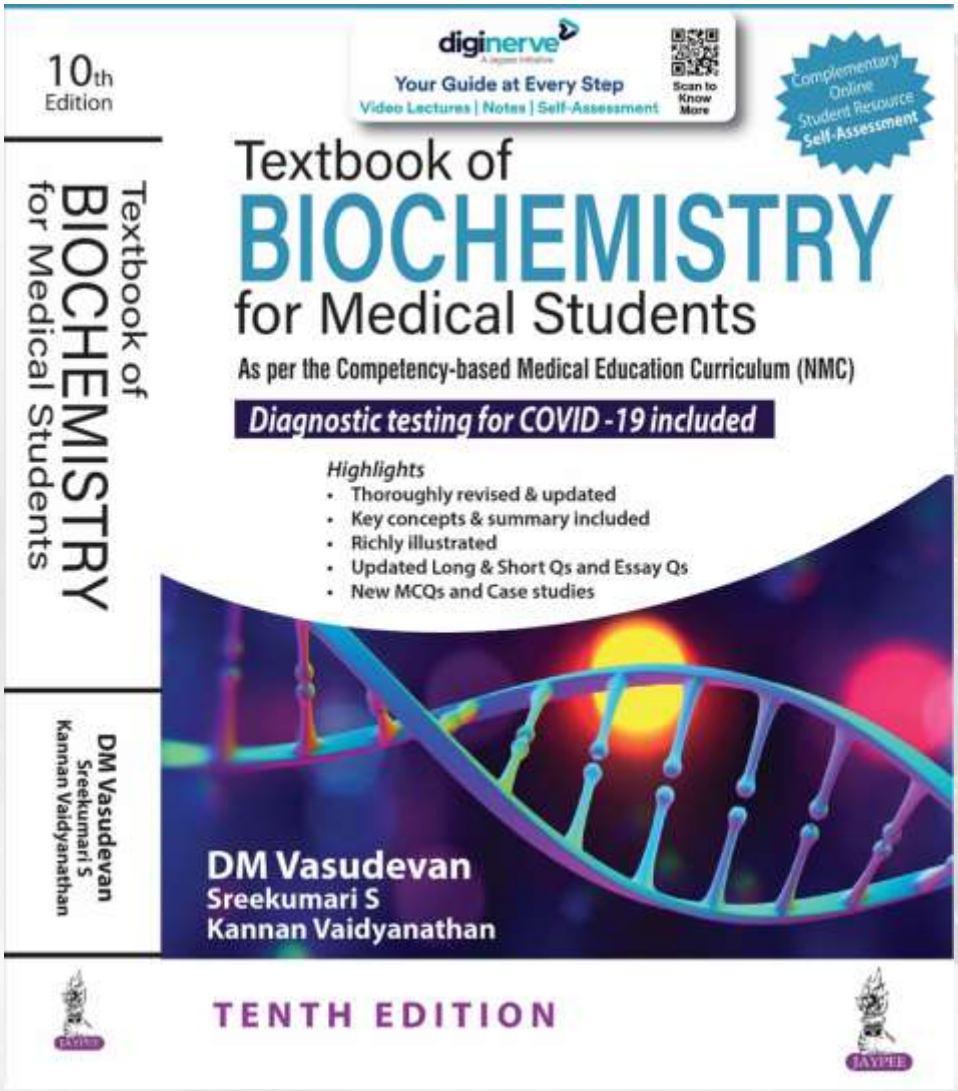
Chapter 40:

Immunochemistry



Textbook of

BIOCHEMISTRY

for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Immunology is one of the rapidly advancing branches of medical science. Small pox has been completely eradicated from the world by 1985; this is a triumph of immunology.

ANTIGENS

The immunocompetent cells could recognize the self from nonself. Any substance which invokes an immunological response is an **antigen** or immunogen.

Antibody response will usually be selective against specific spatial configurations on the antigen, which are called antigenic determinant sites, known as **epitopes**.

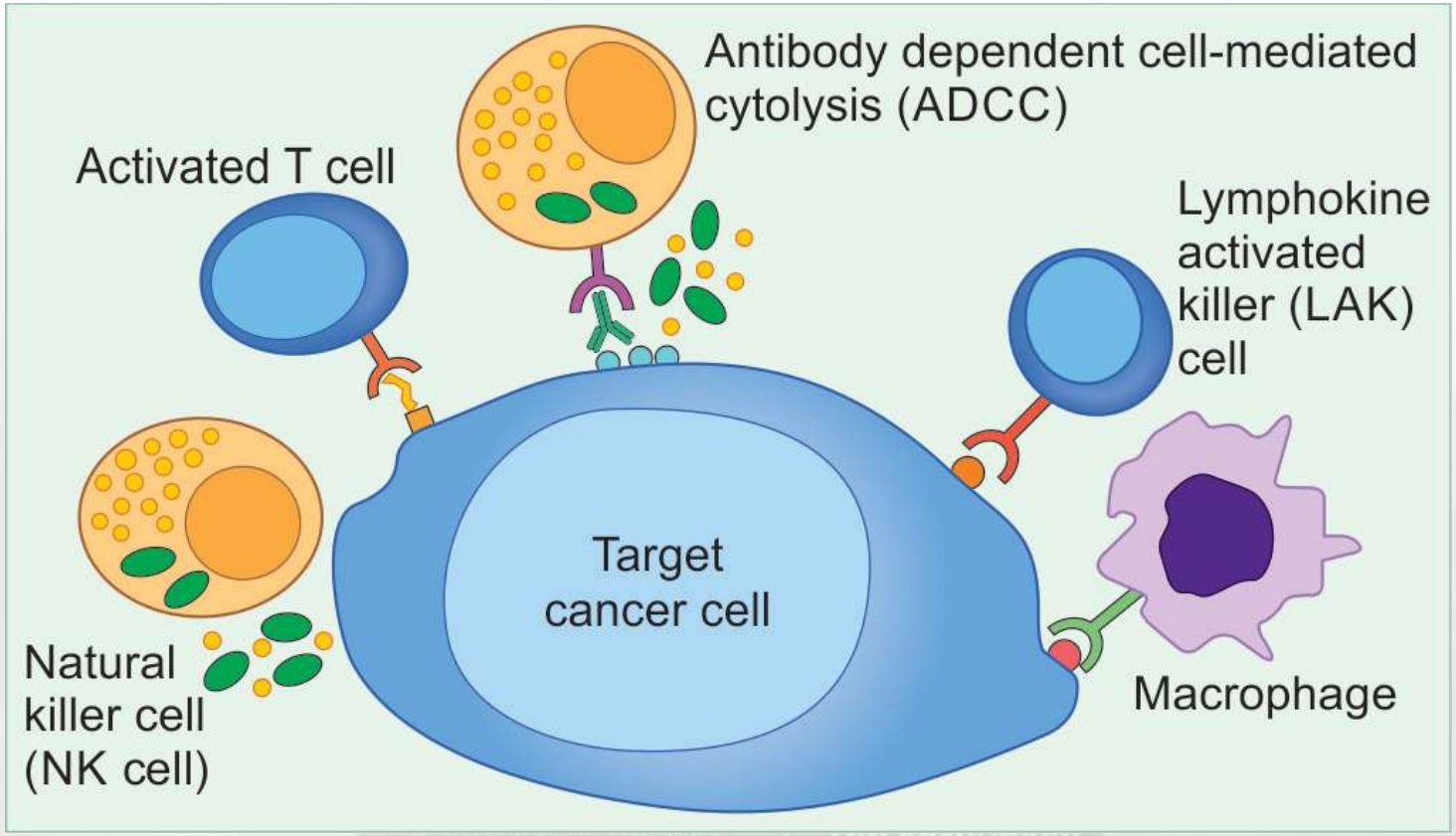
Immune Response

The lymphocytes generated from the bone marrow, passed through and processed by the thymus gland, are then called **T lymphocytes**. They can directly kill the target cells and are the effector cells for the **cell-mediated immunity** (CMI).

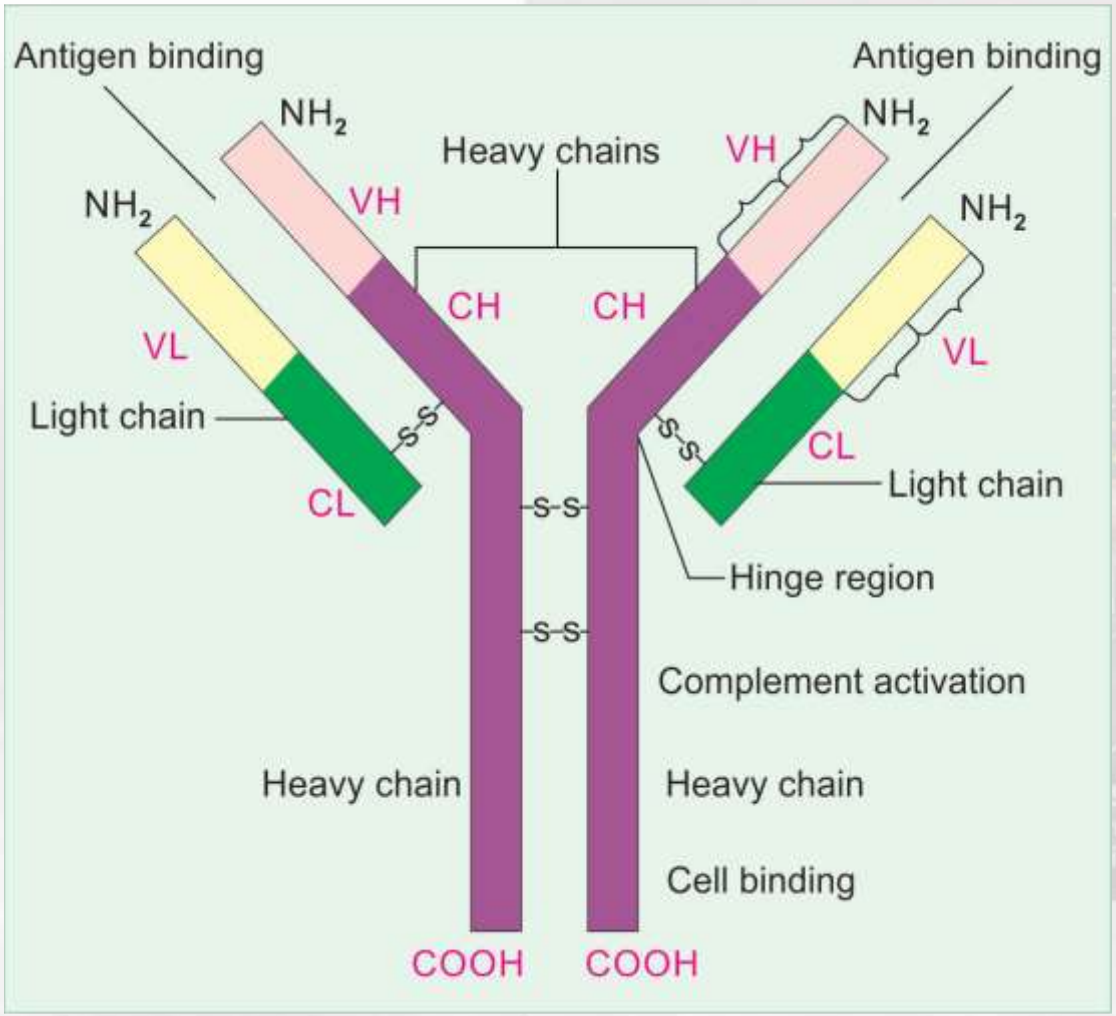
In peripheral blood 80% lymphocytes are T cells and 15% are B cells.

Certain other cells originated from bone marrow and processed by the Bursa of Fabricius in avians, are called **B cells**. The Bursa equivalent organs in human beings are gut associated (including Peyer's patches) and lung associated lymphoid organs. Immunoglobulins are secreted by the **plasma cells** belonging to the B lymphocytes. The B cells govern the **humoral immunity**.

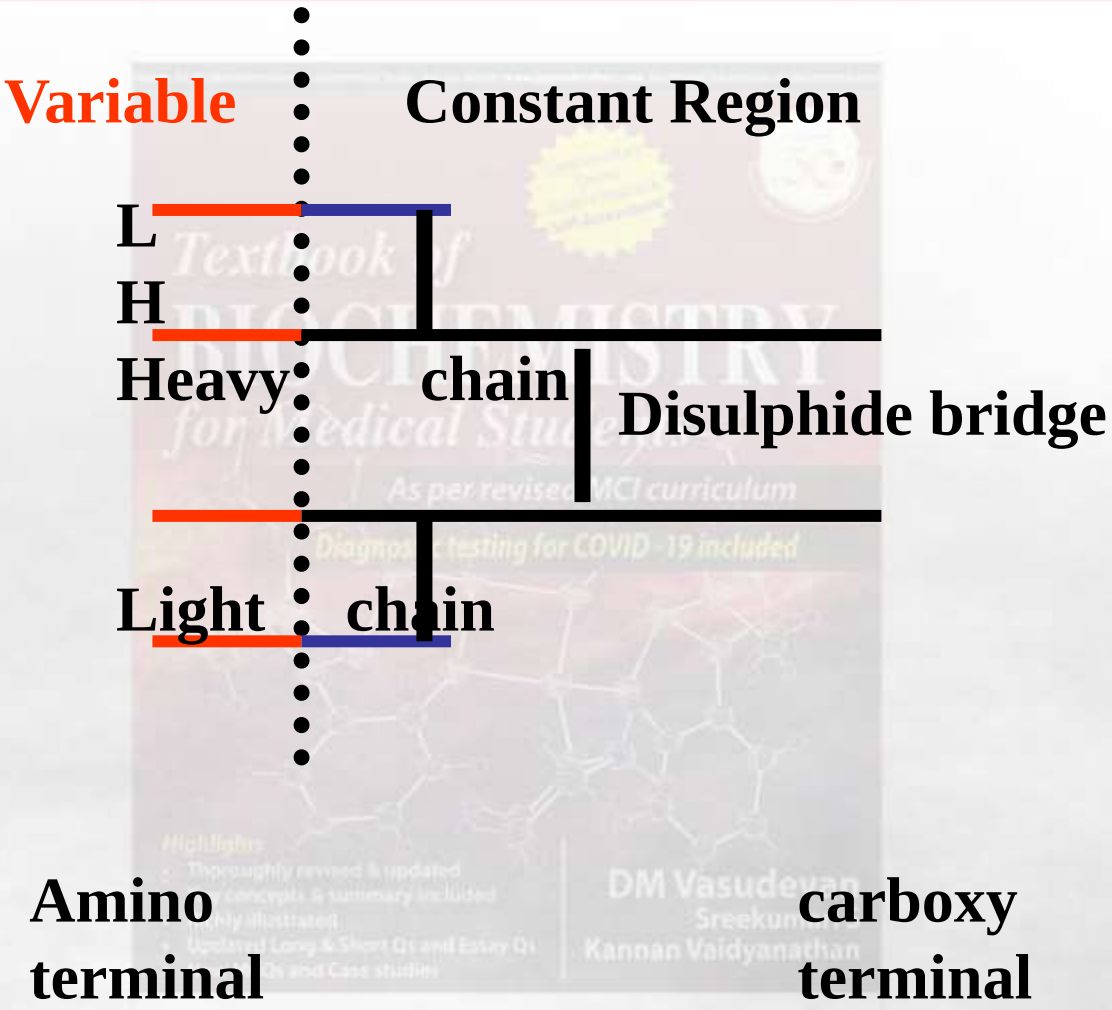
Cell mediated Immunity	Humoral immunity
T cell (Thymus)	B cells (Bursa of Fabricius (Peyer's Patches)
T cells directly kill the target	Plasma cells secrete Immunoglobulins



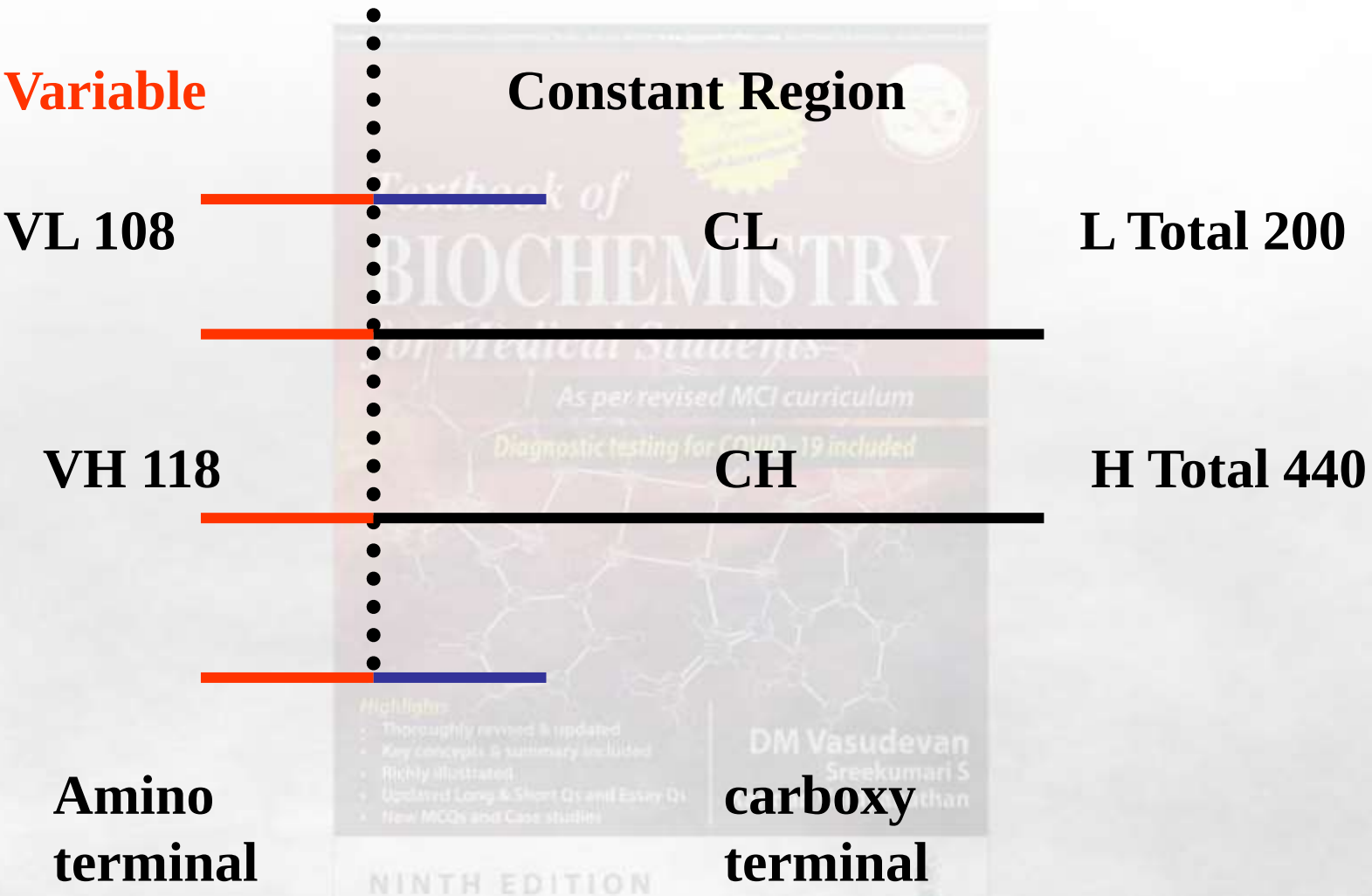
Immune effector cells.



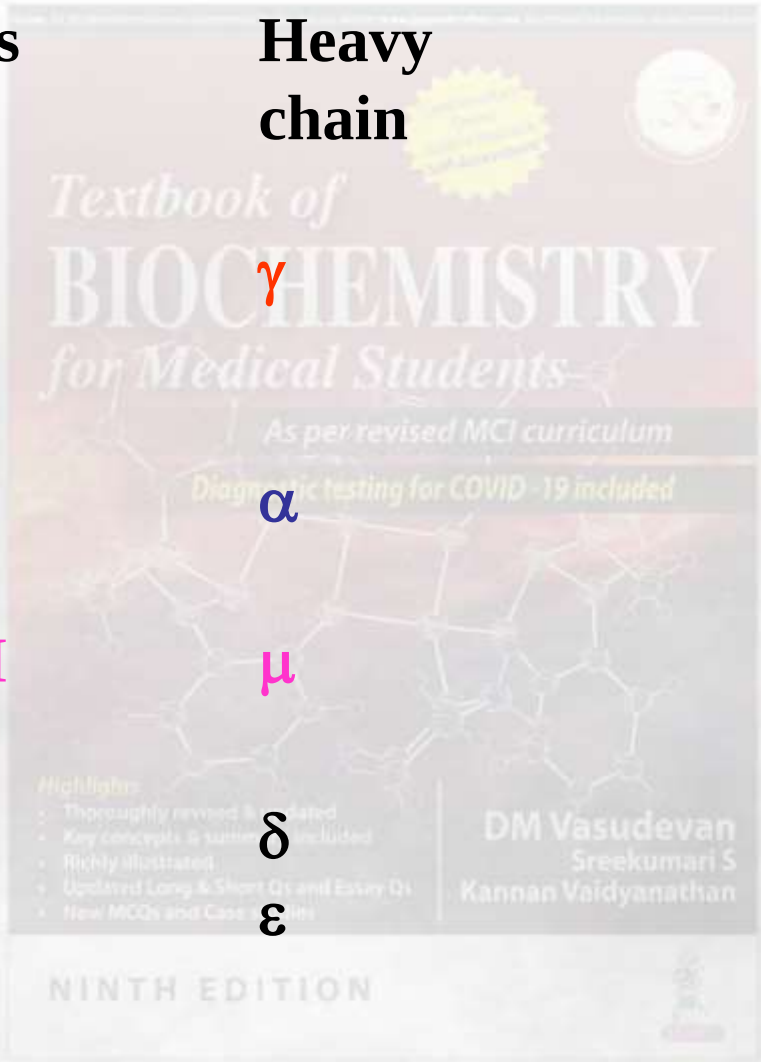
Immunglobulin molecule. Chains are connected by disulfide bridges, shown as -S-S-linkages.
NH2: amino terminal end; COOH: carboxy terminal end;
Constant regions are shown as dark;
VH: variable heavy region;
VL: variable light chain;
CH: constant heavy region;
CL: constant light region.

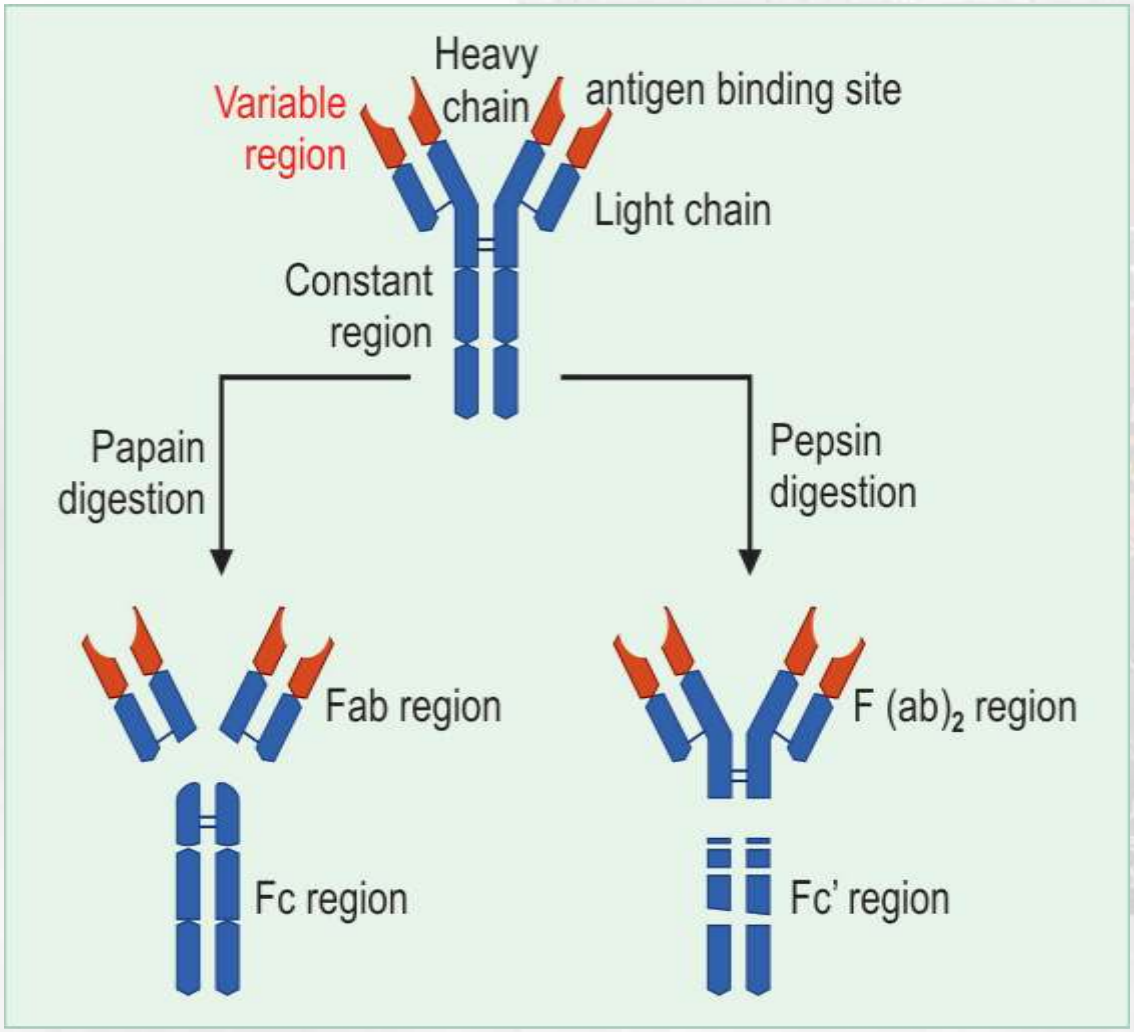


Immunoglobulin G


Variable
Constant Region
VL 108
CL
L Total 200
VH 118
CH
H Total 440
Amino terminal
carboxy terminal

Class	Heavy chain	Light chain
Ig G	γ	κ, λ
Ig A	α	κ, λ
Ig M	μ	κ, λ
Ig D	δ	κ, λ
Ig E	ε	κ, λ





Papain cuts the immunoglobulin molecule at a site toward the amino terminal part of the disulfide linkages. So, two Fab (fraction antibody) and one Fc (fraction crystallizable) portions are produced.

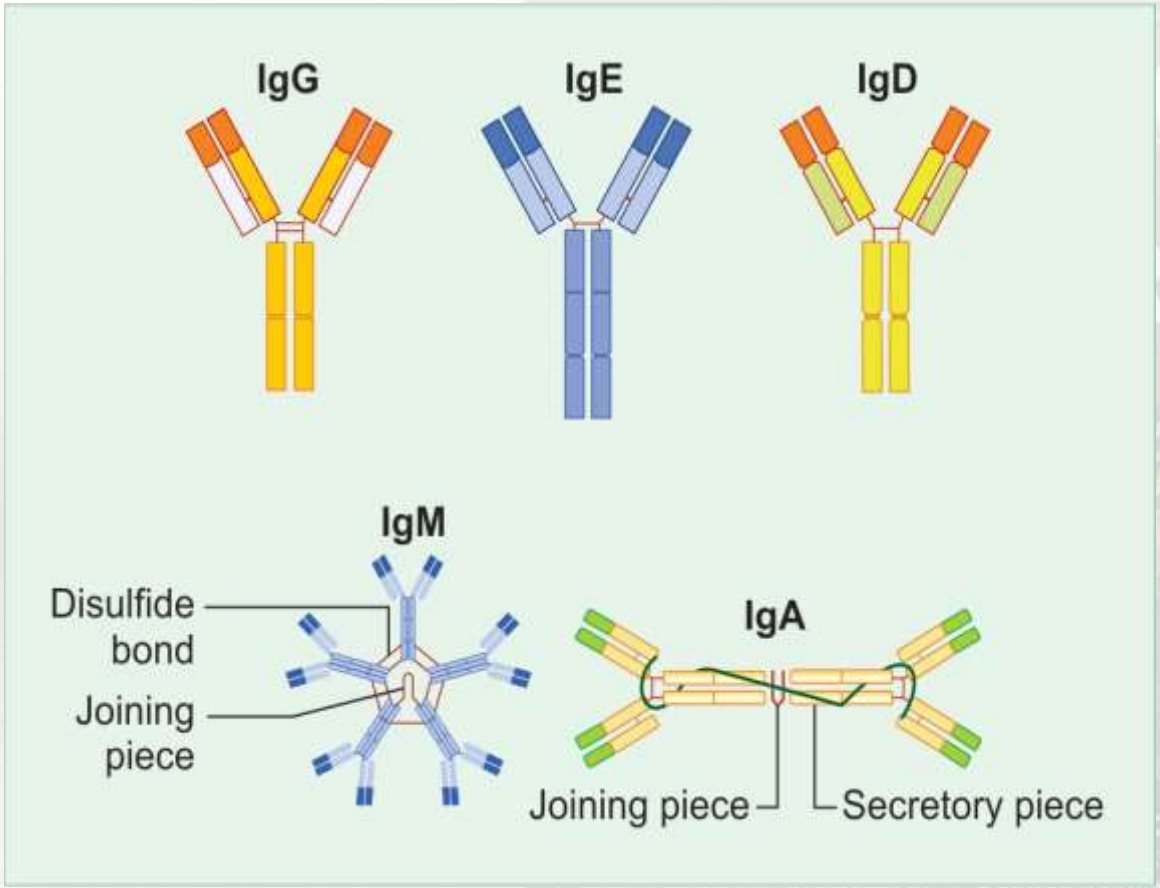
Pepsin cleaves the molecule toward the carboxy terminal part of the disulfide linkages, so that one F(ab)2 and one Fc portion are produced.

Characteristics of Different Immunoglobulin Classes

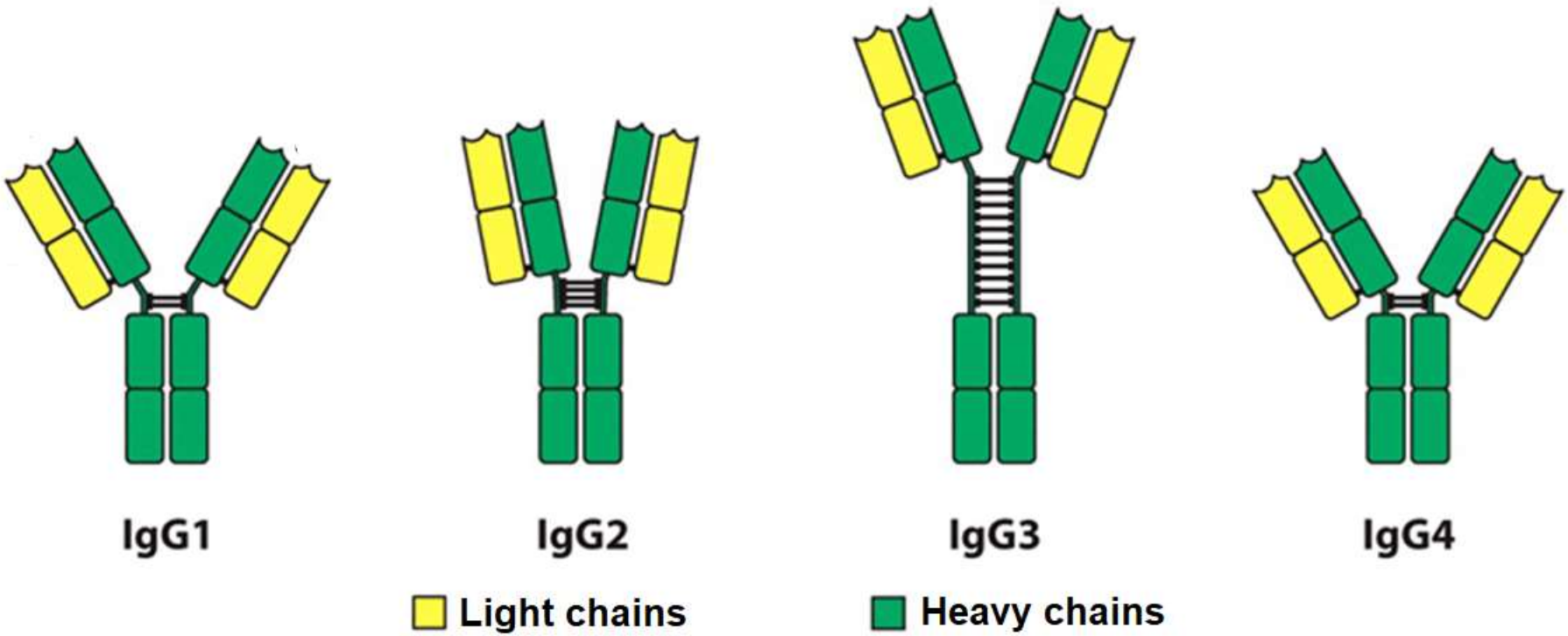
	IgG	IgA	IgM	I
Name of the heavy chain	γ	α	μ	δ
Number of basic 4-peptide units (2L + 2H)	1	2	5	1
Additional unit	—	S and J	J piece	–
Molecular weight (Daltons)	1,46,000	3,85,000	9,70,000	1 0
Sedimentation coefficient	7 S	11 S	19 S	7
Concentration in normal serum / 100 ml	800-1200 mg	150-300 mg	50-200 mg	

Functions of Immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Placental transfer	+	–	–	–	–
Complement fixation	+	+	++	–	–
Agglutination	+	++	+++	–	–
Fixation to mast cells	–	–	–	–	+
Primary response antibody	–	–	+	–	–
External secretions	–	+	–	–	–
Natural antibodies	–	–	+	–	–

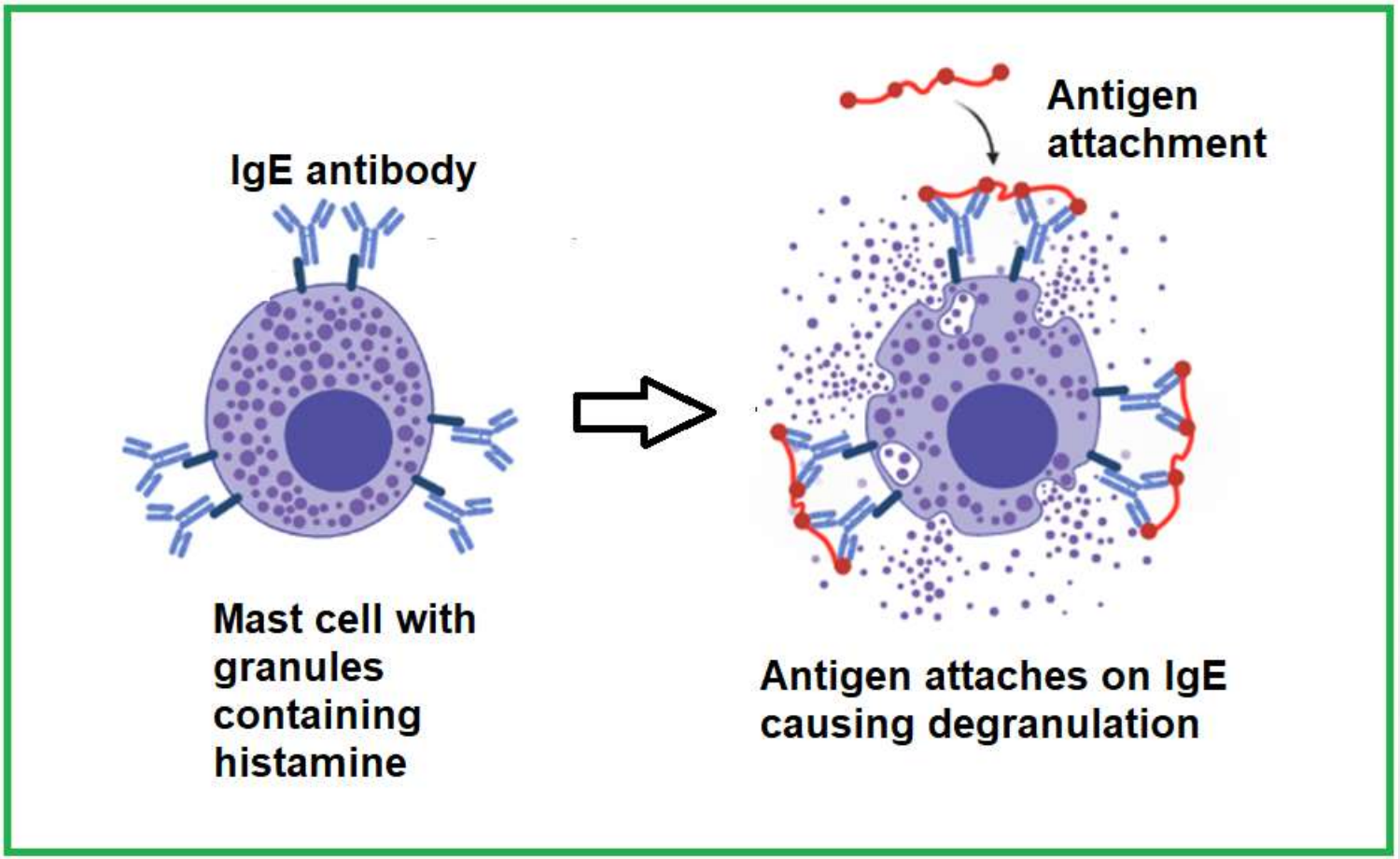


Immunoglobulin G (IgG), IgE, and IgD have one basic unit each, IgM has five basic units and IgA has two basic units. In IgM and IgA, the chains are connected by the joining (J) piece. In IgA, there is an extra secretory piece.



IgG subclasses

Ig E



Cytophilic antibodies fix on mast cells and basophils. These will mediate immediate hypersensitivity, allergy and anaphylaxis.

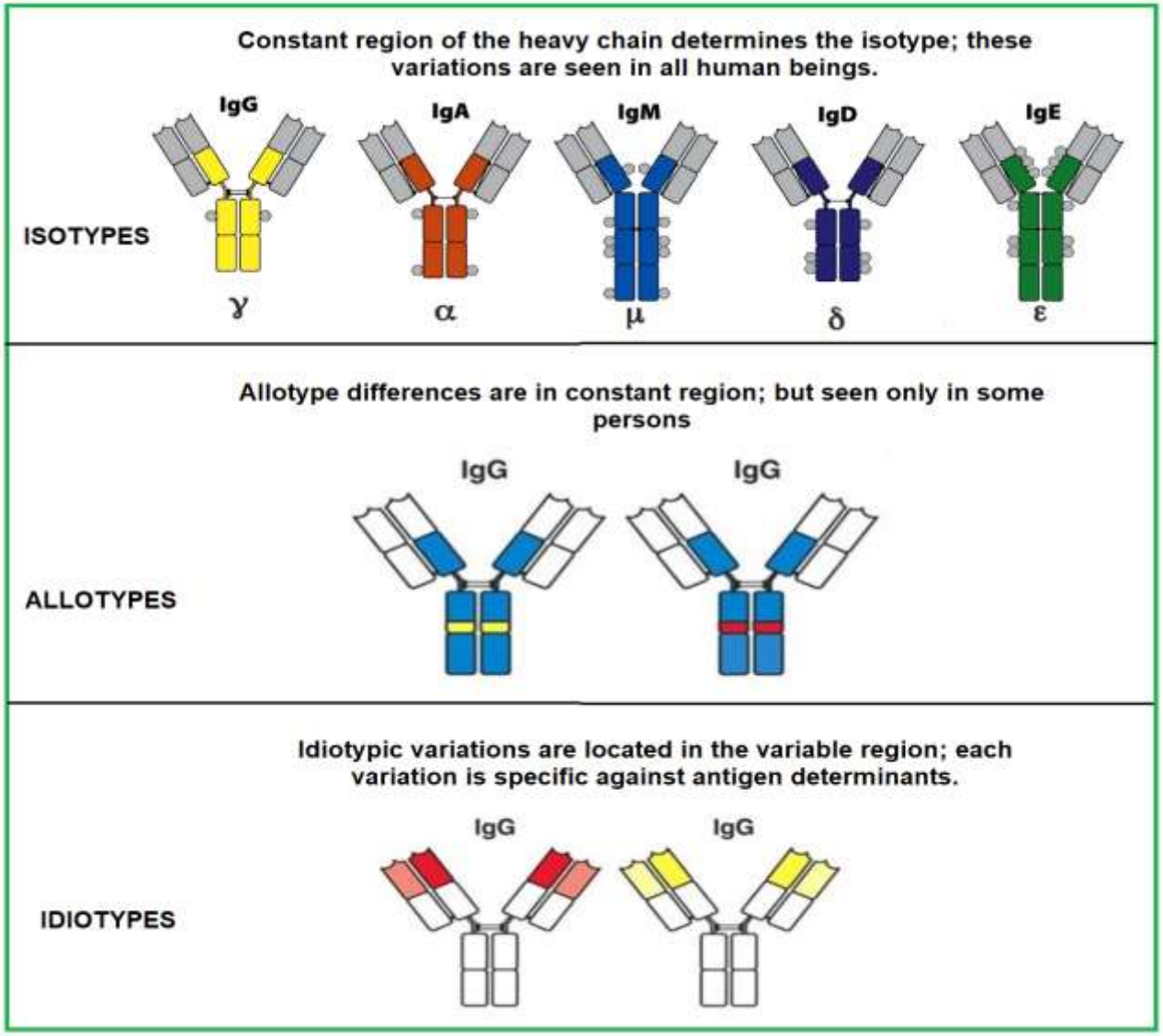
Isotypes, Allotypes, and Ideotypes

Isotypes are variants in molecules seen in all normal persons, e.g. classes and subclasses of immunoglobulins (G, M, A, etc.).

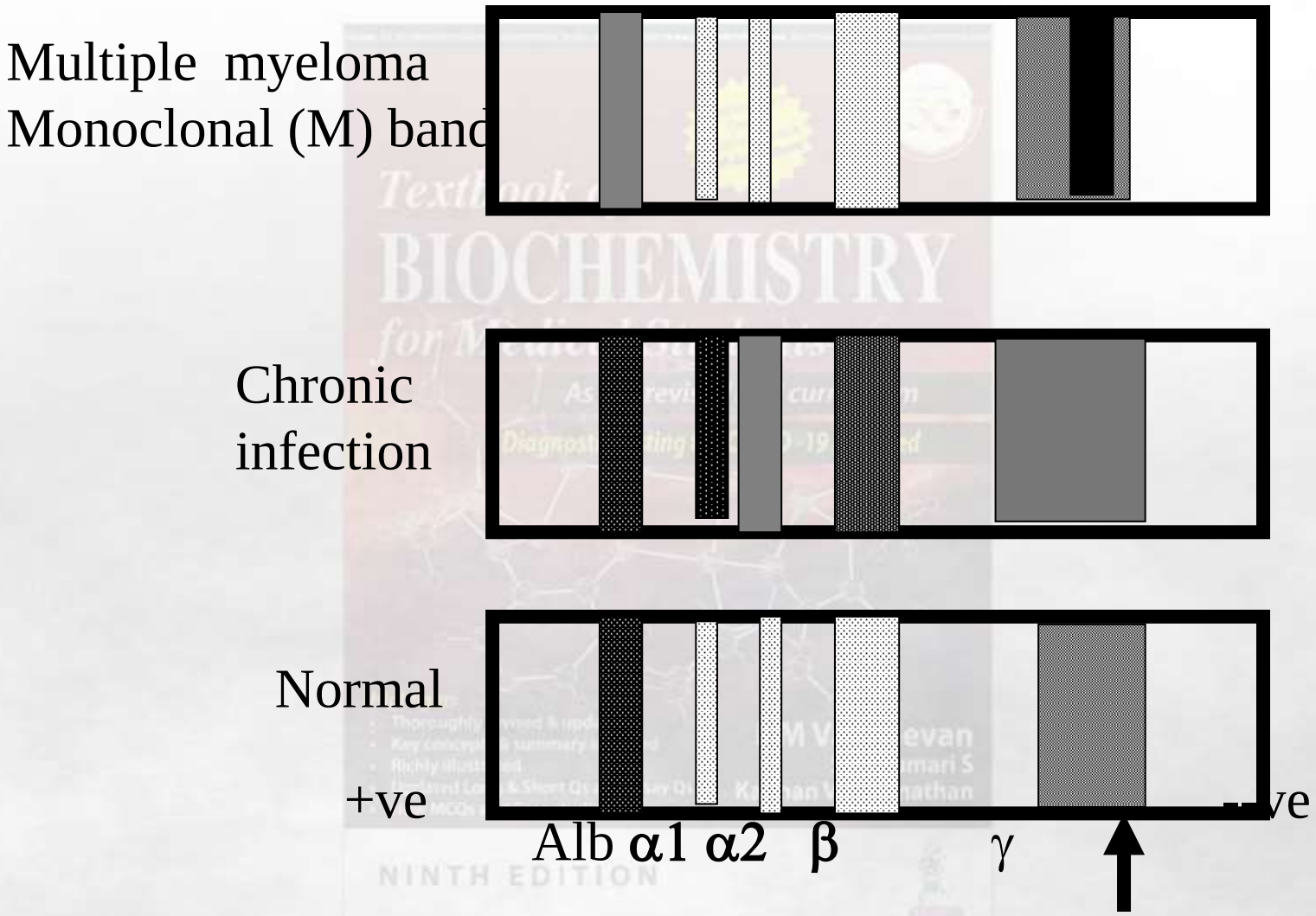
Allotypes are a variation within a subclass, and depend upon the allelic forms; only one form will be seen in one person, e.g. the Gm groups of IgG molecules. If a person is Gma +ve, each of his IgG1 molecules has a sequence Asp-Glu-Leu-Thr. Another person with Gma –ve will have this sequence changed to Met-Glu-Glu-Thr.

Idiotypic variation is individually specific to each immunoglobulin molecule. The idiotypic determinants are located in the variable part of antibody, i.e. the antigen recognition site.

Isotypes, Allotypes, and Ideotypes



Paraproteinemias

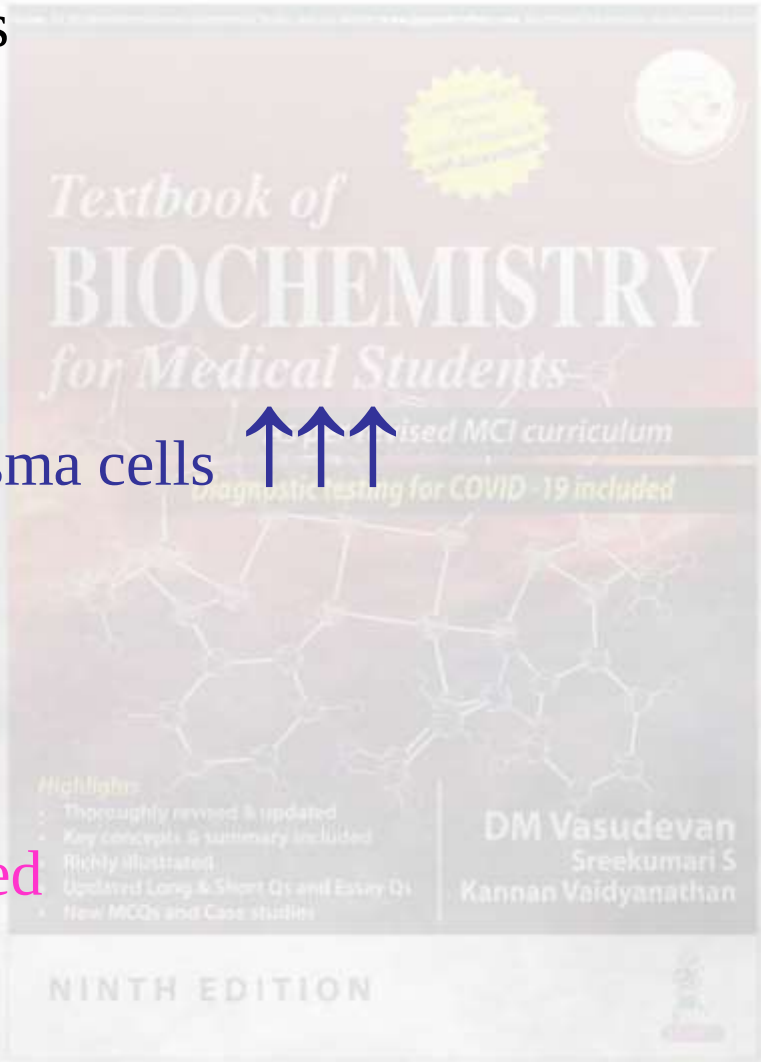


Multiple Myeloma

1% of all cancers
50 - 60 years

Paraproteinemia
M band
Bone marrow plasma cells ↑↑↑

70% IgG;
20% IgA;
10% IgM
Immunity decreased



Lytic lesions in bone

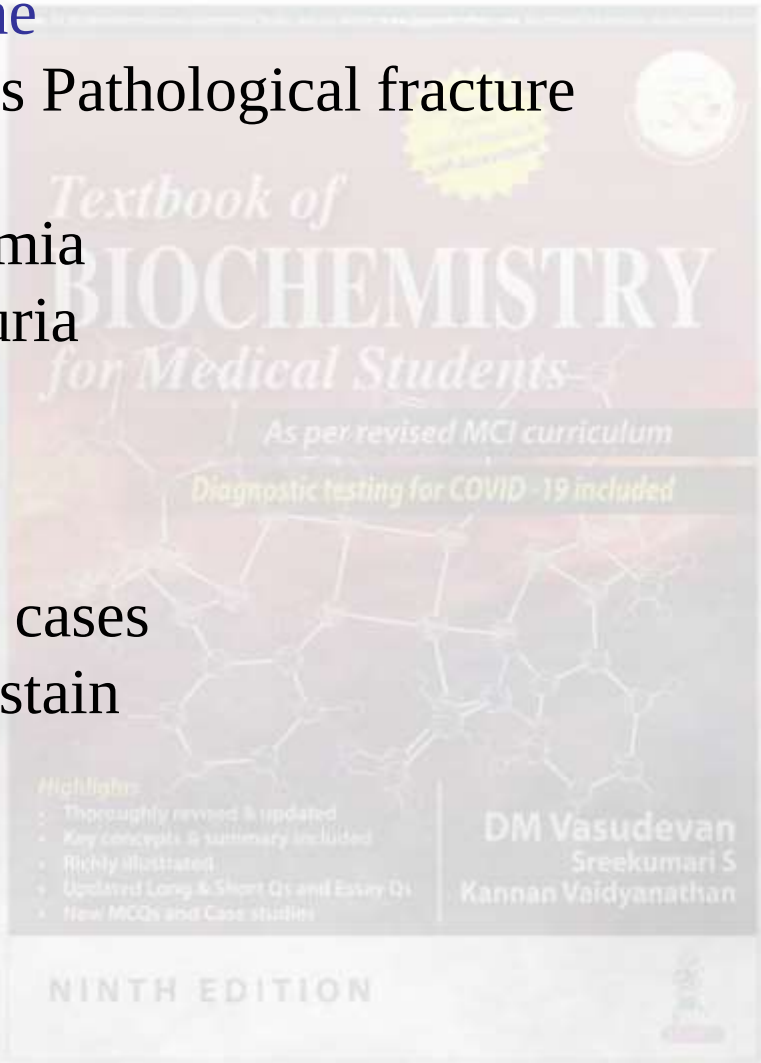
- Spontaneous Pathological fracture
- Pain
- Hypercalcemia
- Hypercalciuria

Anemia

Amyloidosis, 20% cases

- Congo red stain
- VL region

Prognosis good



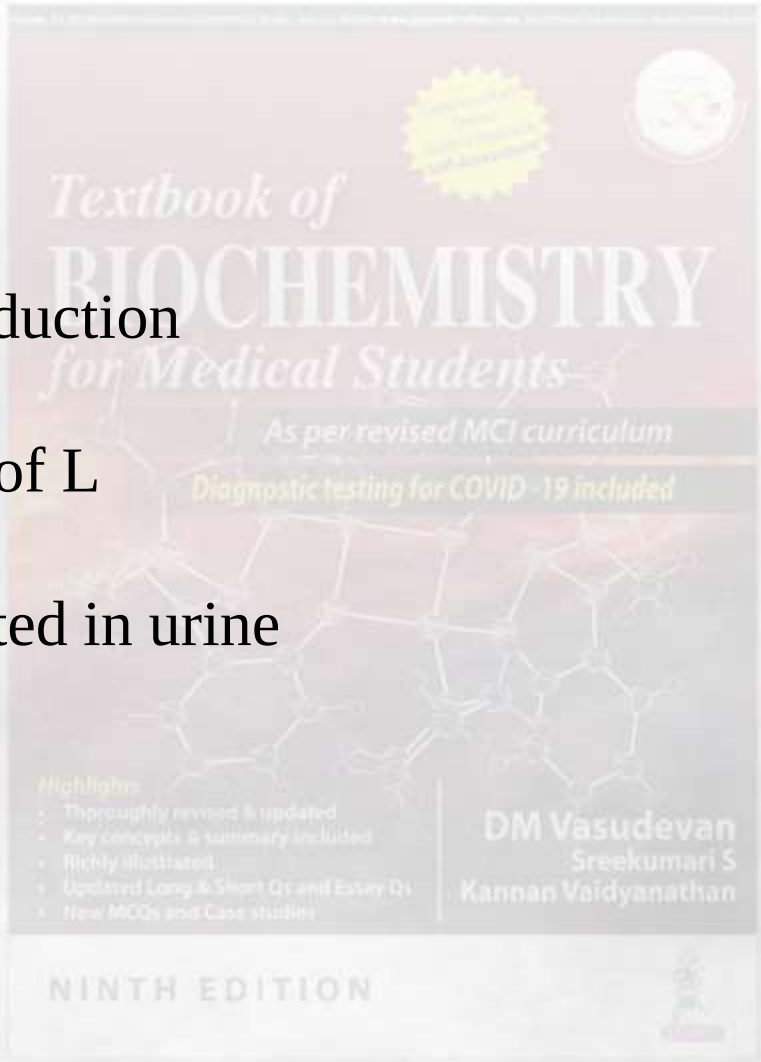
BENCE- JONES Proteinuria

In 20% cases of
Multiple Myeloma

Asynchronous production
of L and H
or deletion of part of L

Light chains excreted in urine

Renal failure
Prognosis bad



Heavy Chain Disease

Some portions of H chains are deleted during synthesis, so that they cannot join with L chains to form normal Ig. These defective heavy chains are excreted through urine. Gamma chain disease is associated with hepatosplenomegaly and lymphadenopathy. Alpha chain disease is associated with abdominal lymphoma and malabsorption.

Waldenstrom's Macroglobulinemia

IgM level in blood is increased considerably with a monoclonal peak. This is due to malignant proliferation of IgM clones. Since IgM are macromolecules, they may form aggregates or cryoprecipitates, serum viscosity is increased.

Amyloidosis

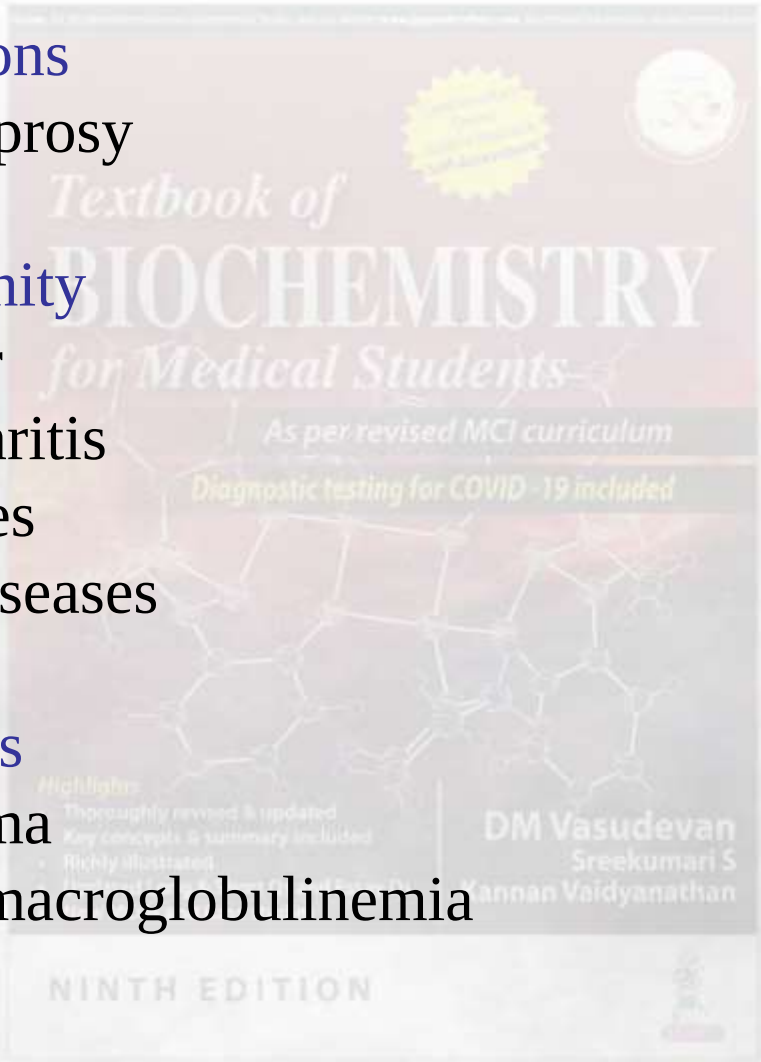
About 20% patients with myeloma develop amyloidosis. Amyloid deposits are seen in liver and kidney. Congo Red will stain amyloid deposits. In the case of myeloma, the amyloid fibrils contain polymerized variable region of light chains of Ig and termed AL.

Hyper Gamma Globulinemias

1. Chronic infections
   TB, Malaria, Leprosy

2. Aberrant immunity
   Rheumatic fever
   Rheumatoid arthritis
   Collagen diseases
   Auto immune diseases

3. Paraproteinemias
   Multiple myeloma
   Waldenstrom's macroglobulinemia



Hypogammaglobulinemia

A primary failure in production may occur as a congenital X-linked disorder (**Bruton's disease**).

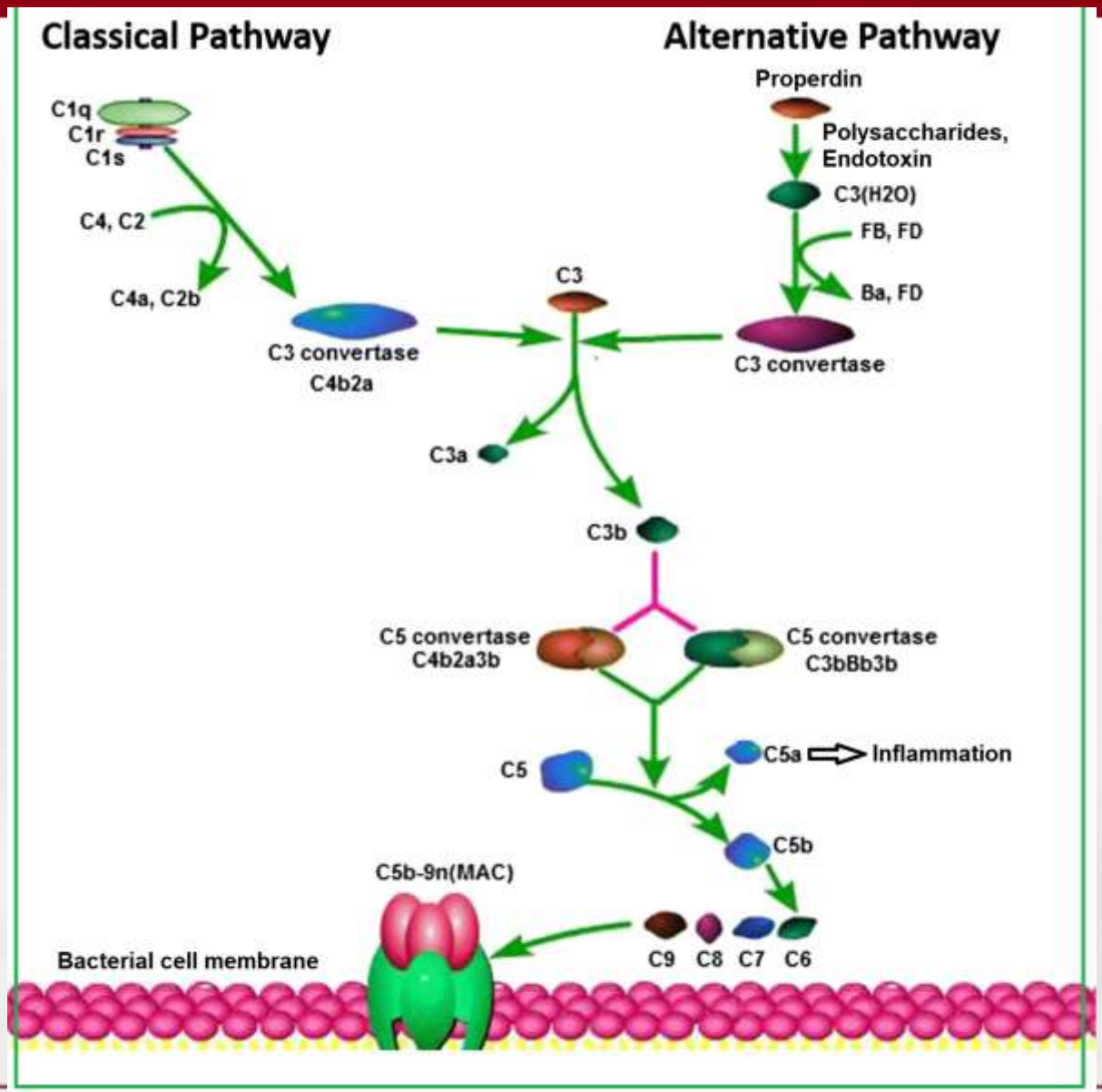
Decreased production may also be secondary to diseases like myeloma or leukemia.

Subacute combined immunodeficiency (SCID) states where both humoral and cell-mediated immunity are affected may be inherited.

Complement Activation

When Ig is bound on the bacteria, the Ig fixes the first component of complement, C1. It has three subunits; C1q, C1r, and C1s. The C1q binds with the antibody. C1s (C-1-esterase) when thus activated, acquires proteolytic activity. It in turn, cleaves and activates the next component, C4. Thus a multienzyme **cascade system** is activated, leading to the chemical amplification of the original message. The final components when activated, create microscopic holes in the target cell membrane. Osmotic entry of water through these pores will cause lysis of the target cell.

Pathways of Complement Activation



Immunodeficiency States

Combined Immunodeficiency

There is defect in maturation of stem cells. Both cellular and humoral immunity are defective. Relatively benign types are Wiskott-Aldrich syndrome (sex-linked) and ataxia telangiectasia (autosomal recessive).

Humoral Immune Deficiency

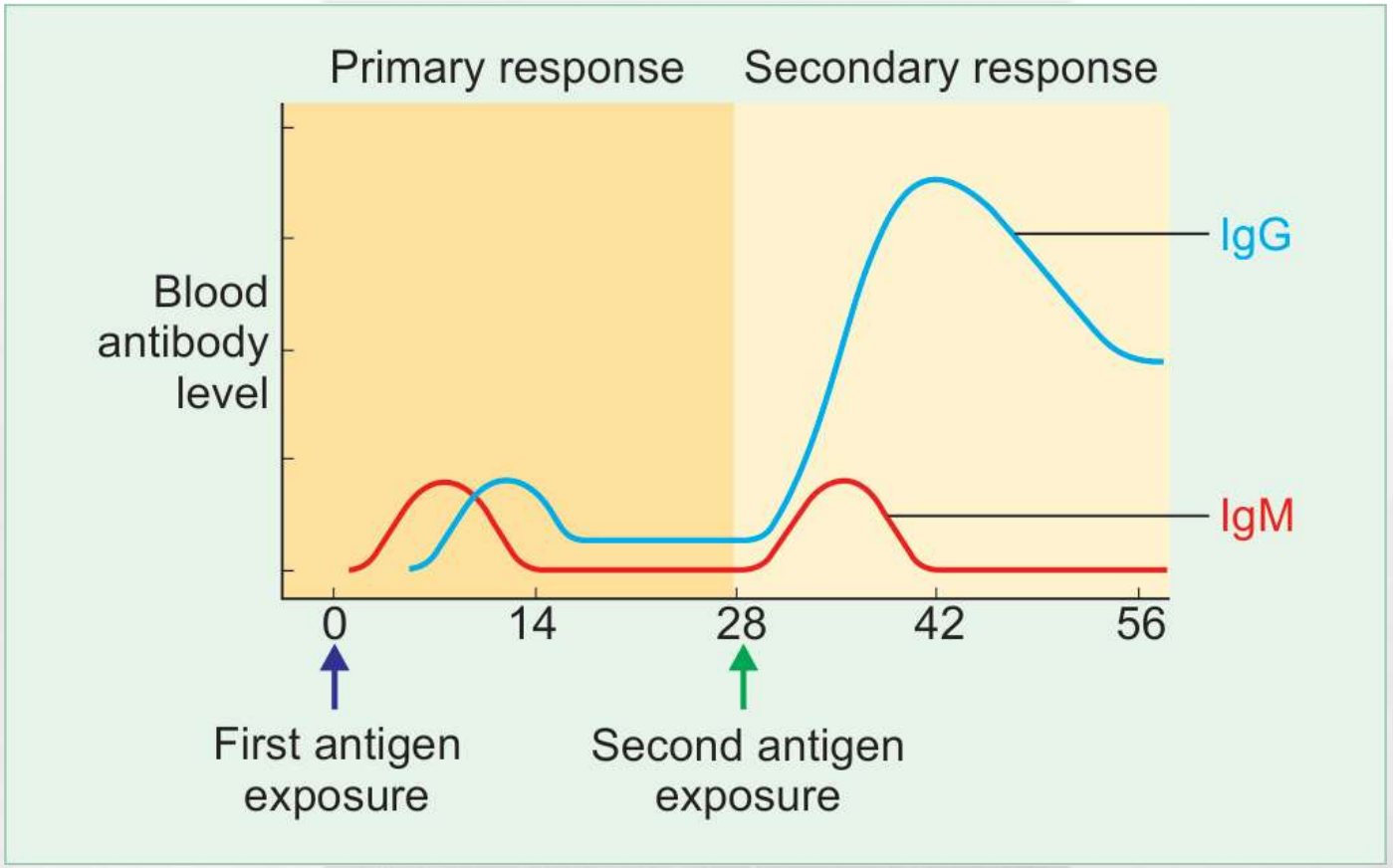
This may be a selective primary deficiency affecting only one of the G, M or A classes of immunoglobulins. In lgG deficiency, the affected persons suffer from repeated pyogenic infections.

Chronic granulomatous disease (CGD)

It is a sex-linked inherited disease, where peroxidase is deficient inside the phagocytes. Macrophages can engulf bacteria, but cannot digest them.

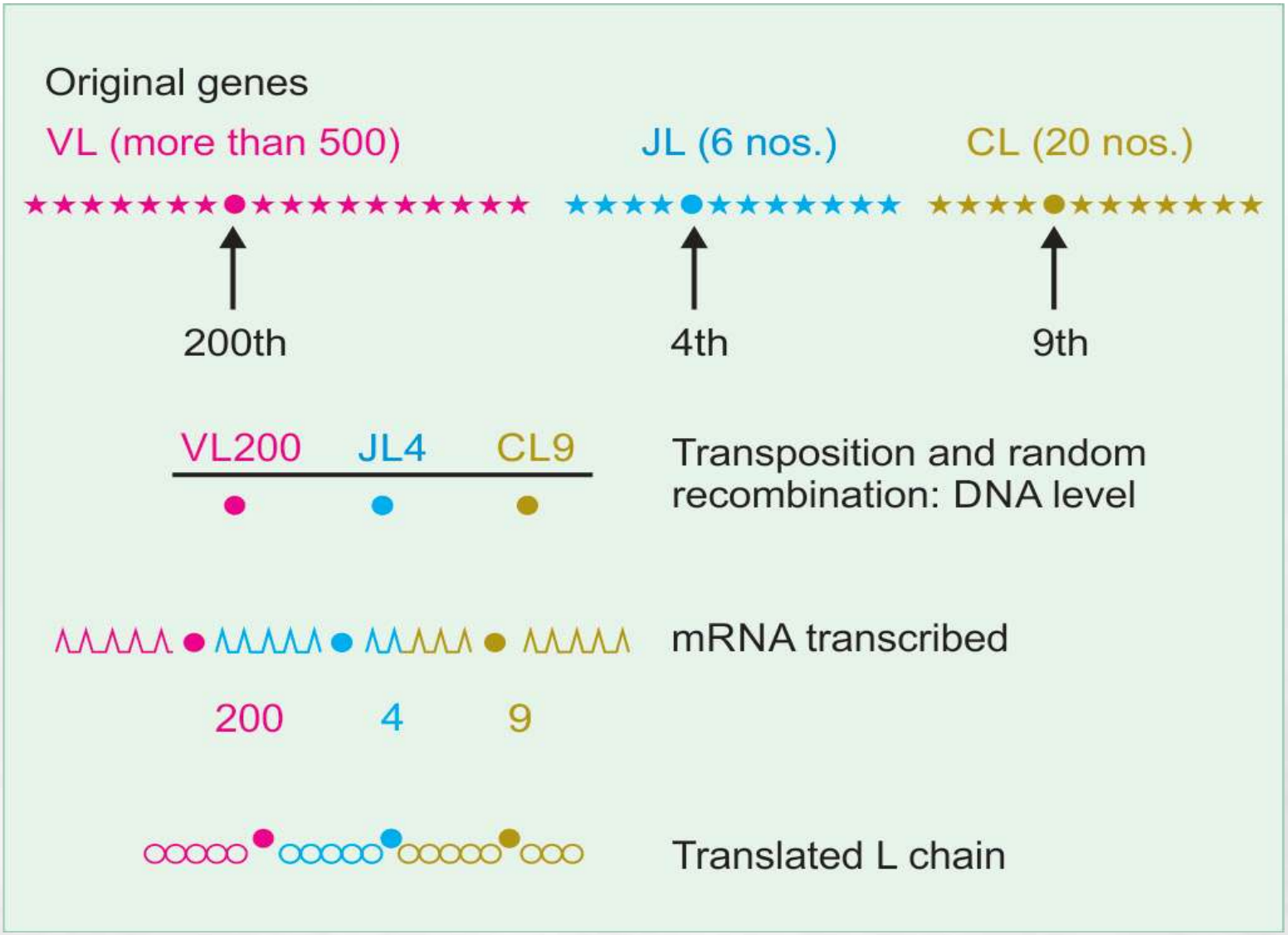
Secondary Immunodeficiency

Seen in malnutrition, leukemias, lymphomas, multiple myeloma and in AIDS.



Primary and secondary immune responses.

Antibody Diversity



Antibody Diversity

TRANSPOSITION OF GENES

It is otherwise called **somatic recombination of DNA**. Each L chain gene contains over 500 VL (variable light) segments, 5–6 JL (joining light) segments and 10–20 CL (constant light) segments. This recombination is similar to the spliceosomes . In this Figure, 200th VL, 4th JL, and 9th CL segments of germline are brought together. In this given example, random rearrange- ment allows VL (200)-JL(4)-CL(9) segments to remain in the gene, while other genes are deleted. These VL-JL-CL segments are transcribed as a single mRNA, and trans-lated into a specific immunoglobulin light chain. Another permutation is taking place in another cell. Thus, millions of cells can produce endlessly diverse light chains.

Molecular Structure of Antigens

RBC, A antigen:

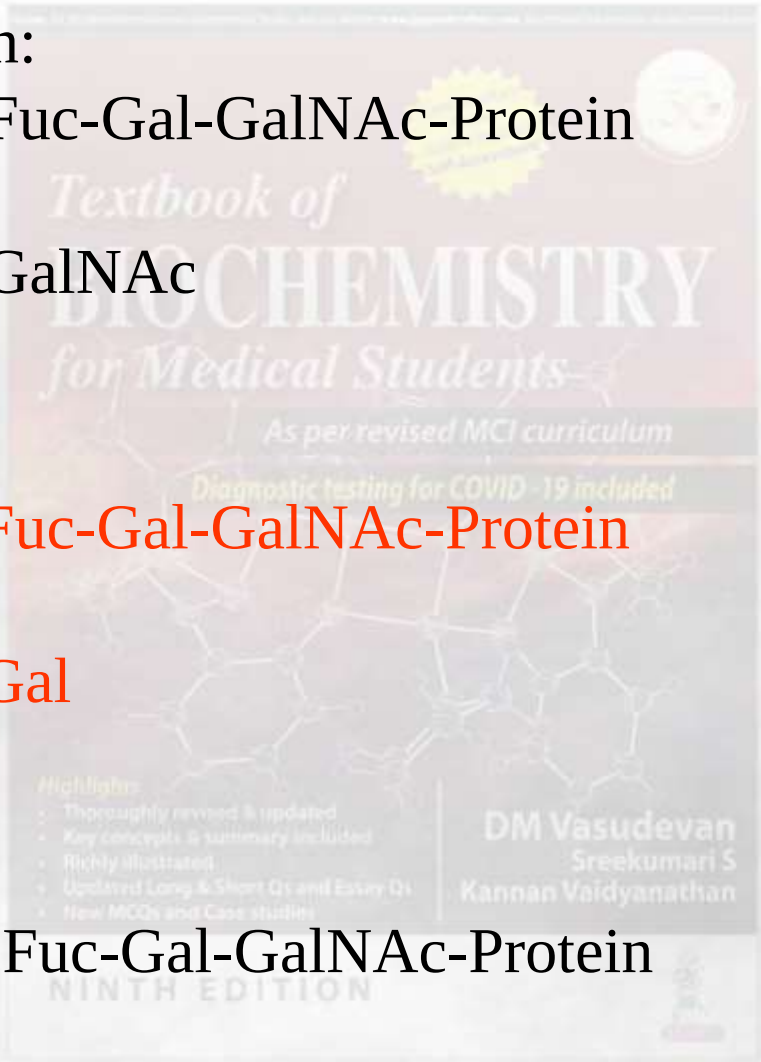
Fuc-Gal-GalNAc-Protein
|
GalNAc

B antigen:

Fuc-Gal-GalNAc-Protein
|
Gal

O antigen:

Fuc-Gal-GalNAc-Protein



The ABO system antigens are glycoproteins present on surface of all cell membranes. The membrane surface will carry a protein into which oligosaccharide unit is attached. The H locus codes for fucosyltransferase, which adds fucose to a terminal galactose unit. This is the precursor for both A and B antigens. Since "h" allele of H locus codes for an inactive fucosyltransferase, a person having "hh" combination will not produce the precursor; neither A nor B substances are added; hence the person's blood **group becomes O**.

Those individuals having Hh or HH allele combinations can produce precursor molecules. Such persons having **BB or BO alleles** will generate a specific transferase which adds a galactose unit to the fucose. Persons having **AA or AO alleles** can generate another transferase, which adds N-acetyl galactosamine to the fucose. Thus, the molecular difference between A group and B group is only with regard to the N-acetyl unit.

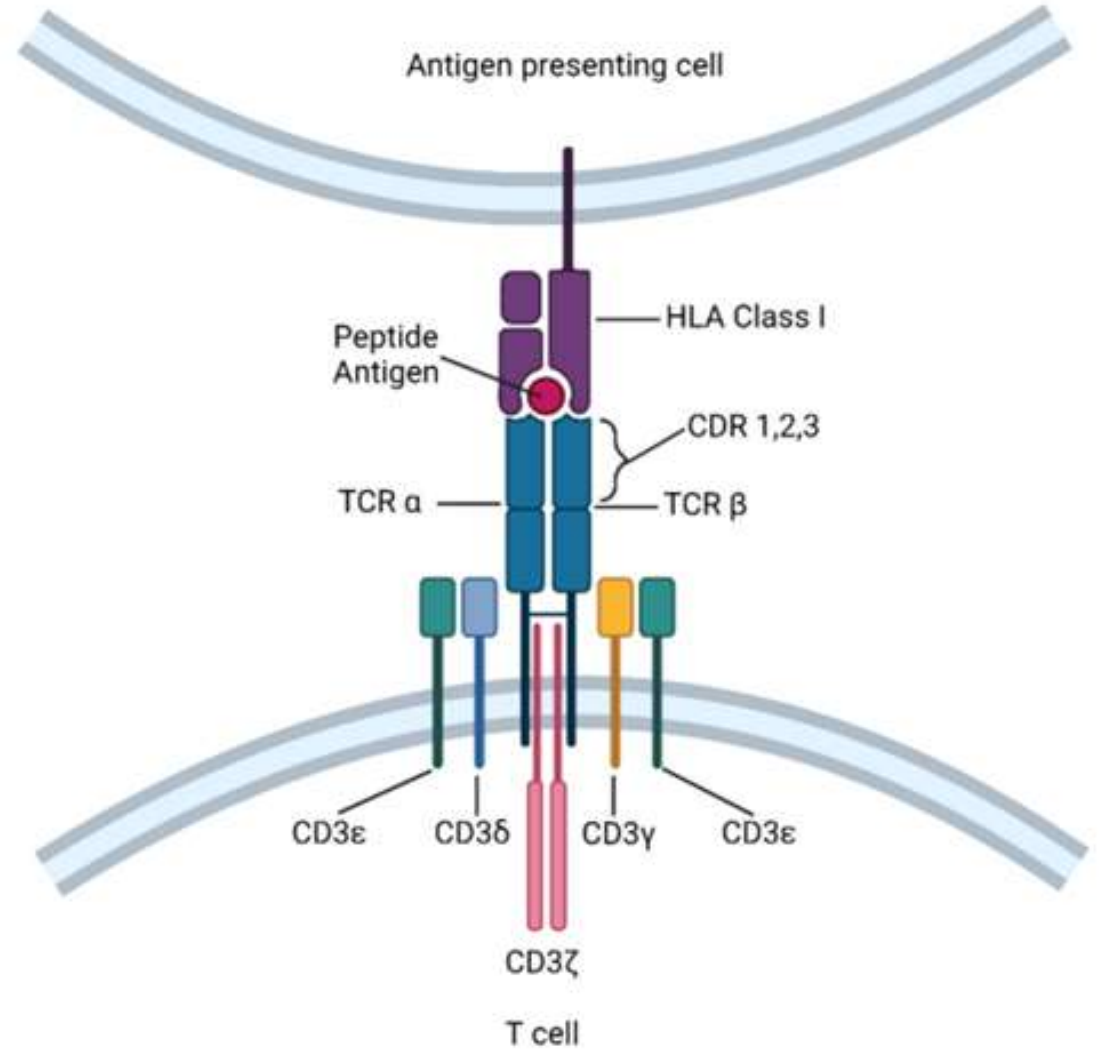
HLA Antigens

When organs are transplanted, the donor and receiver are matched for HLA (human leukocyte antigen) system.

The genes of major histocompatibility complex (MHC) are involved in the recognition between self and nonself antigens. In human beings, the MHC genes are present on chromosome 6. There are A, C, B, D, and DR loci. All these loci together contain more than 150 alleles. Permutation and combination of them could produce an astronomical number of variations.

Hence, the antigenic constitution of one person will be entirely different from another one. These are main transplantation antigens, responsible for rejection of allograft.

T cell receptor



Interleukins

They are a group of cytokines (signaling molecules) that were first seen to be expressed by white blood cells. The majority of IL are synthesized by the helper CD4+ T lymphocytes, monocytes, macrophages, and endothelial cells. About 20 ILs are isolated.

The **IL-1** stimulates production of receptors for IL-2 on lymphocytes.

The **IL-2** stimulates T cells and NK cells and differentiates them into the effector cells capable of killing the cancer cells. The IL-2 is therefore useful to produce lymphokine-activated killer **(LAK) cells**. Lymphocytes cultured with cancer antigens and stimulated by IL-2 can act as specific (LAK) cells.

Interferons

They inhibit viral multiplication in host cells, modulate cell differentiation and inhibit oncogene expression. Interferons (alpha, beta, and gamma) are proteins made and released by the cells in response to the presence of pathogens (viruses, bacteria, parasites) or tumor cells. They are named after their ability to "interfere" with viral replication within host cells. The IFNs activate immune cells, such as natural killer cells and macrophages and they increase the ability of uninfected host cells to resist new infection by virus. About seven distinct IFNs have been identified in humans.

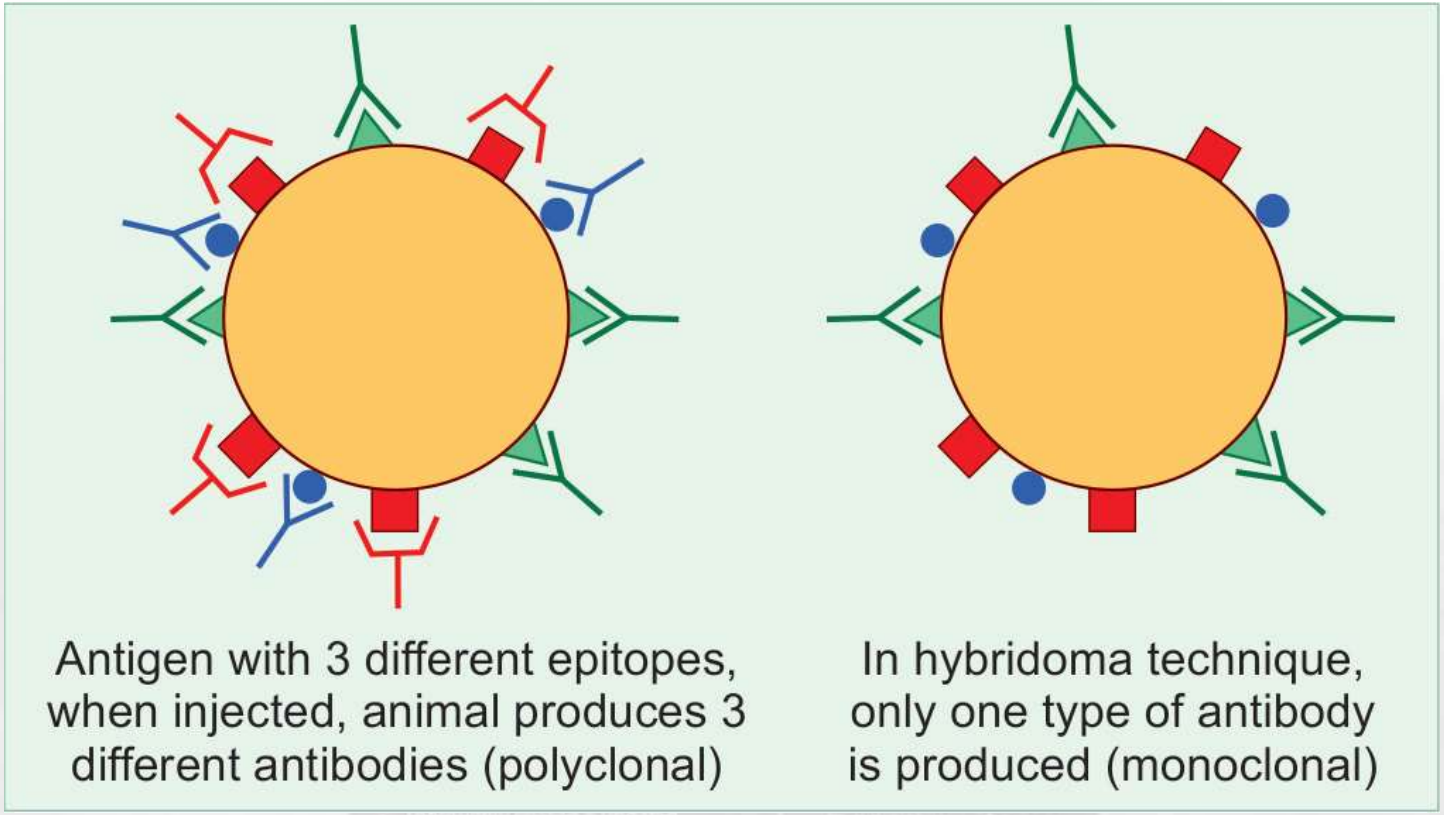
IFN b-1a and IFN b-1b are used to treat and control multiple sclerosis. The IFN is also effective for treating hematological malignancy; leukemia and lymphomas including hairy cell leukemia, chronic myeloid leukemia, and lymphoma. Both hepatitis B and hepatitis C are treated with IFN-a, often in combination with other antiviral drugs.

Lymphokines

Name	Secreted by	Target cell/Function
IL-1	Monocyte, Leucocyte	Induces IL-2 receptors; induces acute phase proteins
IL-2	T helper cells	Maturation of T and NK cells into LAK cells
IL-6	Leukocytes	B cell differentiation; induces acute phase proteins
IFN-alpha	Leukocytes	Proliferation of macrophages
IFN-beta	Fibroblast	Antiviral
IFN-gamma	T and NK	Antiviral; differentiates cells
TNF-alpha	Macrophages	Inflammation, fibrosis, pyrexia, acute phase proteins
TNF-beta	T cells	Inflammation
G-CSF	Macrophages	Stem cell stimulation of granulocytes
GM-CSF	T cells and macro	Stem cell stimulation of granulocytes and macrophages
MIF	T cells	Activation and inhibition of mobility of macrophages

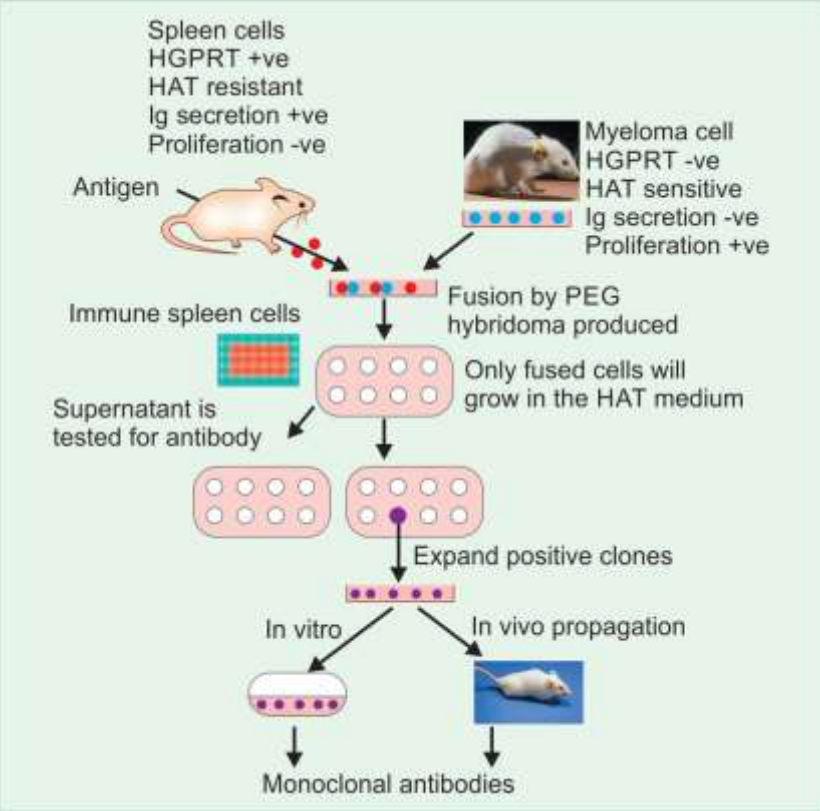
IL= Interleukin. IFN = Interferon. TNF= Tumor necrosis factor; G-CSF = Granulocyte colony stimulating factor. GM-CSF = Granulocyte macrophage colony stimulating factor. MIF = Macrophage migration inhibition factor. CRP = C-reactive protein.

Monoclonal antibodies



Difference between polyclonal and monoclonal antibodies.

Monoclonal antibodies



Principle of production of monoclonal antibodies by hybridoma technique. Immune spleen cells and myeloma cells are fused. The cells are incubated in HAT medium. Unfused lymphocytes will die as they do not have the proliferation capacity. Unfused myeloma cells lack in *HGPRT* gene, so alternate pathway for DNA synthesis is not available. The main pathway for DNA synthesis is blocked by aminopterin in the HAT medium. So, unfused myeloma cells die. Only fused cells can grow in HAT medium.

Monoclonal antibodies

Applications of Monoclonal Antibody

- Enumeration of lymphocyte subpopulations
- Quantitative preparation of specific cells
- As immunosuppressant in clinical conditions
- Nephelometric assays of blood components
- Specific antibodies in ELISA tests
- Quantitative preparation of pure antigens
- Cancer chemotherapy.

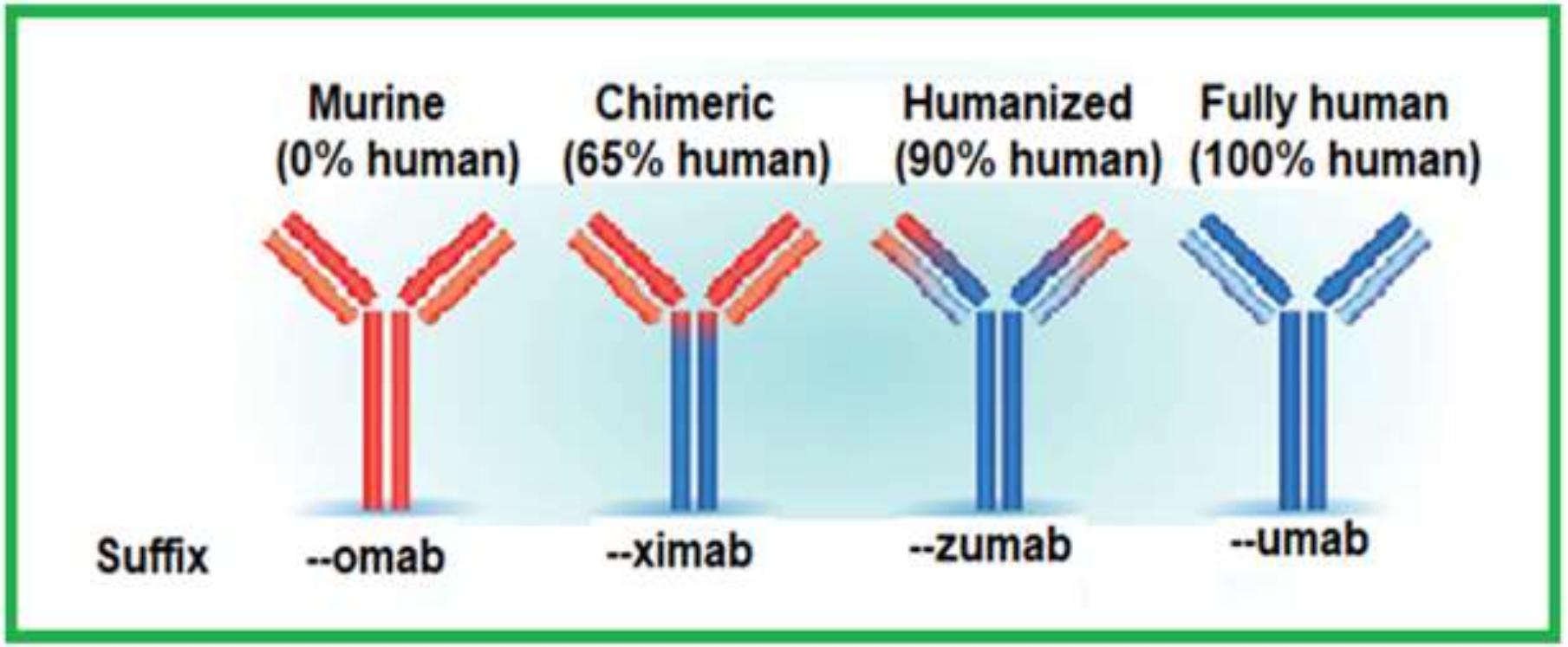
Advantages of Monoclonal Antibody

In a monoclonal preparation, all the antibody molecules are specific against a particular antigen. They have increased **avidity** for attaching with antigen. So in a reaction where polyclonal antibodies require 1 mL, the monoclonals require only microliter quantity. The initial cost for production is high; but when once produced, it could be harvested continuously.

Therapeutic uses of monoclonal antibodies

Useful in disease	Target molecule	Name of drug
Alzheimer's disease	Alzheimer protein beta	Bapineuzumab and Solanezumab
Renal transplant rejection	IL2-receptor	Basiliximab, Daclizumab
Autoimmune diseases,	TNF-alpha	Adalimumab, Golimumab, Infliximab
Rheumatoid arthritis	IL-6 receptor	Tocilizumab, Sarilumab
Systemic lupus erythematosus	B lymphocyte stimulator (BlyS)	Belimumab
Multiple sclerosis	IL-2 receptor	Daclizumab
Multiple sclerosis	CD20	Ocrelizumab
Allergy	Immunoglobulin E	Omalizumab
Allergy and asthma	IL-5 receptor	Benralizumab, Reslisumab
Asthma	IL-4 receptor	Dupilumab

Nomenclature of monoclonal antibodies



The suffix **-omab** means the antibody is completely murine;
suffix **-xima**b means it is 60-65% humanized,
suffix **-zumab** means it is 90% humanized and
suffix **-umab** means it is 100% humanized

Vaccines

Vaccination provides adaptive immunity to resist the attack of specific infective agents. Basic requirements for any vaccine are:

- Safety
- Induce correct type of immunity
- Affordability.

The vaccines improve the immunity against a particular disease. It mimics a pathogen and produces antibody and thereby immunity. Different types of vaccines are

1) live, attenuated vaccines,
2) killed inactivated vaccines,
3) subcellular fragments,
4) toxoid vaccines, and
5) recombinant vaccines.

Mechanism of Action of Vaccines

The pathogen-mimicking vaccine molecule is captured by antigen-presenting cells (APC) and activates immune cells like T-cells and B cells. They produce antibodies against the antigen. Ultimately memory T and B cells are activated.

Hence, the body's response to a subsequent attack by the actual pathogen will be stronger than when there was no vaccine molecule in the first place. The vaccines have the ability to produce herd (community) immunity and this can help prevent outbreak of contagious disease. Diseases like smallpox have been eradicated using vaccination.

Types of Vaccines

	Type of vaccine	Disease prevented
1.	Live attenuated organism	Polio, MMR, Yellow fever, Tuberculosis, hepatitis A
2.	Killed intact organism	Polio, Rabies, Influenza, Hepatitis A, Pertussis, Typhoid, Cholera, Q fever
3.	Subcellular fragments Capsular polysaccharide	Meningitis
4.	Toxin based	Tetanus, Cholera, Diphtheria
5.	rDNA based	Hepatitis B, Rabies, Influenza, Streptococcal pneumonia