembrane segment and acytoplasmic tail.
- α-chain and β-chain are made up of two domains (α1 and α2) and (β1 and β2) respectively.

- The peptide biding cleft is a open ended groove formed between α-chain and β-chain at proximal end. The cleft can bind antigenic peptide of 13-18 aminoacids long.
-

Functions of MHC class II:

- Major function of MHC-II is to bind peptide antigen and present to CD4 T cells.
- MHC-II are found on surface of Antigen presenting cells (APCs).
- CD4+T-cells are specific for MHC-II
- Activates B cells for antibody production
- MHC-II plays a significant role in graft versus host response and in mixed lymphocyte reaction (MLR) because the immune response gene is identical to MHC-II in human.

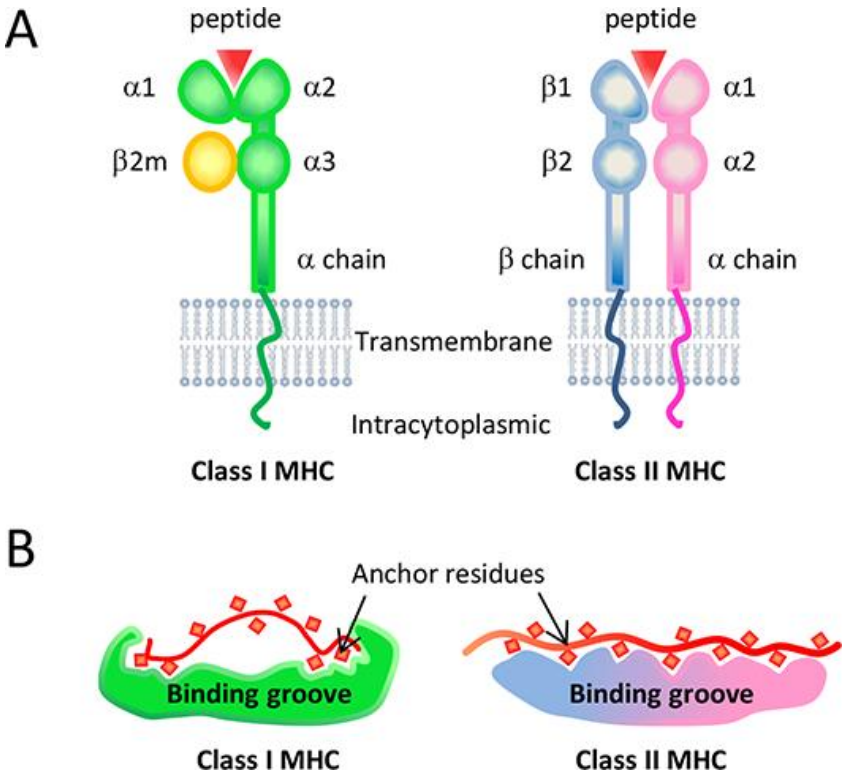


Figure 3 : Structure of MHC molecules and its binding sites.

MHC class III

- MHC III are a diverse group of molecules that serves a wide variety of functions in the immune system.
- MHC III are not a mark on the cell surface.

Functions of MHC class-III:

- Involved in complement activation
- Involved in inflammation caused by cytokines, tumor necrosis factors etc

Major histocompatibility complex (MHC) genes

MHC genes are expressed in an individual that means the alleles of the gene are inherited from both the parents. MHC class I molecules display peptides to the CD8+ lymphocytes to activate cell mediated immune response, and MHC class II molecules display the peptides to CD4+ lymphocytes to activate a humoral mediated immune response. The diversity of the immune system has made MHC class I and II genes to be the most polymorphic genes present in the human genome. In humans, the gene responsible for encoding MHC molecule is located in the chromosome 6 (chromosome 17 in mice). The human MHC class I is encoded by three class of genes namely, HLA-A, HLA-B, and HLA-C. Similarly MHC class II is encoded by genes HLA-DP, HLA-DQ, and HLA-DR. In mice nomenclature for MHC changed to H-2K, H-2D, and H-2L for class I and I-A and I-E for class II (only 2 genes in mice). The set of MHC alleles present on each chromosome are called MHC **haplotype**.

Genetic variation in the MHC influences resistance and susceptibility to a wide variety of infectious diseases, including viruses, bacteria, yeasts, protozoans and intestinal worms. For example, certain MHC alleles in a feral population of sheep in Scotland are associated with increased resistance to an intestinal parasitic nematode, and this resistance to the parasite is associated with increased survivorship. Humans also show many MHC– disease associations, such as with malaria, hepatitis, and human immunodeficiency virus (HIV). Interestingly, most of the diseases associated with certain MHC alleles are autoimmune diseases, such as lupus and arthritis, rather than infectious diseases.

Human MHC Genes (Human Leukocyte Antigen HLA) Genes

1- Human MHC Class I Genes

- **Classical MHC class genes (Major MHC class I)**

HLA-A, HLA -B, and HLA -C are single genes encoding the human classical MHC class I α chains. Each of the HLA-A, HLA-B, and HLA-C genes on both parental chromosomes encodes an α chain that can combine with the invariant β2m molecule to form complete MHC class I α β2m heterodimers, meaning that six different MHC class I molecules can be co-dominantly expressed on any given cell. The human β 2m is located outside the MHC region on chromosome 15.

- **Non-classical class genes (Minor MHC class I)**

The *HLA-E*, *HLA -F* and *HLA -G* genes are encode "nonclassical" MHC products . These genes have a similar structure to the classical class I genes but lack their polymorphism.

2- Human MHC Class II Genes

The classical MHC class II _ and _ chain genes are encoded by separate genes within at least three of the five D families (DP, DQ, DR, DO, and DM). The HLA-DR genes resemble the murine H-2E genes, while those of HLADQ resemble the murine H-2A genes.

Major MHC class II:

1. HLA-DP

- α-chain encoded by HLA-DPA1 locus
- β-chain encoded by HLA-DPB1 locus

2. HLA-DQ

- α-chain encoded by HLA-DQA1 locus
- β-chain encoded by HLA-DQB1 locus

3. HLA-DR

- α-chain encoded by HLA-DRA locus
- β-chains (only three possible per person),

They are encoded by HLA-DRB1, DRB3, DRB4, and DRB5 loci.

The genes of the class II combine to form heterodimeric (αβ) protein receptors that are typically expressed on the surface of antigen-presenting cells.

Minor MHC class II:

- DM
- DO

They are used in the internal processing of antigens, loading the antigenic peptides generated from pathogens onto the HLA molecules of antigen-presenting cell

3- Human Class III Genes

Between the class II and class I genes of the human MHC lies the class III region, which contains additional genes of immunological importance. The human class III region is analogous to the S region of the mouse MHC and contains the corresponding (mostly complement-related and proinflammatory) genes.

The gene cluster was discovered when genes (specifically those of complement components C2, C4, and factor B) were found in between class I and class II genes on the

short (p) arm of human chromosome 6. It was later found that it contains many genes for different signalling molecules such as tumour necrosis factors (TNFs) and heat shock proteins.

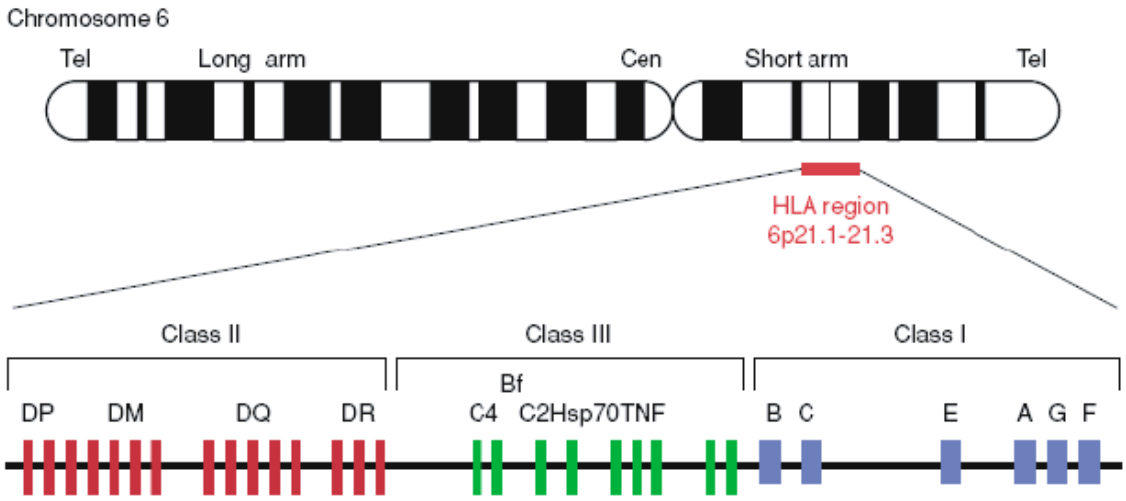


Figure 1: HLA complex is found on chromosome 6 in humans

Nomenclature of HLA Alleles

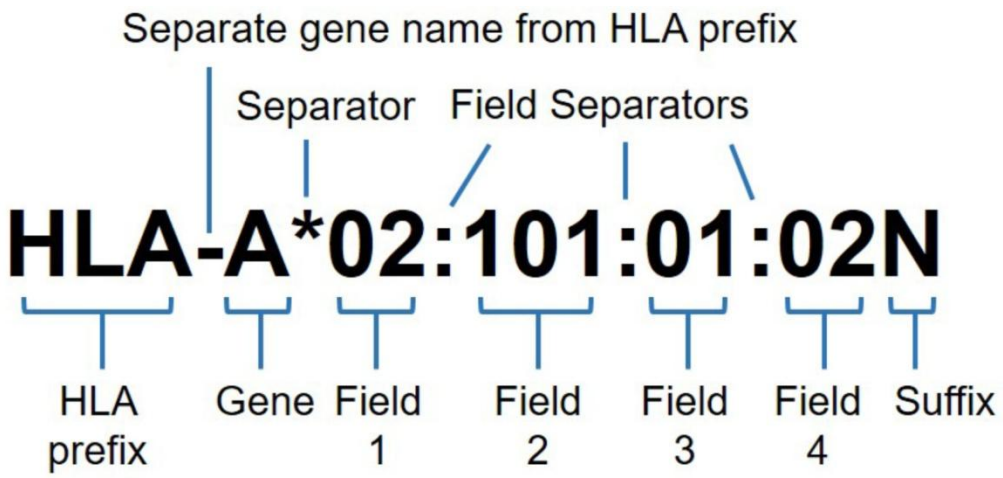
Each HLA allele name has a unique number corresponding to up to four sets of digits separated by colons. The length of the allele designation is dependent on the sequence of the allele and that of its nearest relative. All alleles receive at least a four digit name, which corresponds to the first two sets of digits, longer names are only assigned when necessary.

The digits before the first colon describe the type, which often corresponds to the serological antigen carried by an allotype. The next set of digits are used to list the subtypes, numbers being assigned in the order in which DNA sequences have been determined. Alleles whose numbers differ in the two sets of digits must differ in one or more nucleotide substitutions that change the amino acid sequence of the encoded protein. Alleles that differ only by synonymous nucleotide substitutions (also called silent or non-coding substitutions) within the coding sequence are distinguished by the use of the third set of digits. Alleles that only differ by sequence polymorphisms in the introns, or in the 5' or 3' untranslated regions that flank the exons and introns, are distinguished by the use of the fourth set of digits.

In addition to the unique allele number, there are additional optional suffixes that may be added to an allele to indicate its expression status. Alleles that have been shown not to be expressed - '**N**ull' alleles - have been given the suffix '**N**'. Alleles that have been shown to be alternatively expressed may have the suffix 'L', 'S', 'C', 'A' or 'Q'.

The suffix '**L**' is used to indicate an allele which has been shown to have '**L**ow' cell surface expression when compared to normal levels. The '**S**' suffix is used to denote an allele specifying a protein which is expressed as a soluble, '**S**ecreted' molecule but is not present on the cell surface. The '**C**' suffix is assigned to alleles that produce proteins that are present in the '**C**ytoplasm' and not on the cell surface. An '**A**' suffix indicates an '**A**berrant' expression where there is some doubt as to whether a protein is actually expressed. A '**Q**' suffix is used when the expression of an allele is '**Q**uestionable', given that the mutation seen in the allele has been shown to affect normal expression levels in other alleles.

As of March 2017, no alleles have been named with the '**C**' or '**A**' suffixes.



Field 1: Allele group

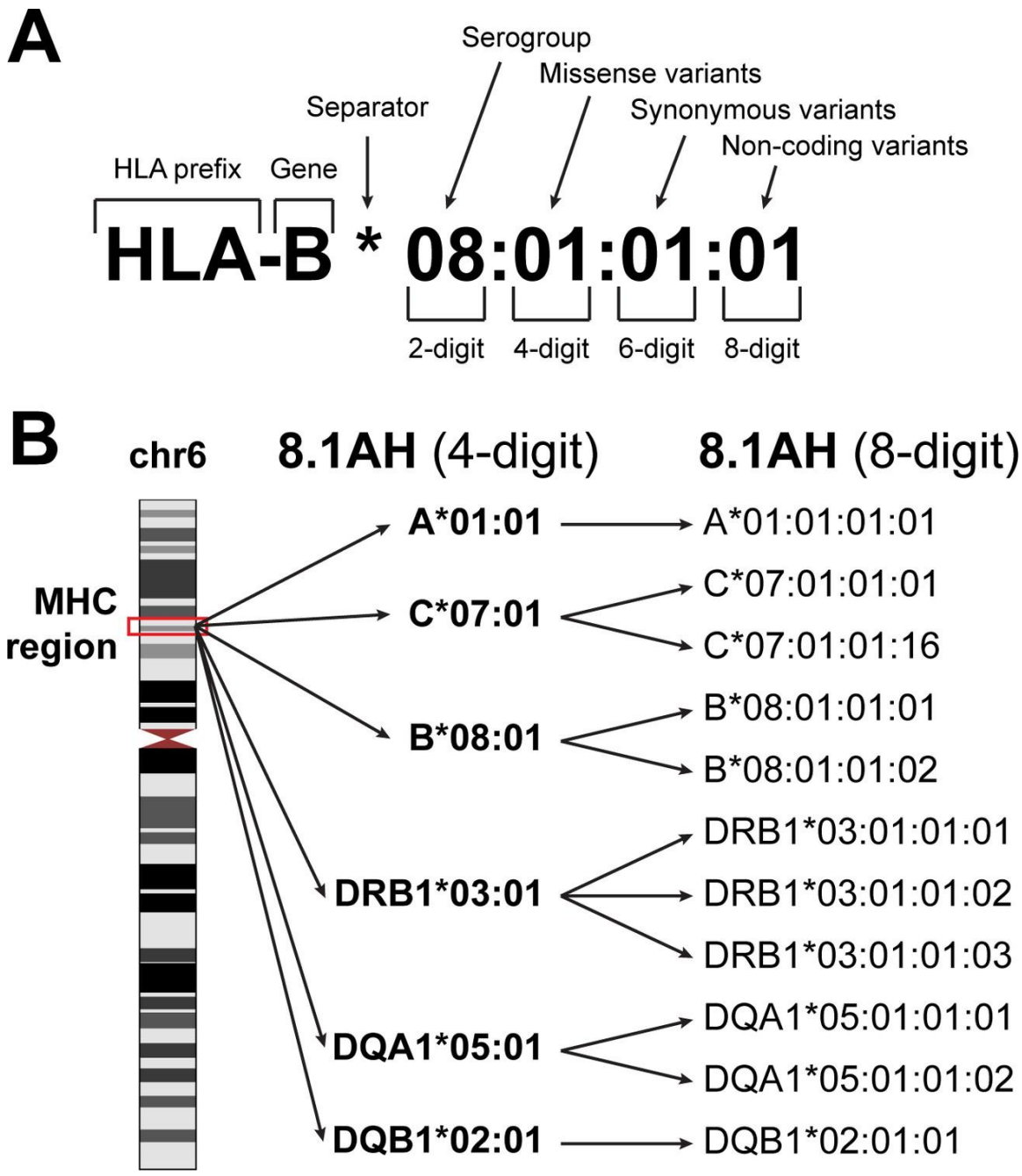
Field 2: Specific HLA protein

Field 3: Synonymous DNA substitution in coding region

Field 4: Changes in non-coding region

Suffix: Denoted changes in expression

Nomenclature	Indicates
HLA	the HLA region and prefix for an HLA gene
HLA-DRB1	a particular HLA locus i.e. DRB1
*HLA-DRB1*13*	a group of alleles that encode the DR13 antigen or sequence homology to other *DRB1*13* alleles
*HLA-DRB1*13:01*	a specific HLA allele
*HLA-DRB1*13:01:02*	an allele that differs by a synonymous mutation from *DRB1*13:01:01*
*HLA-DRB1*13:01:01:02*	an allele which contains a mutation outside the coding region from *DRB1*13:01:01:01*
*HLA-A*24:09N*	a 'Null' allele - an allele that is not expressed
*HLA-A*30:14L*	an allele encoding a protein with significantly reduced or 'Low' cell surface expression
*HLA-A*24:02:01:02L*	an allele encoding a protein with significantly reduced or 'Low' cell surface expression, where the mutation is found outside the coding region
*HLA-B*44:02:01:02S*	an allele encoding a protein which is expressed as a 'Secreted' molecule only
*HLA-A*32:11Q*	an allele which has a mutation that has previously been shown to have a significant effect on cell surface expression, but where this has not been confirmed and its expression remains 'Questionable'


A
Serogroup
Missense variants
Separator
Synonymous variants
Non-coding variants
HLA prefix
Gene
HLA-B * 08:01:01:01
2-digit
4-digit
6-digit
8-digit
B
chr6
8.1AH (4-digit)
8.1AH (8-digit)
MHC region
A*01:01
A*01:01:01:01
C*07:01
C*07:01:01:01
C*07:01:01:16
B*08:01
B*08:01:01:01
B*08:01:01:02
DRB1*03:01
DRB1*03:01:01:01
DRB1*03:01:01:02
DRB1*03:01:01:03
DQA1*05:01
DQA1*05:01:01:01
DQA1*05:01:01:02
DQB1*02:01
DQB1*02:01:01

Genetics of ABO and H Antigens

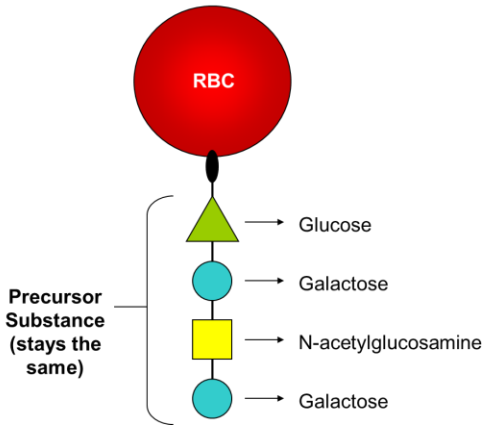
Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three types of alleles inferred from classical genetics: *i*, I^A, and I^B. The *I* designation stands for **isoagglutinogen**, another term for antigen. The gene encodes a glycosyltransferase that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome (9q34).

The I^A allele gives type A, I^B gives type B, and *i* gives type O. As both I^A and I^B are dominant over *i*, only *ii* people have type O blood. Individuals with I^AI^A or I^Ai have type A blood, and individuals with I^BI^B or I^Bi have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship: codominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi,I^Ai). The *cis-AB* phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A or B red blood cells, which can help solve the problem of an apparently genetically impossible blood group.

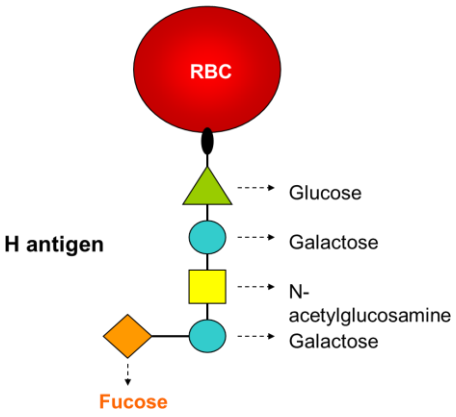
- Inheritance of A and B genes usually results in the expression of A and B gene products (antigens) on RBCs, but H, A and B antigens are not the direct products of the H, A and B genes.
- Each gene codes for the production of a specific transferase enzyme which catalyzes the transfer of a monosaccharide molecule from a donor substrate to a predetermined precursor substance.
- The H gene codes for the production of fucosyl transferase that catalyzes the addition of L-fucose, the immunodominant structure of H antigen, to two slightly different structures, known as the type 1 and type 2 precursor chains. The H gene and its allele h are inherited independently of the allelic A B O genes.
- Once the H gene-specified transferase has acted and the L-fucose has been added to the two chains, the A and B gene-specified products can act to add sugars to the chains that now carry H.
- The A gene codes for production of a galactosaminyl transferase that effects the addition of Nacetylgalactosamine to the preformed H-bearing chains.
- The B gene codes for production of a galactosyl transferase that effects the addition of D-galactose to the same H-bearing structure.
- Thus, the immunodominant structure of the H antigen is L-fucose, of the A antigen Nacetylgalactosamine and of the B antigen, D-galactose.
- In the absence of L-fucose, the immunodominant structure of H, the A and B immunodominant sugars cannot be added. In other words, if an individual does not inherit a functional H gene, the A and B

immunodominant sugars cannot be added to the structures that normally carry those determinants.

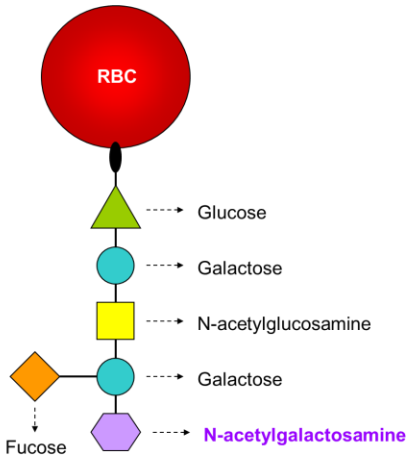
RBC Precursor Structure



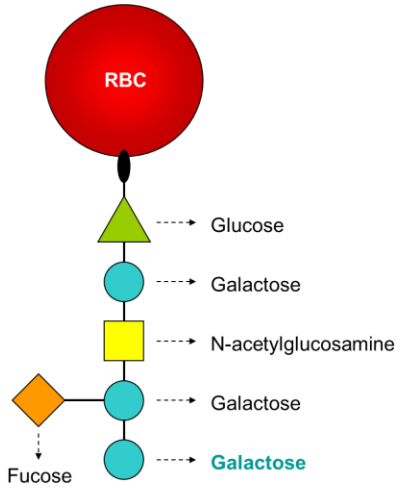
Formation of the H antigen



Formation of the A antigen



Formation of the B antigen



The H antigen, which is found on virtually all RBCs and is the building block for the production of the antigens within the ABO blood group.

H antigen deficiency is known as the "Bombay phenotype" (h/h, also known as Oh) and is found in 1 of 10,000 individuals in India and 1 in a million people in Europe.

In Bombay, India, an individual was discovered to have an interesting blood type that reacted to other blood types in a way that had not been seen before. Serum from this individual contained antibodies that reacted with all RBCs from normal ABO phenotypes (i.e., groups O, A, B, and AB). The individual's RBCs appeared to lack all of the ABO blood group antigens plus an additional antigen that was previously unknown.

Named for the city in which it was first discovered, the "Bombay phenotype" describes individuals whose RBCs lack the H antigen. Because the A and B antigens cannot be formed without the H antigen precursor, their RBCs also lack these antigens. As a result, these individuals produce anti-H, anti-A, and anti-B and can therefore be transfused only with RBCs that also lacks the H, A, and B antigens i.e., they can only receive blood from another person with the Bombay phenotype. Because of the rarity of this blood type, this normally means using blood donations from a suitable relative.

There is no ill effect with being H deficient, but if a blood transfusion is ever needed, people with this blood type can receive blood only from other donors who are also H deficient. (A transfusion of "normal" group O blood can trigger a severe transfusion reaction.)

Because the H antigen is the precursor of the ABO blood group antigens, if it is not produced, the ABO blood group antigens are also not produced.

If patients with anti-H in their circulation receive transfusions of blood that contains the H antigen (e.g., blood group O), they are at risk of suffering an acute hemolytic transfusion reaction.

The H blood group locus (containing FUT1) and the secretor locus (containing FUT2) are located on chromosome 19 at q.13.3. FUT1 and FUT2 are tightly linked, being only 35 kb apart. Because they are highly homologous, they are likely to have been the result of a gene duplication of a common gene ancestor.

The H locus contains the FUT1 gene, which is expressed in RBCs. At least one functioning copy of FUT1 needs to be present (H/H or H/h) for the H antigen to be produced on RBCs. If both copies of FUT1 are inactive (h/h), the Bombay phenotype results.

The Se locus contains the FUT2 gene, which is expressed in secretory glands. Individuals who are "secretors" (Se/Se or Se/se) contain at least one copy of a functioning enzyme. They produce a soluble form of H antigen that is found in saliva and other bodily fluids. "Non-secretors" (se/se) do not produce soluble H antigen. The enzyme encoded by FUT2 is also involved in the synthesis of antigens of the Lewis blood group.

Immunoglobulins

Definition: Glycoprotein molecules that are produced by plasma cells in response to an immunogen and function as antibodies.

Immunoglobulin Structure

1. Basic Structure of Immunoglobulins:

A. Heavy and Light Chains:

All Igs have a four chain structure as their basic unit. One pair of the polypeptide chain contains approximately twice as many amino acids as the other pair. They are called heavy (H) chains (50-70 KDa.) and light (L) chains (25 KDa.) respectively.

B. Disulphide bonds:

1) Inter-Chain: The heavy chain and light chain and the two heavy chains are held together by inter-chain disulphide bonds.

2) Intra-Chain: Within each of the polypeptide chains, there are also intra-chain disulphide bonds.

C. Variable (V) and Constant (C) regions:

Both the H-chain and L-chain can be divided into two regions based on variability in the amino acid sequences.

1) Light Chain: Variable region, V_L (110 amino acids) and constant region, C_L (110 amino acids)
2) Heavy Chain: Variable region, V_H (110 amino acids) and constant region, C_H (330-440 amino acids).

The antibody (Ig) binds with the antigen through the V-region of the heavy and light chains, in other words a part of the Fab- fragment. The Fc – fragment is responsible for the biological activities.

D. Hinge region:

The region of which the arms of the antibody molecule forms a 'Y' is called the hinge region, because there is some flexibility in the molecule at this point.

E. Domains:

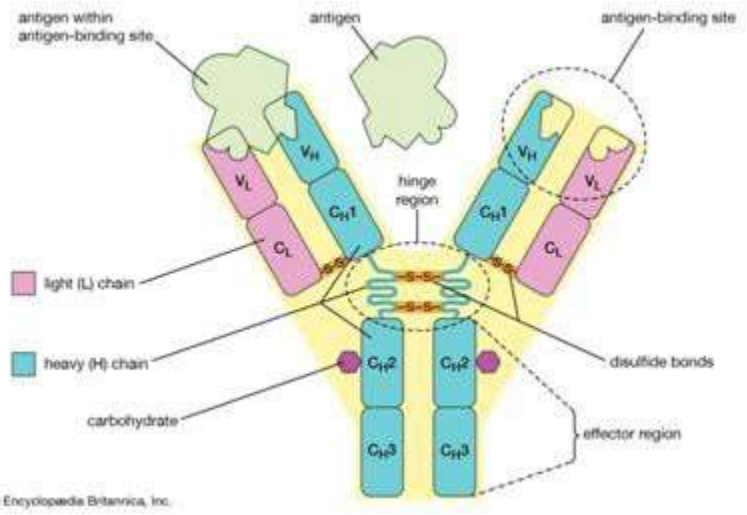
Ig molecule is folded into globular regions, each of which contains an intra-chain disulphide bond. These regions are called domains.

1) Light chain Domains – V_L and C_L
2) Heavy Chain Domains – V_H and C_{H1} – C_{H3}(orC_{H4})

F. Oligosaccharides:

Carbohydrates are attached to the C_{H2} domain in most immunoglobulins.

Immunoglobulin heavy and light chains are each encoded by a separate multigene family.



General Functions of Immunoglobulins

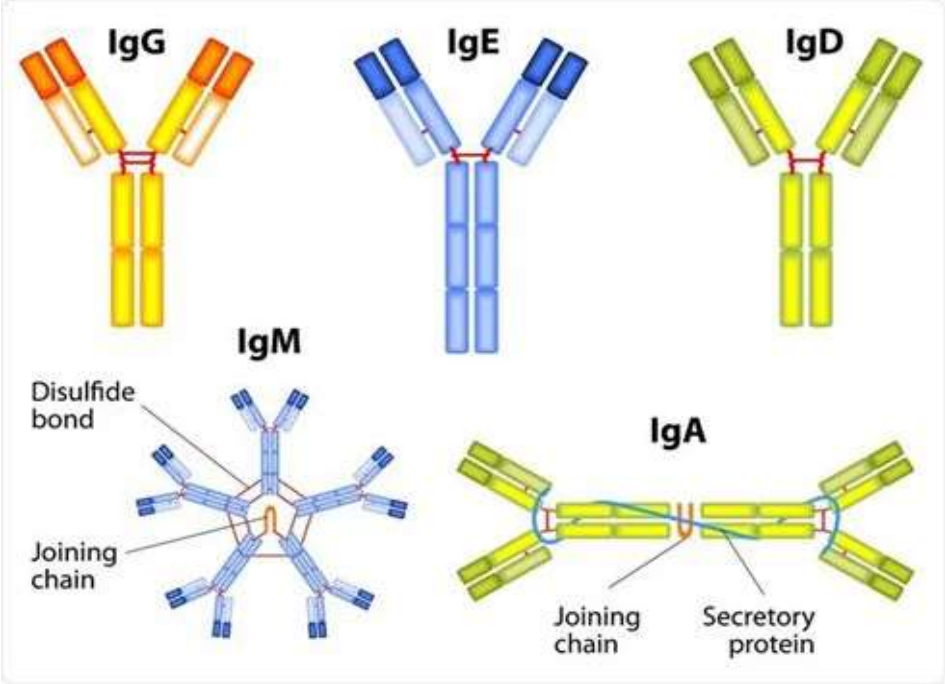
Antigen binding: Can result in protection Effector functions

-Fixation of complement

-Binding to various cells

Human Immunoglobulin Classes

1. IgG - Gamma heavy chain (γ)
2. IgM - Mu heavy chain (μ)
3. IgA - Alpha heavy chain (α)
4. IgD - Delta heavy chain (δ)
5. IgE - Epsilon heavy chain (ε)



Immunoglobulin G (IgG)

-Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in blood circulation.

- IgG antibodies are large globular proteins with a molecular weight of about 150 kDa made of four peptide chains

- IgG molecules are created and released by plasma B cells.

-Each IgG has two antigen binding sites.

-IgG-mediated binding of pathogens causes their immobilization and binding together via agglutination

-IgG activates all the classical pathway of the complement system.

- IgG also binds and neutralizes toxins.

-IgG also plays an important role in antibody-dependent cell-mediated cytotoxicity (ADCC) and intracellular antibody-mediated proteolysis

- IgG is also associated with type II and type III hypersensitivity reactions

Immunoglobulin IgM

-IgM is the largest antibody, and it is the first antibody to appear in the response to initial exposure to an antigen.

- IgM is constructed of five or six units (i.e. mostly as pentamers but also hexamers occur) which are each comprised of two heavy-chains (mu-chains) and two light chains, bound together by disulfide bonds and a so-called J-chain.

- IgM is present on B cells and its main function apparently is the control of B-cell activation. B-cells create IgM antibodies as a first line of defense.

-It is a good complement fixing Ig leading to lyses of microorganisms

- It is also a good agglutinating Ig, hence clumping microorganisms for eventual elimination from the body.

- It is also able to bind some cells via Fc receptors.

Immunoglobulin IgA

-Serum IgA is monomeric, but IgA found in secretions is a dimer having a J chain. Secretory IgA also contains a protein called secretory piece or T- piece, this is made in epithelial cells and added to the IgA as it passes into secretions helping the IgA to move across mucosa without degradation in secretions

- It is the second most abundant Ig in serum

-It is the major class of Ig in secretions- tears, saliva, colostrums,mucus,and is important in mucosal immunity.

-It binds to some cells- PMN cells and lymphocytes

- It does not normally fix complement.

Immunoglobulin IgD

-Is about 1% of proteins in the plasma membranes of immature B-lymphocytes where it is usually co-expressed with another cell surface antibody called IgM.

- IgD is also produced in a secreted form that is found in very small amounts in blood serum , Secreted IgD is produced as a monomeric antibody with two heavy chains of the delta (δ) class, and two Ig light chains.

- IgD has important immunological functions.
- In B cells, the function of IgD is to signal the B cells to be activated. By being activated, B cells are ready to take part in the defense of the body as part of the immune system.
- When a B cell reaches its mature state, it co-expresses both IgM and IgD
- - IgD was found to bind to basophils and mast cells and activate these

cells to produce antimicrobial factors to participate in respiratory immune defense in humans.

Immunoglobulin IgE

- IgE is a type of antibody that has only been found in mammals.

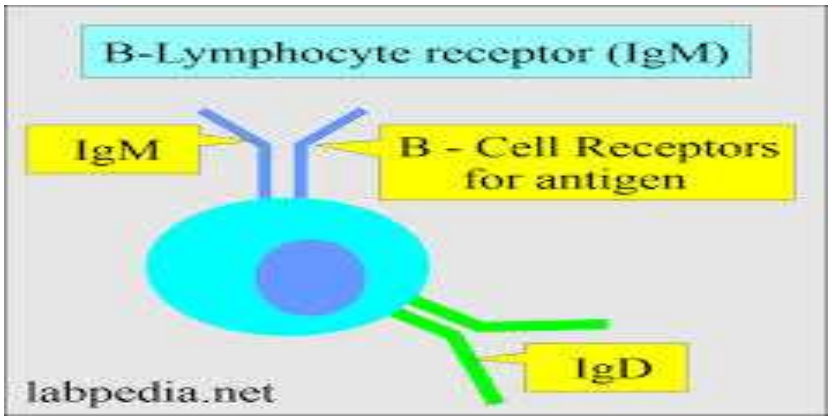
- IgE is synthesised by plasma cells.

- IgE has co-evolved with basophils and mast cells in the defence against parasites like helminths (like Schistosoma)
- effective in bacterial infections.
- IgE may play an important role in the immune system's recognition of cancer

Immunoglobulin Gene Organization

Immunoglobulin exists in two forms: secreted and membrane bound, which are identical except for differences in the C-terminal portion, where one has a transmembrane anchor region and the other does not. These two different forms are generated by differential splicing of messenger ribonucleic acid (mRNA). **Secreted immunoglobulin** is made mainly by plasma cells and may bind to microbes and act to neutralize, opsonize, fix complement, or serve other functions.

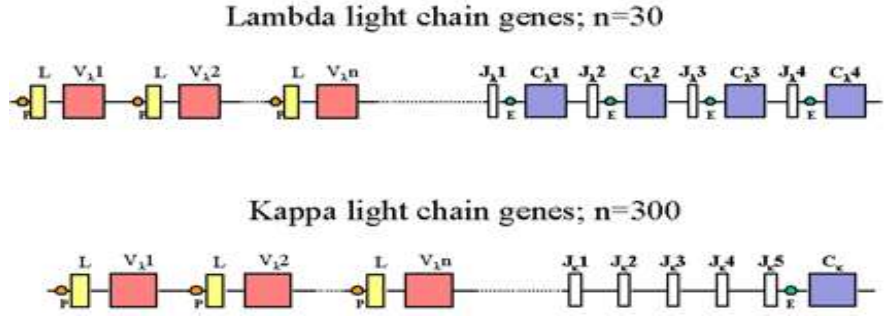
Membrane-bound immunoglobulin, in association with signal-transducing molecules, **Ig_{alpha} and Ig_{beta},** serves as a receptor for antigen on the surface of B cells (BCR). The role of the BCR is to recognize, bind, and present antigen specific for the variable region. This also creates a tonic signal for B cell survival during B cell development(Figure 1)



Immunoglobulin M (IgM): Immunoglobulin: B-lymphocyte receptors(Figure 1)

There are several classes of immunoglobulin that are categorized by a unique heavy chain structure: IgG, IgM, IgA, IgD, and IgE. There are also subclasses of IgG (IgG1 to 4) and of IgA (IgA1 and IgA2). In addition, there are two different types of immunoglobulin light chains, kappa and lambda. Immunoglobulin heavy chains and each type of light chain are encoded by genes in different loci. The table shows the locations of these gene complexes on human chromosomes table (1)

Locus	Chromosome
Heavy chain	14q32
Kappa light chain	2p12
Lambda light chain	22q12



Organization of the *kappa* and lamda light chain genes in the germ line or undifferentiated cells

Genes capable of encoding a complete immunoglobulin heavy or light chain do not exist as such within the deoxyribonucleic acid (DNA) of most cells. The complete genes are assembled by the union of separate gene segments, termed "rearrangement." These segments are widely separated in germ cells and in all somatic cells, except for B lymphocytes. Within B lymphocytes, these genes become rearranged to create a "mature" immunoglobulin gene that can encode a functional protein. This rearrangement process is the core of the immune system's ability to generate antibodies capable of recognizing the tremendous variety of antigenic structures in nature

An immunoglobulin heavy chain gene is assembled from four types of gene segments

- The heavy chain variable region (V_H)
- The heavy chain joining region (J_H)
- The heavy chain constant region (C_H)
- The diversity gene segment (D)

An immunoglobulin light chain gene is assembled from three types of gene segments These are:

- The light chain variable region (V_L)
- The light chain joining region (J_L)
- The light chain constant region (C_L)

Origin of Antibody Diversity History :

Diversity: is the total of all Antibody specificities that an organisms is capable of expressing

-Germ line theory : This theory states that we have different V region for each possible antibody we can make

-Somatic hyper –mutation theory : this theory states that we have only one or a few v region genes and the diversity is generated by somatic mutations which occur in these genes

HLA and Disease Associations

The discoveries of HLA associations with certain diseases have been a major breakthrough in our understanding of the genetics of these diseases. We now know that at least part of the genetic basis of the associated diseases lies in the HLA region of chromosome 6. Furthermore, the "disease susceptibility genes" for hemochromatosis, congenital adrenal hyperplasia, and olivopontocerebellar ataxia (Menzel type) are known to be in linkage with HLA and have been mapped on chromosome 6. However,it is interesting to note that in the beginning these HLA associations generated high hopes and enthusiasm that the etiology of these diseases will soon be resolved. Yet, surprisingly, a decade after the landmark results on HLA-B27 and ankylosing spondylitis we are still searching for the mechanism involved in these associations. Various hypotheses have been proposed but thus far none is supported by clear-cut evidence. The nature of the mechanisms underlying the empirically observed associations between HLA antigens and diseases has been the subject of much speculation. In a discussion of the association mechanism it is important to recapitulate some of the general characteristics of the available HLA data.The primary data showing the associations are increased frequencies of certain HLA antigens in groups of patients as compared with a sample of normal individuals. Some disease studies of families with more than 1 patient have also demonstrated the disease segregation with an HLA haplotype within a family. However, all the reported pedigrees on a particular disease do not show a consistent segregation pattern. Beyond this, at present there are very little data on humans that can be used as bases for proposing mechanisms for the observed associations.Furthermore, none of the observed associations is absolute. The strongest association to date is that of ankylosing spondylitis with B27 with a relative risk of about 70.0 estimated from the pooled data on all Caucasian patients. It should also be noted that the diseases that are associated with HLA antigens do not show simple Mendelian segregation in families, have a very weak or no effect on reproduction, and are of unknown etiology. Heterogeneity is another important characteristic of these diseases

The proposed mechanisms for the diseas associations can be categorized into 2 main groups.

1- Involvement of HLA Antigens

- Receptors

It has been suggested that HLA antigens may act as receptors for pathogenic organisms (e.g., viruses). Thus, it is hypothesized that B27 molecules on the surface of lymphocytes may be the receptors for some organisms responsible for ankylosing spondylitis, Reiter's syndrome, and other B27-associated diseases.

- Molecular Mimicry

The molecular mimicry hypothesis, originally proposed by SnelI, postulates that the molecular structures of infectious agents are similar to those of the HLA antigens on the cell surface. Because of this mimicry the host organism cannot recognize such viruses, bacteria, and parasites as "non-self' and is, therefore, unable to initiate an immune response.

- Interaction of HLA Molecules with Nonimmunologic Ligands

Svejgaard and Ryder have postulated that HLA antigens may interfere with ligand-receptor interactions not directly involved in immune reactions. It was hypothesized that some HLA molecules may have structures similar to those on receptors for certain hormones that could cause competition between HLA and receptor molecules for the hormone. Such interactions may, under certain conditions, lead to nonimmunologic diseases, such as hemochromatosis.

- Viral Modification of HLA Antigens

It has been shown by Doherty and Zinkernagel that cytotoxic T cells from mice infected acutely with lymphocytic choriomeningitis virus interact only with H-2-compatible virus-infected cells. Similar results have been observed for vaccinia virus by Koszinowski and Ertl and for ectromelia virus by Blanden et a1

2- Involvement of Genes Closely Linked with the HLA Complex

The second group of hypotheses proposes that HLA antigens are not involved in the causation of the disease. They are markers for the "disease susceptibility genes" that are very closely linked with the HLA complex. The observed population associations are due to the existence of linkage disequilibria between the HLA antigens and the alleles for the disease susceptibility genes. As an

example, it can be postulated that the association of DR2 with multiple sclerosis (MS) is due to the existence of an MS susceptibility gene very closely linked with the DR locus. The disease susceptibility allele of this locus is in linkage disequilibrium with the DR2 allele of the DR locus.

- Immune Response Genes

It is postulated that disease associations are due to the immune response (Ir) genes closely linked with the HLA complex and in linkage disequilibrium with certain alleles of A, B, C and D/DR loci. Histocompatibility-linked immune response genes have been shown to exist in mouse and other animal species. In the mouse Ir genes have been mapped in the I region of the chromosome. The D/DR lociin humans are believed to be analogous to the I region in the mouse and thus it has been hypothesized that Ir and immune suppression genes also exist in the D/DR region of the HLA complex.

- Metabolic Genes

It is possible that genes other than those involved in immune response and immune suppression are linked with the HLA complex. These genes may be responsible for the diseases with no apparent immunologic basis. These genes may affect some steps in metabolic pathways through enzymes or common precursor substance.

HHLA and INFECTIOUS DISEASES

Although numerous diseases in other sections have some relationship to infections,such as the reactive arthridities that follow Shigella, Salmonella, and Yersinia infections, this particular section will deal with the immediate response to infectious agents.

HLA and Viral infection

1- Infectious Mononucleosis

Infectious Mononucleosis HLA antigen frequencies in numbers of patients with infectious mononucleosis showed higher than controls had low EBvirus titers, or from that of EB-virus antibody-negative controls.'HLA Locus play roles in infection.

2- Congenital Rubella

Studied showed an increased incidence of several HLA antigens. Al showed the greatest alteration with an increase to 40.8% in the entire group of 87 patients compared to 32% of controls.correlate the different frequencies of HLA antigens with seropositivity to rubella in different populations .

3 -Recrudescent Herpes Labialis

The herpes viruses have an unusual propensity for establishing latent infections after the initial primary exposure. Reactivation of the virus may occur at intervals. In man, the cytomegalovirus, varicellar zoster, and especially herpes simplex virus Types 1 and 2 share this characteristic, study of his original observation that there was an increase in those individuals with a susceptibility to recurrent infection with herpes virus Type 1 .This was demonstration of an association of a known viral disease with an HLA antigen.

4-Cytomegalovirus Infection

cytomegalovirus (CMV) infections. An examination of the HLA antigens of those with CMV showed an increase. CMV infections occurred in 8 of 12 HLA identical siblings as well as an identical twin.

HLA and Bacterial infection

1-. Haemophilus Influenza — Type b

More patients with Haemophilus influenza type b infections (epiglottitis or meningitis) showed the following significant differences in HLA antigen

2- Streptococcal

Streptococcal Antigen Responses (including Rheumatic Fever and Heart Disease) . the Lymphocytes from random individuals when tested for their response to Streptococcal antigens (streptokinase-streptodornase [SK/SD]) exhibited a high responder group significantly increased The response

pattern to this purified Streptococcal antigen was found to be associated with HLA haplotypes .

3- Leprosy

Leprosy affects only a small proportion of those who become exposed to Mycobacterium leprae because the great majority of exposed individuals develop effective immunity.independent family studies suggest that a genetic determinant for tuberculoid leprosy is linked to the HLA locus or

chromosome 6. The mode of inheritance appears to be recessive.

4-. Gonococcal Urethritis

HLA-DR2 and HLA-B7 may be association with infection .

5- Syphilis

HLA-B8 and HLA-BI5 may be association with infection .

HLA and Protozoan Infections

1- Malaria.

The association between polymorphism at the hemoglobin S (HbS) locus and susceptibility to severe clinical forms of malaria provides the archetypal example of the selective force of infection in human evolution. Malaria is a complex disease, with different clinical outcomes. there is strong a priori

support for the candidacy of polymorphism at HLA class II and class I in determining susceptibility to human Malaria.

2- Leishmaniasis.

Different species of Leishmania cause a spectrum of clinical diseases in humans, including localized, mucosal, diffuse, and disseminated forms of cutaneous disease, as well as visceral leishmaniasis that can be followed posttreatment by post-Kala-azar dermal leishmaniasis. Early studies of Leishmania donovani in mice showed dramatic differences in visceral disease in livers and spleens in congenic mice with different H-2 haplotypes . Genetic analysis using recombinant congenic mice and functional analysis blocking IA or IE (corresponding to DQ and DR, respectively) molecules in support for the candidacy of polymorphism at HLA class II and class I in determining susceptibility to human visceral leishmaniasis.

HLA and Helminth Infections

A number of studies have demonstrated that host genetics is an important determinant of susceptibility to human helminth infections for Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Schistosoma mansoni and for measures of worm burden and worm biomass, respectively. Immunologically, helminth infections are associated with polarized CD4 Th2 responses and with high immunoglobulin E levels. HLA class II molecules are therefore considered functionally important, and the genes encoding them are potential susceptibility loci

The HLA and the multifactorial genetic disease

Studies of HLA and disease were begun when only the class I antigens were known. Thus, all the first associations discovered were with class I antigens. Subsequently, when the class II antigens were defined, many of these diseases were actually shown to have a stronger association with the class II antigens that are in linkage disequilibrium than with the previously associated class I antigens. HLA antigens was of primary clinical importance in transplantationand of great basic interest in human genetics and anthropology, a rather unexpected bonus has been the determination that HLA antigens are associated with disease susceptibility to a greater extent than any other known genetic marker in man. many genetic polymorphisms have been suspected to be associated with diseases.

The HLA region is characterized by high linkage disequilibrium (LD) levels, which leads to difficulty when genotyping using high-throughput methods. Although HLA is underrepresented on GWAS chip arrays, GWASs have identified massive numbers of variants within the HLA gene that are associated with complex diseases. In particular, alternative uses of HLA imputation methods have allowed the imputation of classical HLA alleles and the use of LD information to establish relationships between alleles and single nucleotide polymorphisms (SNPs) in the HLA region. These approaches can provide guidance and new directions for the study of HLA alleles.

Thalassemia Major

HLA alloimmunization is a potential complication of red blood cell (RBC) transfusion with detrimental consequences for future organ or hematopoietic stem cell transplantation. HLA antibodies(HLA Class I and II antibodies) have a high prevalence in TM patients and may be associated with nonleukoreduced transfusions and older age. For such patients, antibody identification will be useful if subsequent organ or stem cell transplantation is needed.

Tendinous Calcifications

calcification disease reveals the existence of rounded intra- and extracellular crystalline formations. These crystalline formations of calcium and phosphorus are made up of accumulations of tiny crystals shaped , HLA typing of patients with multiple tendinous calcification or single tendinous calcification shows an increased frequency of HLA A2 and BW 35 in comparison with controls.

Calcifying tendinopathy is a common disorder of the shoulder, of unknown etiology, characterized by deposition of calcium crystals inside one or more tendons of the rotator cuff. Some authors have also correlated calcifying tendinopathy with a genetic predisposition, through higher levels of human leukocytic antigen A1 (HLA-A1) in the patients affected, in comparison with the healthy population

Iron-Overload-Associated Myopathy

Disease resulting from massive iron deposits in various organs, including heart, liver, and pancreas, may lead to architectural and functional disturbances of these organs. Even though Iron overload can occur in nonuremic as well as in uremic individuals. the patient has unexplained cardiomyopathy, hepatic cirrhosis, proximal myopethy, diabetes mellitus, arthropathy, or Immune dysfunction such as listeriosis.

HLA-A3, B7, and B14 antigens are associated with idiopathic hemochromatosis and were tested in hemodialysis patients, some of them with myopathy, showing correlation between presence of the HLA-antigens and serum ferritin levels . Patients on maintenance hemodialysis carrying these hemochromatosis-related antigens have an increased risk of iron overload and muscle iron deposition

Dupuytren's Contracture

Dupuytren’s disease (DD) is a familial, fibroproliferative, irreversible, and progressive disease of the palmar fascia. The highly polymorphic human leukocyte antigen (HLA) region is an ideal biomarker target. There have been some coherent data within the literature to suggest a genotype to phenotype association

between certain HLA loci and a number of fibrotic disorders such as keloid and scleroderma, markedly with class II molecules and disease pervasiveness and clinical progression.

Schizophrenia

genetic association of human leucocyte antigens (HLA) and alleles with schizophrenia, A schizophrenia locus on chromosome 6p near the HLA region has also been reported, on the basis of linkage studies. HLA association investigations should employ operational diagnostic criteria, comparison subjects screened for illness and HLA genotyping, and should include both association studies of candidate alleles and transmission disequilibrium and haplotype relative risk studies.

Manic-Depressive Disorder

Bipolar disorder, formerly called manic depression, is a mental health condition that causes extreme mood swings that include emotional highs (mania or hypomania) and lows (depression).
study assess the relationship of HLA to affective disorders; the role of HLA as a marker of susceptibility to affective disorder , a positive association between manic-depressive disorders and HLA-A3, HLA-B7, and HLA-Bw16

Alzheimer's Disease

immunore-activity for HLA-DR association Alzheimer's disease

Multiple Infarct Dementia

the genetic factors in demented patients in Japan, HLA antigens were examined HLA B16

Narcotic Dependence

The occurrence of HLA-B5- and HLA-CW4-antigens Potential use of these antigens as genetic markers of predisposition to narcotization is discussed.

Tourette Syndrome

multiple individuals manifesting Tourette syndrome (TS) or related abnormal movements were evaluated for linkage between TS and HLA-A, B, C and DR antigens.

Polycystic Kidneys

HLA-B8 sugested association

Retroperitoneal Fibrosis

histocompatibility antigen HLA-B27 were present association

Human leukocyte antigen and tumors

Immune surveillance and immune escape are closely related to the onset of tumors. The pathogenesis of tumors has been found to be associated with the abnormal regulation of HLA class I and II molecules. Tumorigenesis is closely connected with T cell-based immune surveillance. Tumor cells can escape $CD8^+$ T cell surveillance and death when HLA I gene variations lead to the loss of HLA I antigen expression or reductions in HLA I antigen density, resulting in tumor formation and development. Because of the genetic instability of tumors, neoantigens, called tumor-associated antigens, are generated. Classically, tumor-associated antigens are generated from apoptotic or necrotic cells and are presented by class II molecules to $CD4^+$ T cells, ultimately activating $CD8^+$ cytotoxic T cells that discriminate tumor cells via HLA class I molecules. In addition, solid neoplasias can secrete HLA class II antigens and serve as nonprofessional antigen-presenting cells (APCs) that imitate the proliferation of autologous $CD4^+$ T cells, intuitively.

HLA and autoimmun disease

The human leukocyte antigen (HLA) system comprises closely linked genes controlling highly polymorphic proteins involved in the presentation of peptides to the T-cell receptor. Specific alleles at HLA loci are associated with diseases, often those suspected to be of autoimmune aetiology. Many of these associations result from linkage disequilibrium between the HLA gene studied and other HLA genes or non-HLA genes close by. Owing to its high level of polymorphism and its candidate role in many diseases, HLA was the first system used in many techniques of genetic mapping, such as affected-sib-pair analysis and association (linkage disequilibrium) studies. Much remains unknown about the reasons why diseases are associated with HLA. Experience gained from HLA has, however, shown how other loci involved in complex traits can be identified by studying families with multiple affected cases or sib pairs, followed by linkage-disequilibrium mapping and then analysis of candidate genes.

The molecular mechanisms identified to date that influence HLA–peptide–TCR interactions and that have been implicated in autoimmune disease development include alternate TCR docking, low-affinity-mediated thymic escape, TCR stabilization of weak peptide–HLA complexes, altered binding registers, 'hotspot' molecular mimicry, post-translational modification of antigenic peptides, hybrid peptides and differential HLA expression and stability.

Autoimmune diseases (ADs) are chronic complex inflammatory diseases. They are considered to be either specific or systemic and characterized by inducing immune humoral (B cell) or cell (T cells) responses. Although their etiology is unknown, they are well known to have environmental and polygenic components that are involved in defining susceptibility or protection. Thus, the sum of the genes involved in ADs makes up the genetic component that defines

them. ADs are presented with a multifactorial genetic inheritance pattern which does not completely follow a classical Mendelian model.

Furthermore, studies in monozygotic and dizygotic twins have estimated the relative contribution of genetic effects. Among the most relevant and studied genetic factors for ADs are genes located in the Major Histocompatibility Complex (MHC) and, in particular, loci from Human Leukocyte Antigen (HLA) class I and class II. An extensive list of ADs has been associated with different variants of the HLA genes, particularly, class II genes. Note that the set of alleles associated with various ADs may vary from one population to another, and within the same population, different alleles might be associated with different ADs. Moreover, the genetic effect of HLA might also be involved in changing and defining the relationship between the environmental factors associated with ADs

Evaluation of the role of the HLA molecules and alleles in susceptibility or protection against any disease, particularly ADs, is a key component in the definition of the population at risk. Therefore, conventional epidemiological analysis of age, sex, ethnicity, and geographical origin might elucidate multiple environmental factors associated with these diseases. Furthermore, these variables should go hand in hand with molecular epidemiology which seeks to understand the genetic distribution in a population and its correlation with the distribution of the disease and associated alleles.

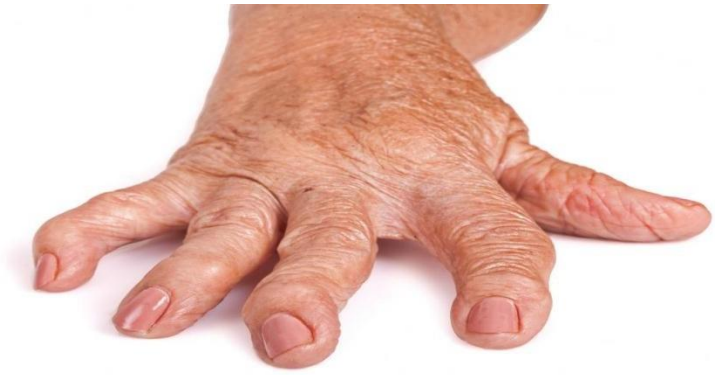
Musculoskeletal Diseases

Musculoskeletal conditions are a major cause of impairment, disability, health care utilization, and loss of economic productivity throughout the world. It is considered to be of autoimmune aetiology, with a strong genetic component. The incidence and severity are associated with a specific amino acid sequence on several human leukocyte antigen (HLA) DRB1 alleles.

rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mostly affects the joints, although systemic manifestations are frequent. It starts with inflammation of the synovial membrane and often leads to the erosive destruction of adjacent cartilage and bone. HLA-DRB1 alleles increase RA risk and severity; however, the underlying mechanisms of action remain unclear.

Relevant HLA genes involved in susceptibility to RA are *HLA-DRB1*04:01,* in Caucasians; *HLA-DRB1*04:05* in Spaniards and Japanese; *HLA-DRB1*01:01* ; *HLA-DRB1*14:02* in some Native Americans . Although the pathogenic mechanisms of these alleles in RA are still unresolved, different hypotheses have been postulated as follows: first, presentation of arthritogenic antigens; second, alterations of peptide affinity during T cell repertoire selection; and third, molecular mimicry with microorganism peptide residues.

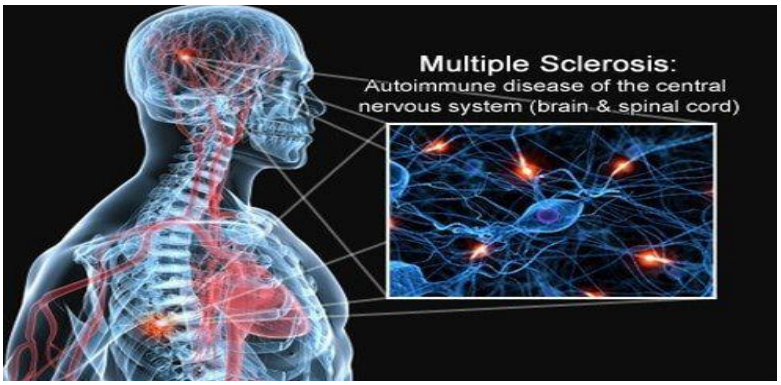


RA Disease

Multiple sclerosis (MS)

This condition corresponds to an autoimmune pathology with a predominant immune cell response characterized by the presence of autoreactive T cells that react against the myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and the proteolipid protein (PLP). The *HLA-DRB1*15:01* and *HLA-DQB1*06:02* alleles, which are in LD, are the main alleles associated with risk for .

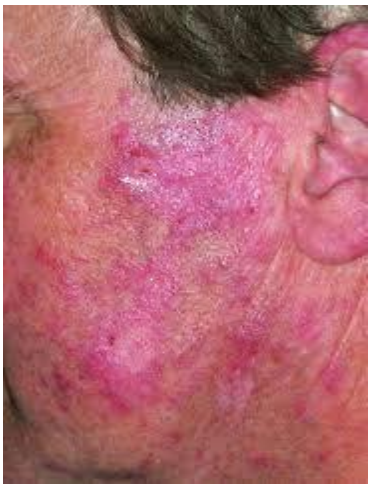
Several studies have explored phenotype-genotype correlation for associated HLA alleles in MS and reported that *HLA-DRB1*15* has been associated with younger age.



Systemic lupus erythematosus (SLE)

The HLA has been shown to exert the strongest genetic association and effect on SLE to date. The top association was found at *HLA-DRB1*.

GWAS in both European and Asian populations has shown that the strongest contribution to risk for SLE resides in the HLA region and consists of multiple genetic effects. The long-range LD within the HLA region has made assessing the relative contribution of each component gene to disease susceptibility difficult. However, the available evidence suggests that genetic variants such as *HLA-DR2* and *HLA-DR3*, *HLA-DPB1*, *HLA-G* and class III genes, in particular, predispose an individual to SLE.

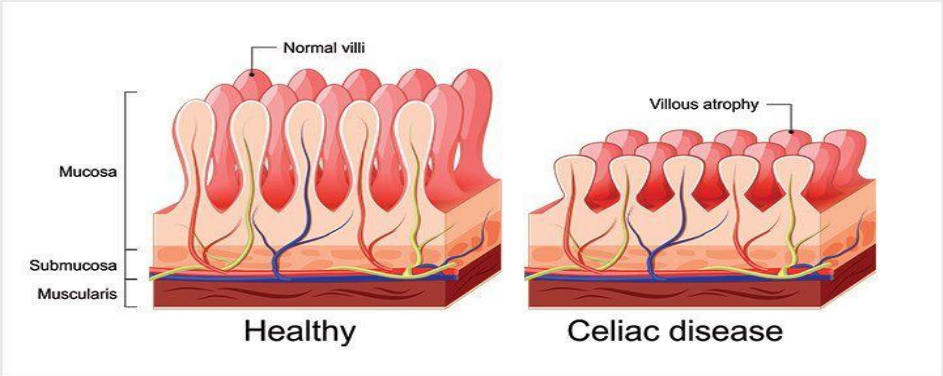


Type 1 diabetes mellitus (T1D)

Several alleles have been associated with and linked to susceptibility to T1D including *HLA-DQB1*03:02* and *DQB1*02:01* the quantity of alleles found associated with T1D in the *DR* and *DQ* loci is high, the role of the locus or loci conferring susceptibility/protection is unclear given that they are presented with low effects when compared to the identified susceptibility haplotypes of T1D , This can be explained by the variety of existing alleles in the HLA, population changes, and the pattern of inheritance for both susceptibility and protection alleles.

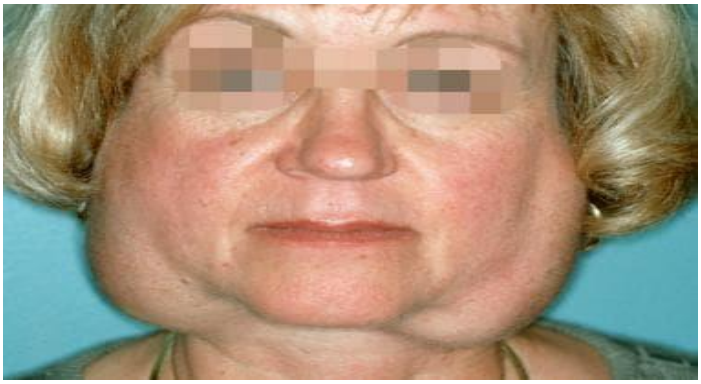
Celiac disease (CD)

CD is a complex disorder of the small intestine caused by an inappropriate immune response to ingested wheat gluten . CD has a strong genetic component as illustrated by a monozygotic twin concordance of nearly 90% compared to 10% in first-degree relatives . A significant proportion of the genetic predisposition comes from HLA genes. HLA-DQ2 or HLA-DQ8 is expressed in 30%–35% of the populations where CD. This implicates other genetic as well as environmental factors as contributors to the manifestation of CD.



Sjögren's syndrome (SS)

SS is an autoimmune exocrinopathy characterized by a lymphocytic and plasma cell infiltration of the salivary and lachrymal glands. This is accompanied by *de novo* production of autoantibodies leading to keratoconjuntivitis sicca and xerostomia. A recent meta-analysis of association studies from around the world identified associations between HLA Class II and SS.the allelic level, *DQA1*, *DQB1*, and *DRB1* alleles were found to be risk factors for the disease.



Sjögren's syndrome

Immunogenetics applications

Methods and Applications in Clinical Practice seeks to serve both the immunogenetics community and the wider scientific community with a collection of detailed information.

Application to Clinical Medicine

Inevitably the relationship between immunogenetics and disease has been explored most widely in animal models and this is reflected in the book by articles on murine models of systemic lupus erythematosus, myasthenia gravis, autoimmune thyroiditis, and allergic encephalitis. Nevertheless, headway is being made with human diseases as exemplified by HLA associations with immune response genes to streptococcal and cedar pollen antigens.

Clinical Application of Immunogenetics to Precision Medicine

precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. This approach will allow doctors and researchers to predict more accurately which treatment and prevention strategies for a particular disease will work in which groups of people to learn about human genetic disorders and rare diseases and what they reveal about our immune system.

Its application in autoimmune disease treatment will bring the required breakthrough in medicine. The precision medicine of selected autoimmune diseases was discussed, and the different

biomarkers utilized in the diagnosis, prognosis, stratification and response monitoring of such condition were considered.

Immunogenetics in the fields of forensic medicine

The way to medico legal identification was open at the end of the twenty-first century by the "digital fingerprinting" represented by the multifactorial phenotypical trait, determined by both polygenic and environmental factors, followed by group-specific antigens, or with specificity for blood and tissue, and ending with the DNA molecule in use today. Because of this aspect, the framework of modern forensic medicine includes a new field, that of forensic genetics, that mostly involves working with investigations that have human genotype identification as a goal.

Immunogenetics Methods and Applications in Clinical Practice

seeks to serve both the immunogenetics community and the wider scientific community with a collection of detailed information and helpful tips attained by many years of experience in the field.

Immunogenetics to pharmacogenetics and personalized medicine

immunogenetics and pharmacogenetics are merging to bring to the individual patient tailored and personalized treatment. Providing insights into the complexities of predictive, preventive participatory and personalized medicine, the role of the HLA system will be consolidated at the forefront of the newer medicine.

Immunogenetics and systems biology and medicine

provided the computational framework for the formation of electronic public repositories, which have been greatly expanded with the advent of genomics. The development in the recent years of powerful computational resources is proving essential for maintaining the access and analysing the exponentially increasing flow of diverse data types on biomolecules cells, organs and the associated normal and pathological phenotypes. Their implementation in computer grids is providing computing-on-demand cloudbased resources that can be managed externally, enabling researchers to focus their attention on the development and use of mathematical and computational tools.

Immunogenetics as a tool in anthropological studies

Anthropology is the scientific study of humanity, concerned with human behavior, human biology, and societies, in both the present and past, including past human species.

the study of these systems shows great promise for investigating both the peopling history of modern humans in the time since their common origin and human adaptation to past environmental (e.g. pathogenic) changes. Therefore, in addition to mitochondrial DNA, Y-chromosome, microsatellites, single nucleotide polymorphisms and other markers, immunogenetic polymorphisms represent essential and complementary tools for anthropological studies.

The polymorphism of HLA

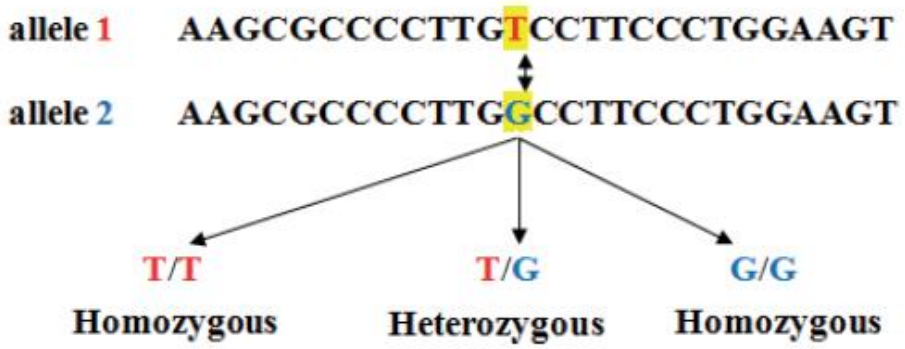
A round 99.9% of the individuals genome among persons is alike, and only 0.1% of it differs in chromosome. This variance is accountable for the diversity in phenotypes and receptiveness of them to environmental effects. DNA variants are happening in numerous formulas. Mutations might be definite as order variants which happen in less than 1% of the populace, whereas the extra prevalent variant is identified as polymorphisms.

Generally, genetic polymorphism can be available in numerous designs, comprising: single nucleotide polymorphisms (SNPs), tandem repeat polymorphisms which include a variable number of tandem repeats (VNTRs) and short tandem repeats (STRs), insertion/deletion polymorphisms, transposable elements (TE) or Alu repeats also known as "jumping genes," structural alterations, and copy number variations (CNV).

1-Single-nucleotide polymorphism (SNP)

Single nucleotide polymorphisms (SNPs) (pronounced: snip) are an alteration in a lone DNA order structure building block unit: (A, T, C, or G) which termed a nucleotide, Figure 1.SNPs are the most frequent occurrence from all genetic variants, which happen usually in a person's DNA. It is a ratio of occurrence near 90% of human genomic variants .

They may be occurring one time in each 300 nucleotides on usual, that is, average is about 10 million SNPs in the individual's genome.. As soon as SNPs happen inside a gene or in an adjusting area nearby a gene, they might show an additional strong impact in disease via stirring the gene's role. However, the SNPs generally have no influence on the general state of health.



2- Polymorphic repetitive sequences

The extension of the human genome threads that include gene sequences or intergenic and include retro (pseudo) genes and transposons are composed of small sequences of nitrogen bases that have repeated in tandem. It can consist of more two-thirds of human DNA. The number of units of these tandems in a specified site is extremely variable between separated persons. Tandem repeat polymorphisms include a variable number of tandem repeats (VNTRs) minisatellites and short tandem repeats (STRs) microsatellites. Both of VNTRs and STRs are the same in the total grounds. The difference between different alleles is consequence to a difference in the number of repeat bases that exist in alleles that are of various lengths. In microsatellites, the order repeat base composes between 2 and 9 units; while mini-satellites composes between 9 and 100 units.

3 -Insertion/deletion polymorphisms

It is a type of genomic difference in which a particular base order of different sizes ranging from one base to several 100 units is inserted or deleted.

The polymorphism of MHC class I and class II molecules

We have 6 gene clusters that encode for MHC class II molecules 3 loci on each chromosome 6; the molecules encoded on these loci are respectively termed MHC class II DR, DP and DQ. There are also 3 MHC class I loci on each chromosome 6

and the molecules encoded at these loci are termed MHC class I A, B and C. Cross-over during meiosis within the MHC region of chromosome 6 is relatively rare; consequently the alleles on one of our chromosome 6 are inherited en bloc from one of our maternal chromosomes and those on our other chromosome 6 from one of our paternal chromosomes. The alleles present on a chromosome 6 are termed the MHC haplotype of that chromosome. There is almost a 1 in 4 chance of two siblings having the same MHC haplotype on both chromosomes; in this situation the siblings are said to be HLA identical.

MHC loci are some of the most genetically variable coding loci in mammals, and the human HLA loci are no exceptions. Six loci have over 100 alleles that have been detected in the human population. Of these, the most variable are HLA B and HLA DRB1. As of 2012, the number of alleles that have been determined are listed in the table below. To interpret this table, it is necessary to consider that an allele is a variant of the nucleotide (DNA) sequence at a locus, such that each allele differs from all other alleles in at least one (single nucleotide polymorphism, SNP) position. Most of these changes result in a change in the amino acid sequences that result in slight to major functional differences in the protein.

There are issues that limit this variation. Certain alleles like DQA1*05:01 and DQA1*05:05 encode proteins with identically processed products. Other alleles like DQB1*0201 and DQB1*0202 produce proteins that are functionally similar. For class II (DR, DP and DQ), amino acid variants within the receptor's peptide-binding cleft tend to produce molecules with different binding capability.

However, the gene frequencies of the most common alleles (>5%) of HLA-A, -B, -C and HLA-DPA1, -DPB1, -DQA1, -DQB1, and -DRB1 from South America have been reported from the typing and sequencing carried out in genetic diversity studies and cases and controls. In addition, information on the allele frequencies of HLA-I and HLA-II genes for the European population has been compiled. In both cases the distribution of allele frequencies reveals a regional variation related with the history of the populations.

MHC class I

locus	#[22][23]
Major Antigens	
HLA A	4,340
HLA B	5,212
HLA C	3,930
Minor Antigens	
HLA E	27
HLA F	31
HLA G	61

MHC class II

HLA	-A1	-B1	-B3 to -B5[1]	Theor. possible combinations
locus	#[23]	#[23]	#[23]	
DM-	7	13		91
DO-	12	13		156
DP-	67	1,014		16,036
DQ-	95	1,257		34,528
DR-	7	2,593	312	11,431

[1]DRB3, DRB4, DRB5 have variable presence in humans

Number of variant alleles at class I and II loci according to the IMGT-HLA database, last updated October 2018:

DETERMINING HLA TYPE

Methods for determining individual HLA polymorphisms or 'HLA typing' have evolved enormously since the discovery of the human major histocompatibility complex and have developed in parallel with, and contributed to, the unravelling of the genetic complexity of this region, such that over 2000 alleles of the classical HLA class I (A, B and C) and class II (DR, DQ and DP) loci are now known.

Every person inherits each of the following antigens from each parent:

- HLA-A antigen
- HLA-B antigen
- HLA-C antigen
- HLA-DR antigen
- HLA-DQ antigen
- HLA-DP antigen

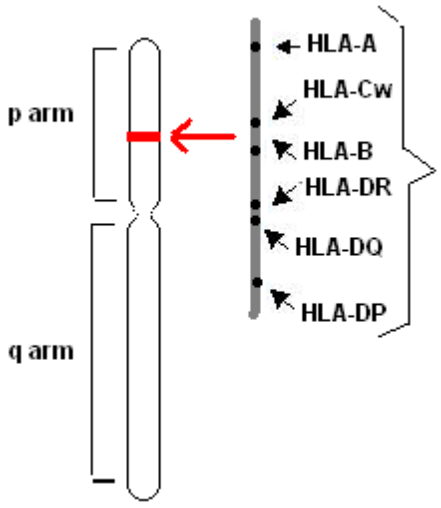
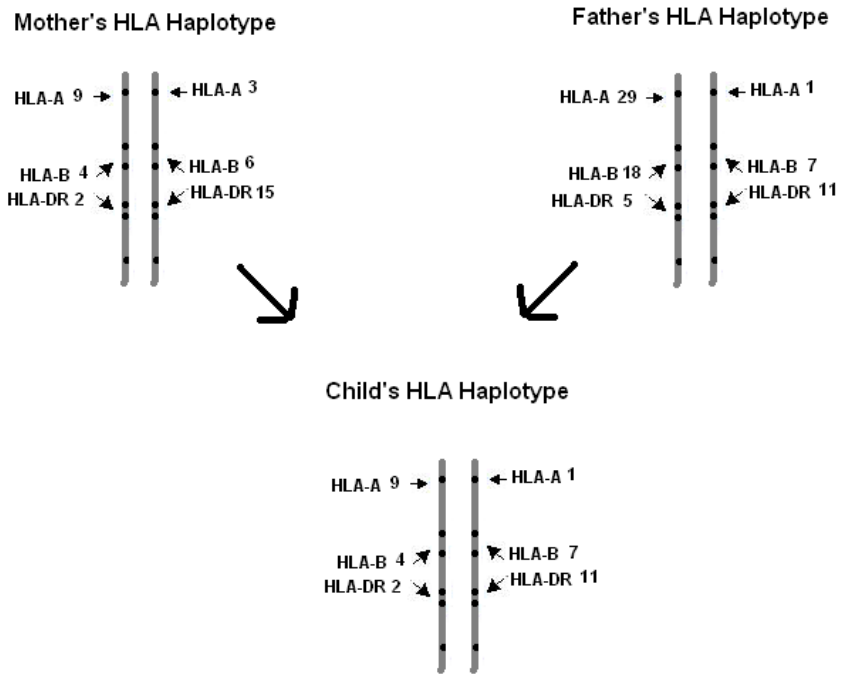


Fig: Major histocompatibility complex at chromosome 6



Each person has two of each of the antigens (one inherited from the mother and one inherited from the father

Methods for HLA typing are either based on detection of genetic variation in the expressed HLA molecules (serological typing), or now almost universally, at DNA sequence level (DNA typing).

1- Serological HLA typing

HLA typing methods were originally based on the detection of expressed HLA molecules on the surface of separated T cells (HLA class I) and B cells (HLA class II) using panels of antisera, usually obtained from multiparous women in a complement dependent cytotoxicity test.

Such 'serological' HLA typing suffers from a number of drawbacks. Live lymphocytes are required, and lymphocyte counts can be low in some transplant

patients. Panels of antisera must be maintained, although commercial kits are now available. Finally, the typing resolution obtainable from serological methods is low. While good serology may provide a level of resolution adequate for renal transplant HLA typing, it is inadequate for stem cell transplant matching and therefore has been largely superseded by DNA-based typing in clinical HLA laboratories. However, serological typing still has a useful role as an adjunct to DNA-based typing, for example to determine whether a particular HLA allele is actually expressed at the cell surface. A number of such non-expressed 'null' HLA alleles are now known.

2- DNA-based HLA typing

DNA-based typing methods offer a number of advantages over serological typing methods. Live lymphocytes are not required and DNA is easily extracted from any nucleated cell, although peripheral blood lymphocytes are the usual source. DNA is easily stored, allowing repeat sample testing when required.A number of different DNA-based HLA typing methods are in everyday use in clinical HLA typing laboratories, all of which are based on PCR amplification of target sequences in the HLA genes under investigation. PCR primers and oligonucleotide probes can be designed and validated in-house, or purchased commercially. As such, unlike antisera, they are a renewable resource.

- **PCR with sequence specific primers (PCReSSP)**

One commonly applied approach is to use panels of 'sequence specific primers' which amplify particular HLA alleles or allele groups. The presence or absence of a particular allele is determined by the presence or absence of DNA amplification by a particular primer pair, as determined by agarose gel electrophoresis.

- **PCR with sequence specific oligonucleotide probes (PCReSSOP)**

Another commonly employed approach is to detect HLA polymorphisms in locus-specific PCR products using short oligonucleotide DNA‘ probes’ in a hybridisation assay.

- **Sequence-based typing**

Sequence-based typing (SBT) can also be used to achieve allelic HLA typing as required for stem cell transplantation programmes. SBT is also required for investigation and confirmation of new allelic sequences.A number of other methods are in use some clinical histocompatibility & immunogenetics laboratories, although none of these are widely used

3- Cellular typing

Not or Rarely used by laboratories these days. Requires panels of homozygous typing cells, Cell culture method therefore takes a long time and Labour intensive involves use of radioisotopes.

Application of HLA typing

1- Organ and tissue transplantation

In organ and tissue transplantation, HLA antigens of the donor identified as invaders by the recipient causing rejection. Careful selection of the matched donor and recipient critically affect the outcome of transplantation.

The major loci important in organ transplantation are HLA A, B and DR. When both alleles for these three antigens are matched, it is termed a 6-antigen match. With a few exceptions (such as the gonads) all nucleated

cells express all HLA antigens, although the quantity on the cell surface at any moment is highly controlled. In transplantation this appears to have 2 major consequences. Firstly, allogeneic HLA molecules are recognised by T cells resulting in a powerful cytotoxic (Th1) inflammatory response (acute rejection of a graft by host T-cells, or acute graft-versus-host disease in the case of engrafted T cells attacking an immunosuppressed host). Secondly, in the case of bone marrow transplantation, antigen presenting cells and T cells are of dissimilar MHC type, so that cellular co-operation is very inefficient resulting in prolonged immunosuppression and downregulation of normal immune responses.

Xenogeneic - tissue transplanted from a different species

Allogeneic - tissue transplanted from a member of the same species

Autologous - tissue transplanted from the same individual

Syngeneic - tissue transplanted from a genotypically identical twin

2- Diagnosing some disease :

In autoimmunity: Many HLA combination are potentially indicative of autoimmune disorders

HLA allele	Associated disease
HLA B27	Ankylosing spondylitis, Reactive arthritis, Reiter's syndrome
DR2	Multiple sclerosis, Good pasture's syndrome
DR3	Myasthenia gravis, SLE
DR3/DR4	Insulin dependent DM
DR4	Rheumatoid arthritis
A3/B14	Hereditary hemochromatosis

3- Paternal testing

HLA typing can be used alongside other test for paternity testing.

4- Infertility (recurrent pregnancy loss):

Infertility due to recurrent pregnancy loss can be attributed to immune factors (40%) one of which is presence of certain common HLA antigens between the parents.

5- Phylogenetic studies:

Some HLA haplotypes have distinctive geographical distribution and are found only in some population. These haplotypes can be used to trace human migration.