IMMUNOLOGY :

Immunity involves the resistance shown and protection offered by the host organism against the infectious disease.

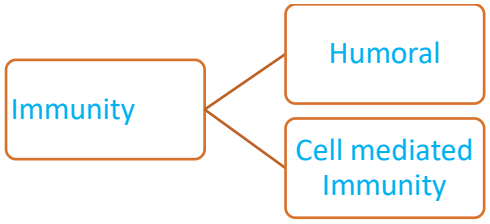


Figure number 1- Types of immunity

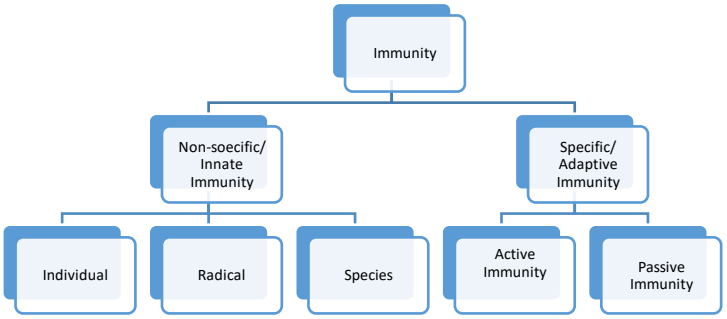


Figure number 2 -Types of immunity

A)Cell Mediated Immunity:

It is associated with T-lymphocytes. The maturation of T-lymphocytes occurs in thymus.

T-lymphocytes identify the viruses and micro-organisms from the antigen displayed on their surface.

There are 4 types of T-lymphocytes:

1) Inducer T cells: Mediate development of T-cells in thymus.
2) Cytotoxic T-cells: Reorganise and kill infected or abnormal cells.
3) Helper T-cells: Initiate immune responses
4) Suppressor T-cells: Suppress immune response.

B)Humoral Immunity :

B-Lymphocytes are responsible humoral immunity.

Bone marrow is the site of maturation of B-lymphocytes

B-lymphocytes mature into plasma cells and secrete antibodies (Immunoglobulins).

Immunoglobulins:

Humoral Immunity is mediated by special group of proteins called immunoglobulins or antibodies produced by B-lymphocytes.

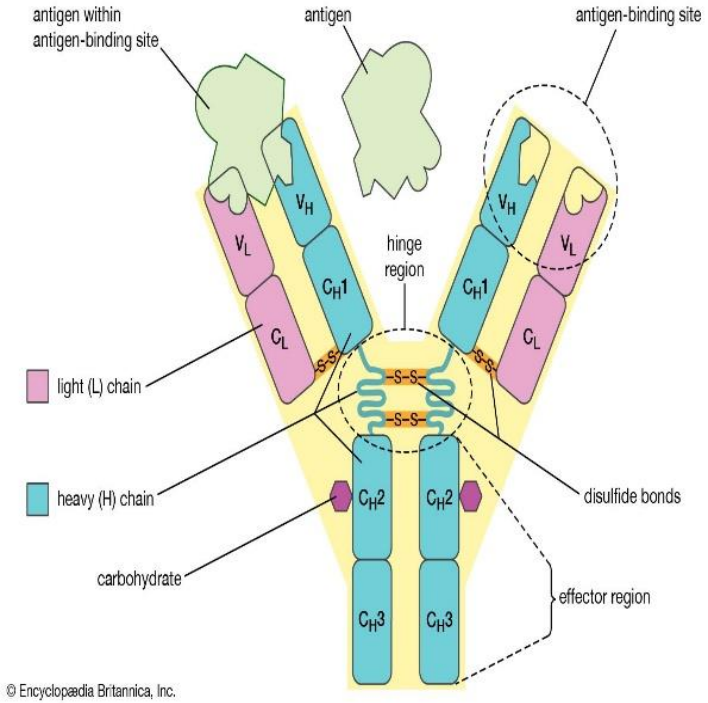
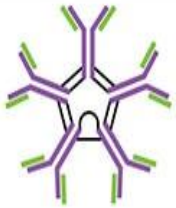
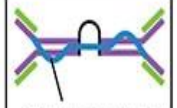
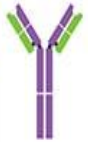
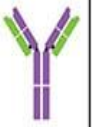


Figure number 3- immunoglobulin

Table number 1- Types of immunoglobulins

The Five Immunoglobulin (Ig) Classes					
	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
					
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	no	yes	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor

Major Histocompatability Complex

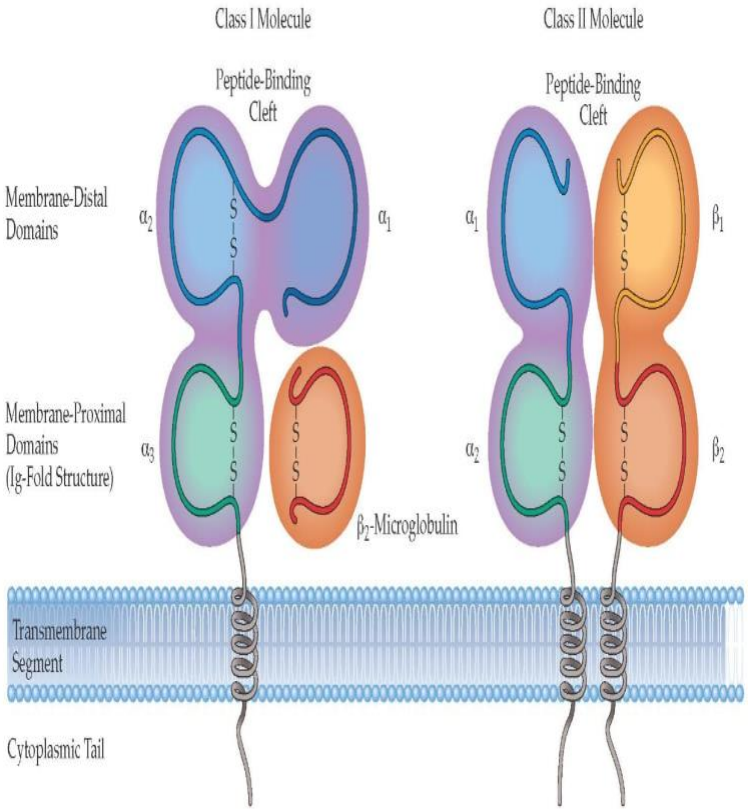


Figure number 4 - Major Histocompatability Complex

Functions of MHC:

1) MHC provides cell mediated as well as humoral mediated immune response. The antibodies selectively bind with with antigens. T cells recognize only those antigens combine with MHC molecules are antigen presenting structure

2) Class 1 and Class 2 MHC molecules provides process endogenous antigen CD8 T-cells Class2 molecules provides exogenous antigen CD4 T-Class cells
3) 1 molecules identifies all the body cells as "shelf" and induced antibodies synthesis that enters the post having different class 1 molecules
4) Class 2 molecules have D group MHC responsible for stimulating antibody formation
5) Class 2 molecules participate in T-cell communication with macrophage and B-cells

Immune Stimulation:

Stimulation of immune system by an external source is termed immune stimulation or immunostimulation. This stimulation builds up a protective mechanisms against a micro-organisms. Substance that induce that elevate the activity of one or more immune system components are termed as immunostimulants.

Examples of immunostimulalting agent:

1) Levamisole

2) Thalidomide

4) Recombinant cytokines

a) Interferons
b) Interleukins

Immune Suppression:

A phenomenon in which an organisms ability to form antibodies in response to an antigens. Stimulus is reduced or suppressed is the termed as immune suppression or immunosuppression

Immunosuppressive drugs that are used for prolonging the life expectancy organ. Examples are heart, kidney, and eyes.

Examples of immunosuppressive agents:

1) Antiproliferative agents : Cyclophosphamide
2) DNA Base analogue : 6-mercaptopurine
3) Antibiotic : ActinomycinD
4) Mitotic Poison : Mitomycin

HYPERSENSITIVITY REACTIONS:

Table number 2-Types of Hypersensitivity Reactions

Type	Descriptive Names	Mechanism
1	Inge mediated/ anaphylactic hypersensitivity	Antigen induces cross linkage Inge bound to mast cells and basophils along with the release of vasoactive mediators
2	Antibody dependent cytotoxic Hypersensitivity	Antibody directed against cell surface “against the mediated cell destruction by complement activation or ADCC
3	Immune Complex mediated hypersensitivity	Ag-Ab complexes deposited in various tissue induce complement activation and anti-inflammatory responses
4	Cell mediated/ Delayed type hypersensitivity	Sensitized T DTH cells release cytokines that activates macrophages or Tc cells that mediate direct cellular damage
5	Stimulatory hypersensitivity	-

HYBRIDOMA TECHNOLOGY:

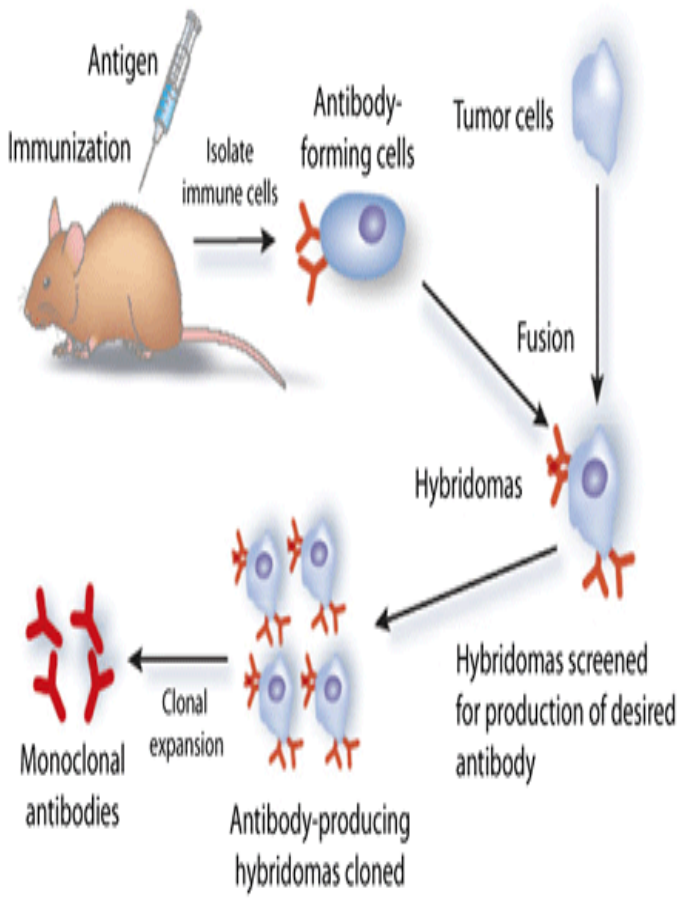


Figure number 5 - Hybridoma Technology

Monoclonal antibody (Mab) is a single type of antibody that is directed against a specific antigenic determinant (epitope).

Gorge Kohler and Cesar Milstein successfully hybridize antibody producing B-lymphocytes with myeloma cells in vitro and create a hybridoma.

The production of monoclonal antibodies by the hybrid cells is referred to as hybridoma technology.

Principle:

The myeloma cells used in the hybridoma technology must not be capable of synthesizing their own antibodies.

The selection of hybridoma cells is based on inhibiting nucleotide DNA synthesizing machinery.

The mammalian cells can synthesize nucleotide by 2 pathways:

1) De novo synthesis
2) Salvage Pathway

The artificially immortalized B-lymphocytes can multiply indefinitely in vitro and produced MAbs. The hybridoma cells possess the growth and multiplying properties of myeloma cells but secrete Ab of B-lymphocytes

Production Of Monoclonal Antibodies:

Steps:

1) Immunization
2) Cell function
3) Selection of products
4) Screening the products
5) Cloning and propagation
6) Characterization and storage

Immunization of animals(mouse) with appropriate antigen and adjuvant (freund's complete or incomplete adjuvant) is injected subcutaneously multiple times

Increased stimulation of B-lymphocytes. Three days prior to killing of the animal, a final dose of antigen is intravenously administered.

The spleen is aseptically removed and disrupted of sacrificed animal by mechanical enzymatic methods to release cells

Lymphocytes are mixed with HGPRT defective myeloma cells

The mixture of cells is exposed to PEG (polyethylene glycol) for short period

PEG is removed by washing and cells are kept in the fresh medium (These cells are composed of mixture of hybridoma, free myeloma cells and free lymphocytes)

Only hybridoma cells grow in HAT medium while the rest will slowly disappear (7-10 days of culture)

Single antibody producing hybrid cells are isolated and grown individually (cultured and desired antibodies are produced)

The hybridomas are screened for secretion of antibodies of desired specificity by using ELISA and RIA techniques. The antibody secreted by hybrid cells is referred as monoclonal antibodies

Cloning and propagation of single hybrid cells producing desired antibodies by using following cells

1) Limiting dilution method
2) Soft agar method

The monoclonal antibodies has to be subjected to biochemical and biophysical characterization for desired specificity

Purification Methods:

1) Centrifugation : For removing cells, cell debris, lipids and clotted materials
2) Filtration : By using 0.45um filter
3) Ultrafiltration or dialysis
4) Ion exchange chromatography
5) Size exclusion chromatography
6) Affinity purification

Application of monoclonal antibodies :

1) Diagnostic applications
   a) Biochemical analysis for the diagnosis of pregnancy, cancer, hormonal disorders
   b) Diagnostic imaging for the detection of myocardial infarction, deep vein thrombosis
2) Therapeutic applications:

a) Direct use as therapeutic agent to destroy disease causing organisms, in the treatment of cancers, AIDS
b) As targeting agents in the therapy as immunotoxin, in drug delivery, for dissolving blood clots
3) Protein purification by immunoaffinity techniques
4) Miscellaneous applications: as catalytic agents, in autoantibody fingerprinting

Storage conditions and stability of official vaccines:

1) BCG Vaccine: Stored at 2-8˚C and protected from light
2) Cholera vaccine: Stored at 2-8˚C and should not frozen
3) Tetanus toxoid : Stored at 2-8˚C and should not be frozen
4) Rabies vaccine : Stored at 2-8˚C and should not be frozen
5) Polio vaccine : Stored at -20˚C
6) Hepatitis vaccine : Stored at 5-8˚C and should not be frozen

General method of preparation of Bacterial vaccines, Toxoids, Viral vaccines, antitoxins, serum immune blood derivatives:

Selected strains of bacteria are cultivated on solid medium for 1-2 days and then wash with sterile with normal saline

The suspension obtained is shaken for uniform distribution

The fragments on the medium are removed by centrifugation

The suspension is sterilized by heat treatment or by using alcohol

Vaccine prepared from cultured of non-sporing bacteria are sterilized in a vaccine bath for an hour at 56-60˚C. to kill the bacteria

The heat is applied should killed the micro-organism but should also their antigenic properties.

After sterilization of the suspension bacteria concentration in it is determined, desired dilution is prepared and preservative is added

The product is filled in sterilized containers and sealed under the aseptic conditions

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