UNIT-3

DATE
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Introduction

Types of Immunity - humoral Immunity, Cellular immunity

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(a) Structure of Immunoglobulin.

(b) Structure and function of MHC

(c) Hypersensitivity Reactions, Immune Stimulation and Immune Suppression.

(d) General method of the prepn of bacterial vaccines toxoids viral vaccine. antitoxins, Serum - immune blood derivatives and other products Relative to immunity.

(e) Storage Condition and Stability of official vaccines.

(f) Hybridoma technology - Production, Purification and Application.

(g) Blood products and plasma substitutes.

• Immunity, Types of immunity

→ Immunity is the state of resistance exhibited by an individual to micro-organisms and foreign cells. The ability of a body to defend itself against specific invading agents such as bacteria, toxins viruses and foreign tissue is called Specific resistance or immunity.

→ The immune system includes the cells and tissues that carry out immune response.

Types of Immunity (Immune responses)

→ Immunity consists of two kinds of responses both are triggered by antigen. There are two types:-

1. Cell Mediated Immunity (CMI)
2. Humoral or antibody mediated immunity.

• Immunology → It is a branch of science in which we deals about the immunity immune system.

Types on the basis of cell involved

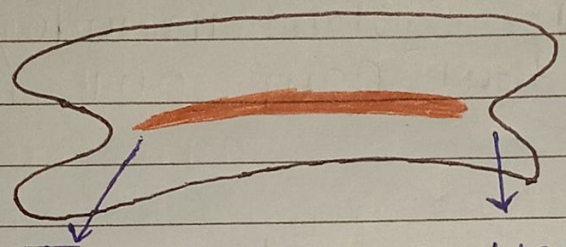
1) Acquired → (artificial means) by vaccines toxoids and other means by gaining immunity.

(2) Innate-> by birth immunity.

1. Cell Mediated Immunity

→ Initiated by T- lymphocytes
→ CD8 T Cells proliferate into cytotoxic T-cells that directly attack the invading agents.

* Red bone Marrow



Pre T-Cells → Thymus → Mature T-cells → CD8 T-cells → Cytotoxic T-cells

Mature B-cells → Activation of B-cells → Plasma cells → antibodies glutinate and react with Ag

CD4 T-cells → Help → B-cells
CD4 T-cells → Helper T cells.

Fig: 01

→ Cell Mediated immune response begins with the activation of small number of T-cells by a specific antigen.

→ once a T-cell has been activated

It undergoes proliferation and differentiation into a clone of effector cells which carry out immune response.

→ THIS CMI effective against:-

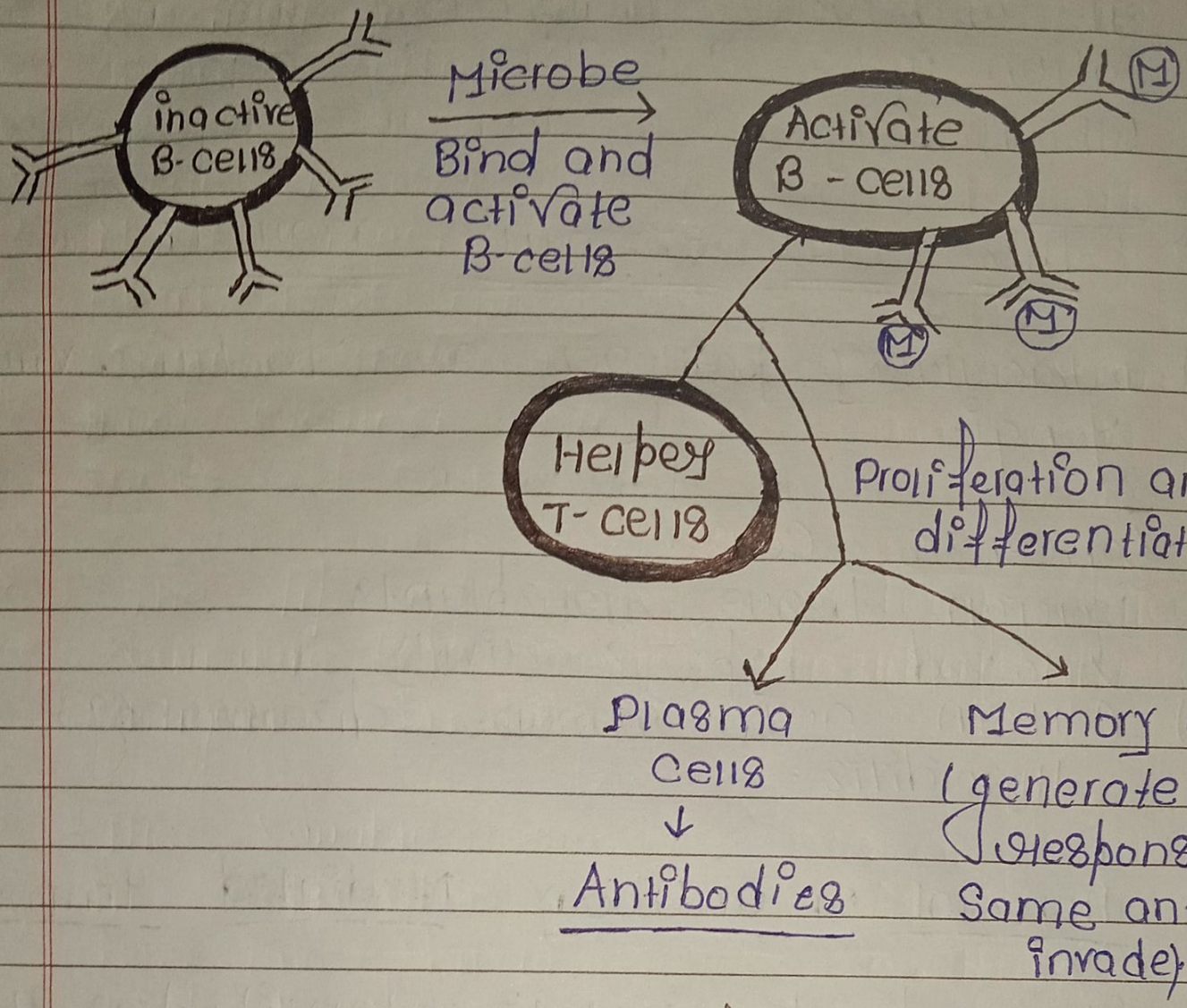
(1) Intracellular pathogen as bacteria, virus fungi.
(2) Cancerous cells.
(3) Foreign tissue transplants
(4) Delayed Hypersensitivity
(5) Certain auto immune diseases eg:- thyroiditis.

2. Humoral or antibody Mediated Immunity

→ It is due to circulating antibodies. It is a major defense against bacterial infection (Extracellular infection) B cells mainly involve in this.

→ Same as diagram on earlier page.

→ B cells can bind antigen directly but they are contact T- Helper cells to produce full activation and antibody formation. The activated B cells proliferate and transform into memory B-cells and plasma cells. The plasma cells secrete large quantities of antibodies into general circulation.



* Humoral immunity effective against:-

1) Antigen present in body fluid.

2) Extra-cellular pathogen or bacteria virus, fungi.

(3) Play major role in immediate type of hypersensitivity.

Structure of Immunoglobulin

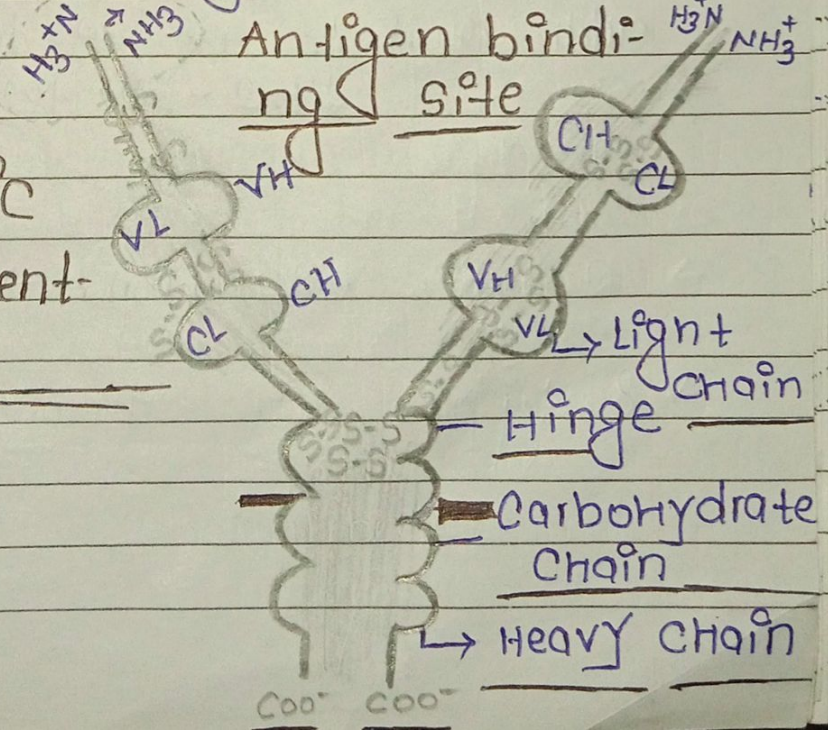
→ Antibodies are protein which produce in response to the entry of an antigen. There are 4 different varieties of plasma protein named as:- Albumin, globulin, Prothrombin and fibrinogen. Globulins are of 3 types:- alpha globulin, β-globulin, gamma globulin since they play their role in immunity so called Immunoglobulin (Ig)

→ As we know that generally immunoglobulins are of five types are as follows:- (a) IgG (b) IgM (c) IgD (d) IgA (e) IgE

→ Most of the Antibodies contain 4 polypeptide chain ie:- (i) 2 heavy chain → high mole. each 450 amino acids cule weight

low molecul- ← (ii) 2 light chain
ar weight. each 220 amino acids.

Diagramatic Representation



→ A disulfide bond (S-S) Hold each light chain with heavy Chain.

→ Two disulfide bonds also link with the Mid region of the two heavy Chain ie hinge region. This hinge region show flexibility, so from this region antibodies arms can move somewhat.

→ The tip of Heavy and light called variable (V) region constitute the antigen binding site.

→ The Variable regions (V) are different in in each Class of Ig. Most of abs Have two binding sites So, they are said to be bivalent. The remainder of each H and L chain are called constant region (C) This region is nearly same in all types of antibodies. Variable region - at N terminal and constant region at C-terminal

→ 1962 - Porter - Proposed Structure of Ig

→ 2 types of light chain → lambda, kappa.

→ 8 types of heavy Chain.

→ These are surface proteins present on the plasma membrane on W.B.C and other nucleotide cells that are unique for each person except identical twins.

→ MHC in human is also known as human leucocyte antigen (HLA) complex.

→ These are also known as self-antigen which are found on the membrane of almost all the cells of human body and vertebrates - also termed - Major Histocompatibility antigens.

1980 - George Snell, Dausset, Benacerraf of Nobel prize.

→ American mouse geneticist - George Snell.

. The cluster of gene was named as Major histo compatibility Complex (MHC) - cause of rejection

→ Major histocompatibility Complex (Genes) (Encode) Major histocompatibility antigen

→ Histo - Tissue, compatibility - agreeable

→ MHC are cluster of genes whose product play important role to differentiate

between self and non self

→ Generally there are three types:-

(i) CLASS-I

Glycoprotein in nature. Transmembrane molecular present on all nucleated cells It consist of 1000 polypeptide chains
(1) α chain (2) β_2 microglobulin

→ α-chain 3 external domain Molecular weight of 45KDA

α_1 α_2 α_3

Encoded by chromosome-6

→ Each domain approximately 90 amino acid long.

→ Transmembrane domain - 25 amino acid.

→ ~~A~~ Short cytoplasmic tail - Intracellular domain.

→ α_3 domain Contains binding site for the T- cell Co- receptor CD8.

→ α_1, α_2 domains form - the antigen binding pocket.

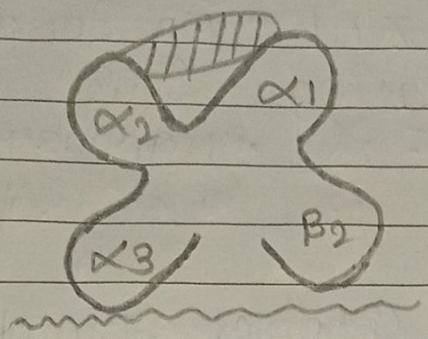
• They has large enough to bind a peptide of 8-10 amino acids.

→ β_2 - microglobulin - Molecular weight - 12KD - encoded by a single gene on

Chromosome - 15

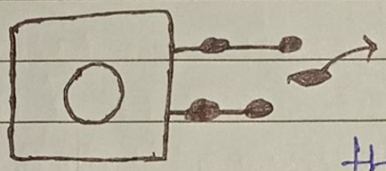
→ It bound non-covalently to α-chain, it do not have transmembrane region. It is identical in all cells.

→



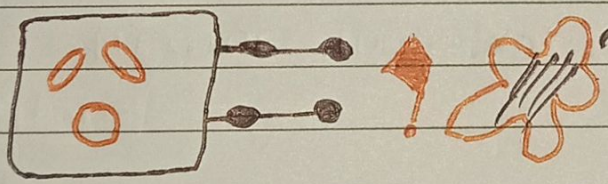
↑ Transmembrane domain
Short cytoplasmic tail (Intracellular domain)

→



Self antigen → so when Leucocyte approach, it recognize that these antigen are self or non self.

→



→ Leucocyte and produce → immune response

Infected cell — Pathogenic antigen

Then leucocyte attach

→ They involved in graft rejection and cell mediated cytolysis.

(ii) Class-II → encoded by HLA-DR, HLA-DQ, HLA-DP.

→ They have a very limited distribution principally found on the surface of -macrophages monocytes activated T-lymphocytes (CD_4) and B-lymphocytes. They are present on surface of antigen presenting cells (APC) - dendritic cell T-lymphocytes macrophages B cells.

→ These protein complex function - To help immune cells to communicate with one another

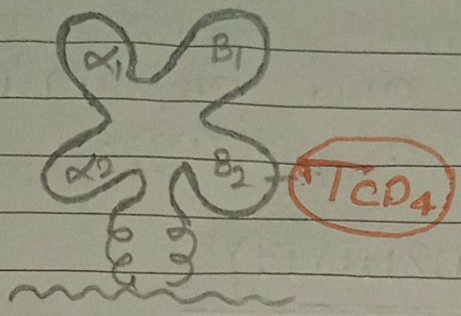
→ Consist of two polypeptide chain - α chain and β-chain.

→ α-chain - α_1, α_2 - External domain
Mol. weight - 33 KDa.

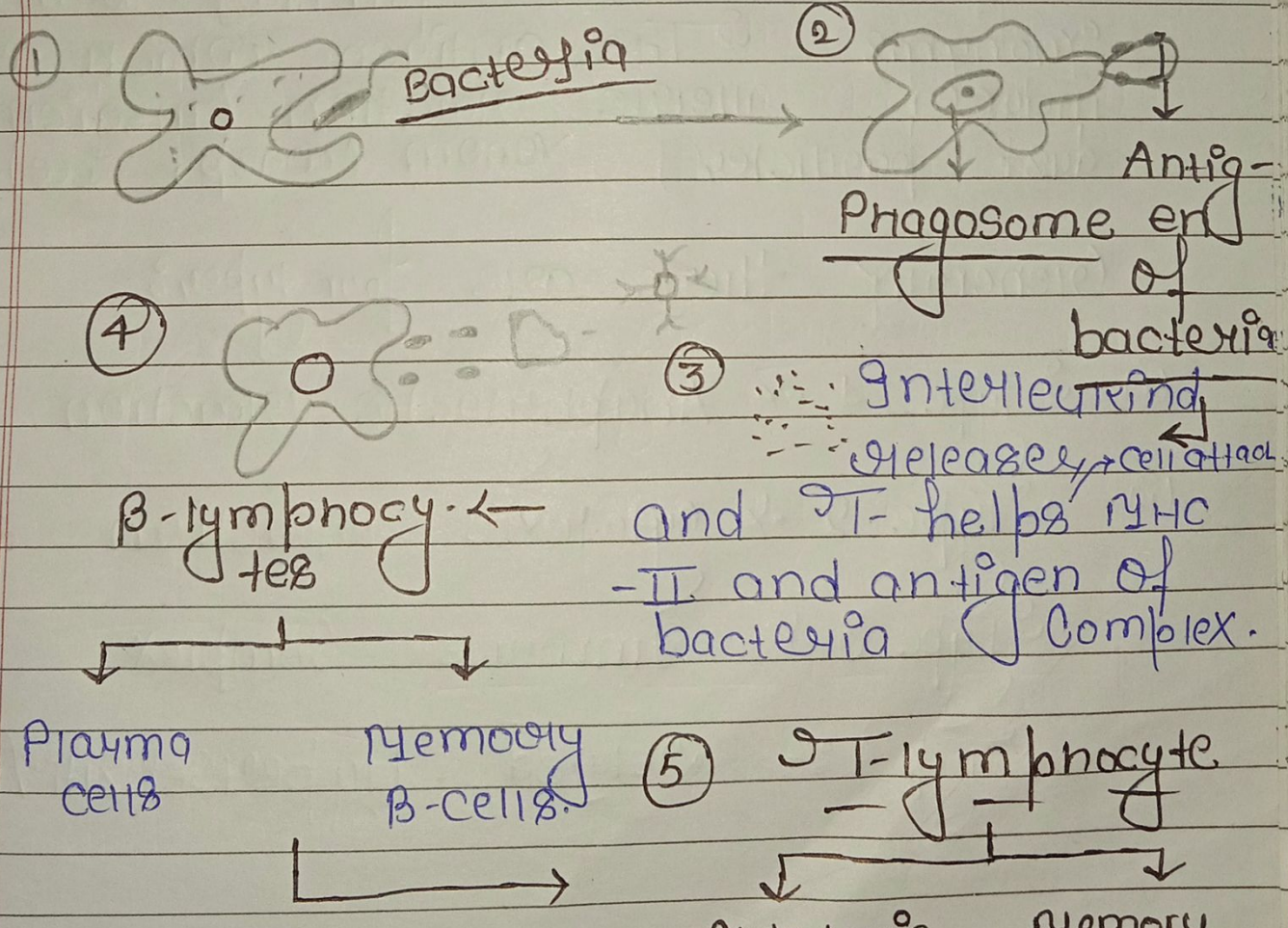
→ β-chain - β_1, β_2 - External domain
Mol. weight

→ β_2-domain contains the binding site for the T-cell - coreceptor CD_4.

→ α_1 and β_1-domain form the antigen binding pocket - 13-18 amino acids Can b/w bind α_1, β_1



◈ Suppose a macrophage engulf a bacterial cell and partially digest on takes a peptide from the bacterial cell (the antigen) and places in on to MHC-Class-II (located on its surface). Then it stimulate T-lymphocyte such as T-helper cells to initiate a set of defensive responses.



(3) Class-III → Class III gene encode C_2, C_4 Complement components of Classical pathway and properidin factor of the alternative pathway.

Hypersensitivity

A person who is overly reactive to a substance that is tolerated by most other people is said to be allergic or hypersensitive. The term hypersensitivity refers to an antigenic response beyond that which is considered normal. The term allergy is more familiar. Both are synonyms. The antigen which are induced allergic reaction (medicines) dust particles, venom (wasp, bee)

→ Generally there are four types:-

(i) Type 1 → Anaphylactic reaction

(ii) Type 2 → Cytotoxic reaction

Type 3 → Immune Complex

Typ 4:- Delayed Hypersensitivity

STRUCTURE AND FUNCTION OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Histocompatibility- histo means tissue compatibility means agreeable.

Complex- these genes are localised to a large genetic region.

Previously it was observed that transplanted tissues from one individual to another member of the same species (allografts) were rejected sometimes. Former in 1930 identified that the antigen responsible for allograft rejection in mice and this lead to the discovery of MHC.

In 1980 George Snell, Dausset and Benacerraf were awarded the Nobel Prize for discovery of MHC (HLA) complex.

Definition-

These are surface proteins present on the plasma membrane on WBCs and other nucleated cells that are unique for each person except for identical siblings.

These antigens protrude from the plasma membrane into extracellular fluid. These are **cell identified markers** are unique for each person except identical twins, although RBCs possess blood group antigen so they are lacking MHC antigens.

MHC in humans is also known as human leukocyte antigen (MHA) because in humans MHC antigens were first discovered in leukocyte cells.

HLA Complex- Major histocompatible complex means the cluster of genes which are responsible for rejection or acceptance for the transplanted or grafted tissue.

Histocompatible genes present on chromosome 6 in humans.

In the case of mice these genes are present at chromosome 17 and known as H-2 complex.

Major histocompatibility complex

---Encode proteins---

Major histocompatibility antigens.

Major histocompatibility complex--Tightly linked cluster of genes whose products play an important role in intercellular recognition and differentiation between self and nonself.

Classification of MHC

MHC has three classes class I class II and class III

Class I- it is encoded by HLA-A, HLA-B, HLA- C genes. The MHC class I antigens are present on the surface of all nucleated cells. They are involved in graft rejection and cell mediated cytolysis.

Structure-

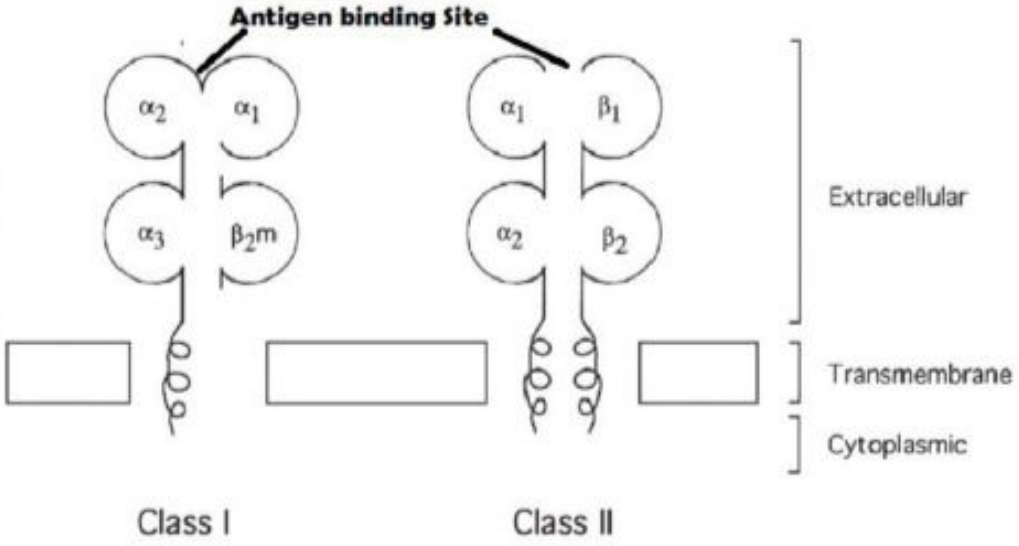
It consists of two polypeptide chains

1) A (alpha) chain
2) B (beta) 2 microglobulin chain.

1. α-chain: **Genes for alpha chain are encoded on chromosome 6.**
    - It is encoded in MHC on chromosome 6 its molecular weight is 45 kilo Dalton. Alpha chain consists of three external domain alpha 1 alpha 2 and alpha 3. Each domain is approximately 90 amino acids long.

- Alpha chain is embedded into the membrane through transmembrane domains which consist of 25 hydrophobic amino acids.
- This transmembrane domain is embedded into the cell membrane through a short cytoplasmic tail which consists of hydrophilic amino acids.
- Alpha 3 domain is attached with a transmembrane domain, and contains the binding site for T cell receptor CD8
- alpha 2 and alpha 1 provide the antigen binding site.
- Alpha 1 and alpha 2 are able to bind peptides (antigen) of 8 to 10 amino acids.

2. Beta 2 microglobulin:
   - Its molecular weight is 20 kilo Dalton and it is encoded by a single gene which is present on chromosome 15.
   - Beta 2 microglobulin bound non covalently to the alpha chain and it is essential for proper folding of the alpha chain so it provides flexibility or stability of the alpha domain.
   - It does not have any transmembrane region and it is identical in all cells.



Working-Healthy cell will bind one of its normal peptides or self-antigen on MHC class 1. When leukocyte approaches, it can recognise the healthy cell by the self-antigen and the leucocytes will therefore leave it alone.

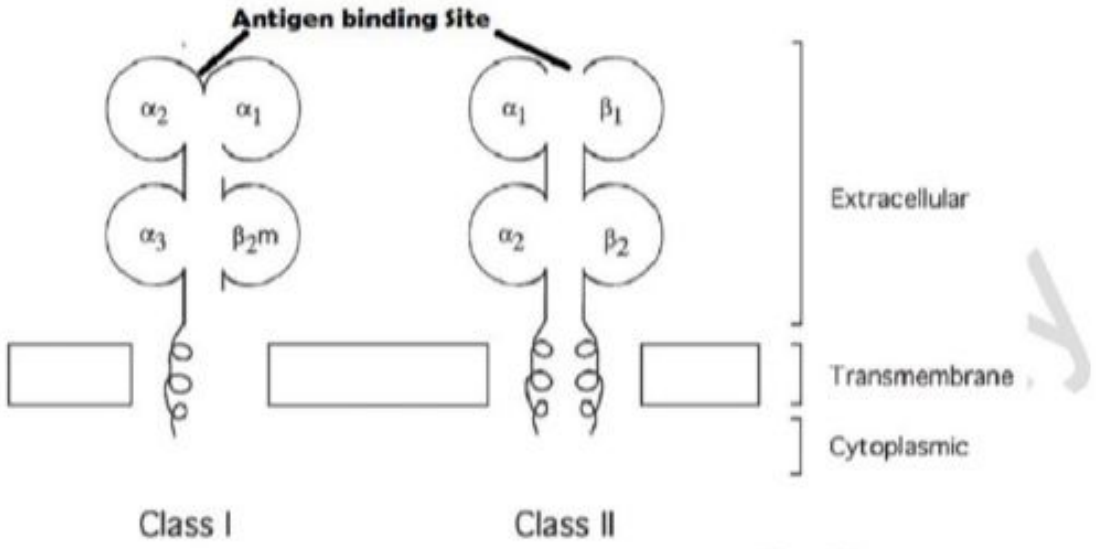
In case of an infected cell the cell will get viral peptide or bacterial peptide or we can say antigen. These viral or bacterial antigens will be placed on MHC class 1.

Leukocyte can recognise those antigens are not from cells, but they are foreign antigens so T cell bind to them and initiate a defensive mechanism that can destroy the infected cell.

Class II: They have encoded by HLA- DR, HLA- DQ, HLA- DP . They have a very limited distribution and principally found on the surface of macrophages, monocytes, activated T-lymphocytes, CD4 and B lymphocytes. They are primarily responsible for the graft versus host response and the mixed leukocyte reaction (MLR).

Structure-

- It consists of two polypeptide chains alpha chain and beta chain.
- Alpha chain has molecular weight 33 kilo Dalton they have to external domains alpha 1 and alpha 2.
- Beta chain has molecular weight 28 kilo Dalton. It has two external domains beta 1 and beta 2.
- beta 2 domain contains the binding site for T cell receptor CD4.
- Alpha 1 and beta 1 domain form the antigen binding site. They can bind up to 13 to 18 amino acids.
- MHC 2 complex functions in helping immune cells to communicate with one another.



For example- suppose a macrophage or antigen-presenting cell engulfs a bacterial cell or viral cell. Then it is partially digested in it. The bacterial partially digested peptide behaves as antigen and placed on MHC class II molecules which are already located on macrophage's surface. So interleukin-1 is released and it calls the T-helper cell. these T-helper cell attach with bacterial antigen and releases interleukin-2. This interleukin-2 calls B lymphocytes and T lymphocytes and activates them. B lymphocytes differentiate into plasma cells and memory B cells whereas T lymphocytes differentiate into cytotoxic T cells and memory T cells.

Plasma cells and cytotoxic cells start to release antibodies against the bacterial or viral antigen so by this method immune cells provide immunity.

Class III - class III gene encodes C2 and C4 complement components of the classical pathway and properdin factor B of the alternative pathway.

Hypersensitive Reaction

Type 1 Hypersensitivity Reaction

A person who is overly reactive a substance that is tolerated by most other people is said to be allergic or hypersensitive.

The term **hypersensitivity** refers to an antigenic response beyond that which is considered normal, the term allergy is more familiar and is essentially synonymous. Hypersensitivity responses occur in Individuals who have been sensitized by previous exposure to an antigen, which in this context it is sometimes called an **allergen**. When an individual who was previously sensitized is exposed to that antigen again his or her, the immune response react to it in a damaging manner.

The four principal types of hypersensitivity reaction-

Type 1- hypersensitivity reaction (anaphylactic reaction)-

It often occurs within 30 minute after a person sensitized to an antigen is really exposed to that antigen anaphylaxis means the opposite of protected anaphylaxis is an inclusive term for the reaction caused when certain antigen combine with **IgE** antibodies.

Anaphylactic responses can be systematic reaction, which produce shock and breathing difficulties and are sometime fatal, or localized reaction which include some common allergic conditions such as hay fever ,asthma, hives (slightly raised of an itchy and red and area of the skin).

IgE antibodies produced in response to an antigen such as insect venom or plant pollen. These antibodies bind to the **mast cells and basophil cells surface.**

Mast cells and basophils can have as many as 500000 sites for IgE attachment.

After attachment of IgE antibodies to mast cells and basophil cells **degranulation** reaction triggers, which releases the granules inside the cells and also the mediators which they contain.

These mediators cause the unpleasant and damaging effect of an allergic reaction.

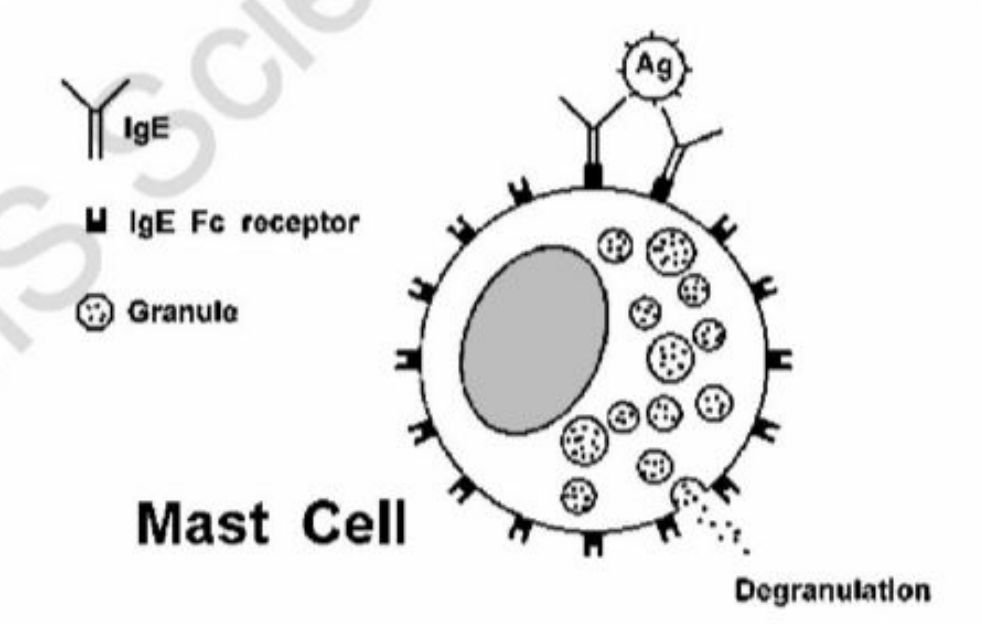
Mediators are as follows-

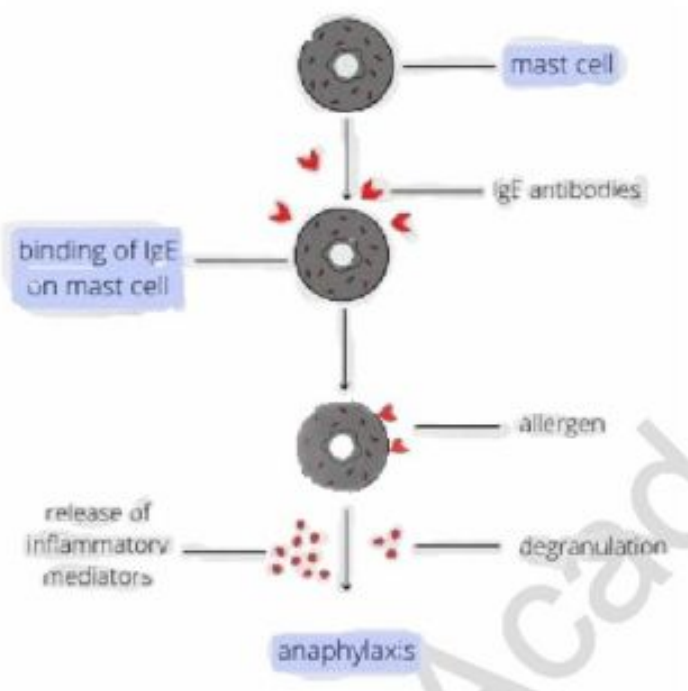
Histamine- this is most important in human anaphylaxis. It causes vasodilation increased capillary permeability and contraction of the smooth muscles.

Serotonin - it causes vasoconstriction increased capillary permeability and smooth muscle contractions.

Prostaglandins and thromboxanes- Both of these are derivatives from arachidonic acid which is formed from disrupted cell membrane of mast cell and leukocytes prostaglandin is a bronchoconstrictor and thromboxane is a powerful but transient bronchoconstrictor.

Platelet activating factor it is released from basophil which causes aggregation of platelets and release of their vasoactive amines.





Type 1 hypersensitivity reaction

Type 2 Hypersensitivity Reaction

Type 2 hypersensitivity it is also named as **cytotoxic reaction IgG and IgM** antibodies are involved and it takes **5 to 12 hours**.

Macrophages, natural killer cells are neutrophils and eosinophils are also involved.

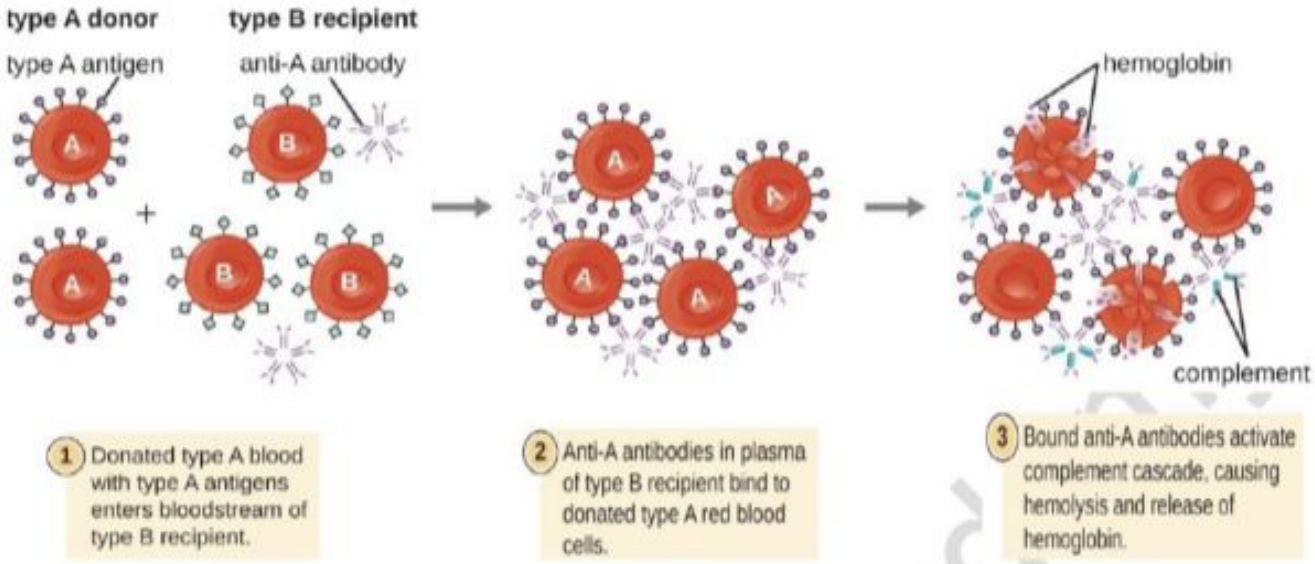
This reaction generally involves the activation of complement by the combination of IgG and IgM antibodies with an antigenic cell. This activation stimulates complement to lyse the affected cell which might be either a foreign cell or host cell that carries a foreign antigenic determinant such as drug on its surface.

The most familiar cytotoxic hypersensitivity reactions are transfusion reaction in which red blood cells are destroyed as a result of reacting with circular antibodies. These involved blood group system that includes the ABO and Rh antigen.

ABO example-

When it transfusion is incompatible as when type B blood is transfused into a person with type A blood. The antigen on the type B blood cell will react with anti B antibodies in the recipient serum. This antigen antibody reaction activates complement which in turn causes lysis of the donor's RBC is as they enter the recipient's system.

	Blood Type			
	A	**B**	**AB**	**O**
Red Blood Cell Type				
Antibodies	 Anti-B	 Anti-A	**None**	 Anti-A and Anti-B
Antigen on Red Blood Cell	A antigen	B antigen	A and B antigens	**None**



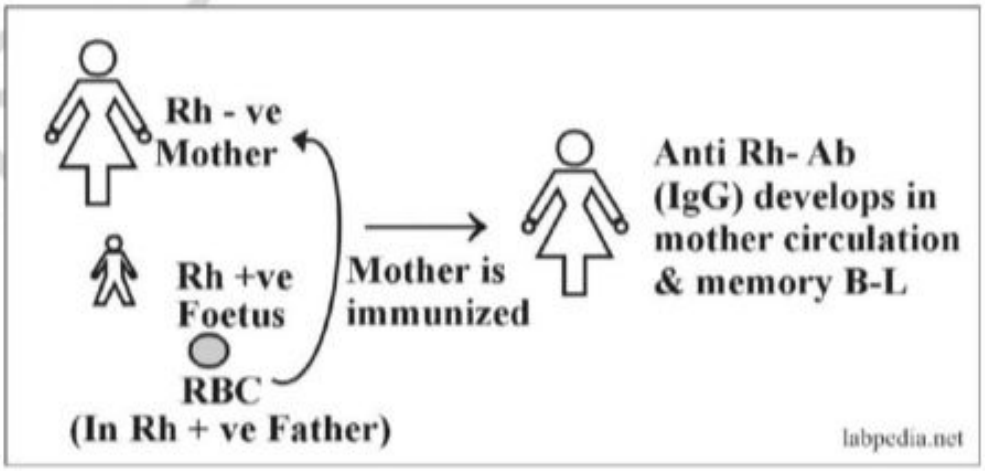
Source: https://courses.lumenlearning.com/microbiology/chapter/hypersensitivities/

Erythroblastosis fetalis-

In this when an **Rh^- woman** has **Rh^+ foetus**, during placental membrane tear the fetal Rh+ RBC enter the maternal circulation causing the mothers body to produce **anti Rh antibodies** of the IgG type.

If mother will get another pregnancy with Rh + foetus her anti Rh antibodies will cross the placenta and destroy the foetal RBC. The fetal body respond to the immune attack by producing large number of immature RBC is called **erythroblast**.

Thus term **erythroblastosis fetalis** it is also termed as **hemolytic disease of newborn** (HDNB).



Type 3 Hypersensitivity Reaction

Type 3 hypersensitivity also termed as **immune complex reaction**. Type 3 reaction involve antibody against soluble antigen circulating in the serum the antigen-antibody complexes are deposited in organ and cause inflammatory damage.

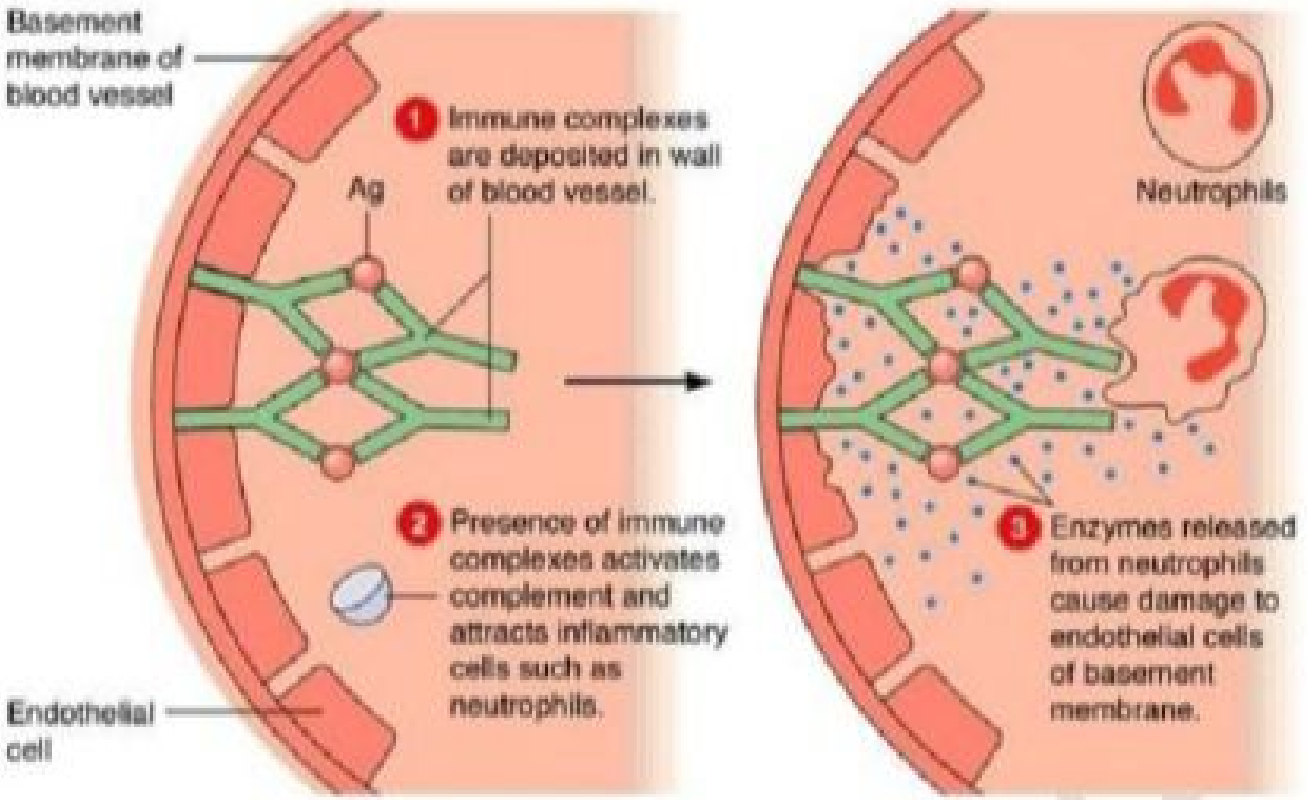
This reaction takes **3-8 hours** and **IgG antibodies** are primarily involved **complement system neutrophil** also take part in this reaction.

Examples of type 3 hypersensitivity are rheumatoid arthritis, serum sickness farmer's lung disease, arthus reaction glomerulonephritis.

Immune complexes form only when certain ratio of antigen and antibody occur. A significant excess of antibody leads to the formation of complement fixing complexes that are rapidly removed from the body by phagocytosis. But in this antigen-antibody complex is too small to phagocytosis.

These complexes circulate in the blood pass between endothelial cells of the blood vessels and become trapped in the basement membrane beneath the cells in this location. They may activate complement and cause a transient inflammatory reaction attracting neutrophil that releases enzymes causing damage to the basement membranes endothelial cell within 2 to 8 hours.

Glomerulonephritis is an immune complex condition usually resulting from an infection that causes inflammatory damage to the kidney glomerular I which are sides of blood filtration.



Source: https://www.onlinebiologynotes.com/type-iii-hypersensitivity-reaction-factors-causing-immune-complex-formation-mechanism-and-types

Type 4 Hypersensitivity Reaction

Type of hypersensitivity it is also named as **delayed or cell mediated hypersensitivity** reaction. These reactions is mediated by sensitized **T lymphocytes** which can contact with specific antigen releases lymphokines that causes biological effect on **macrophages leukocytes and tissue cells**. Type 4 or delayed type of hypersensitivity occurs within **48 to 72 hours** of antigen challenge. As it is not antibody-mediated, it cannot be passively transferred by serum, it can be transferred by lymphocytes or the transfer factor.
Example of delayed hypersensitivity are contact dermatitis, tuberculin infection, and organ rejection in organ transplantation.
CD4 T cells (helper T cells) and CD8 T cells (killer T cells or cytotoxic cells) are involved in this type of hypersensitivity.

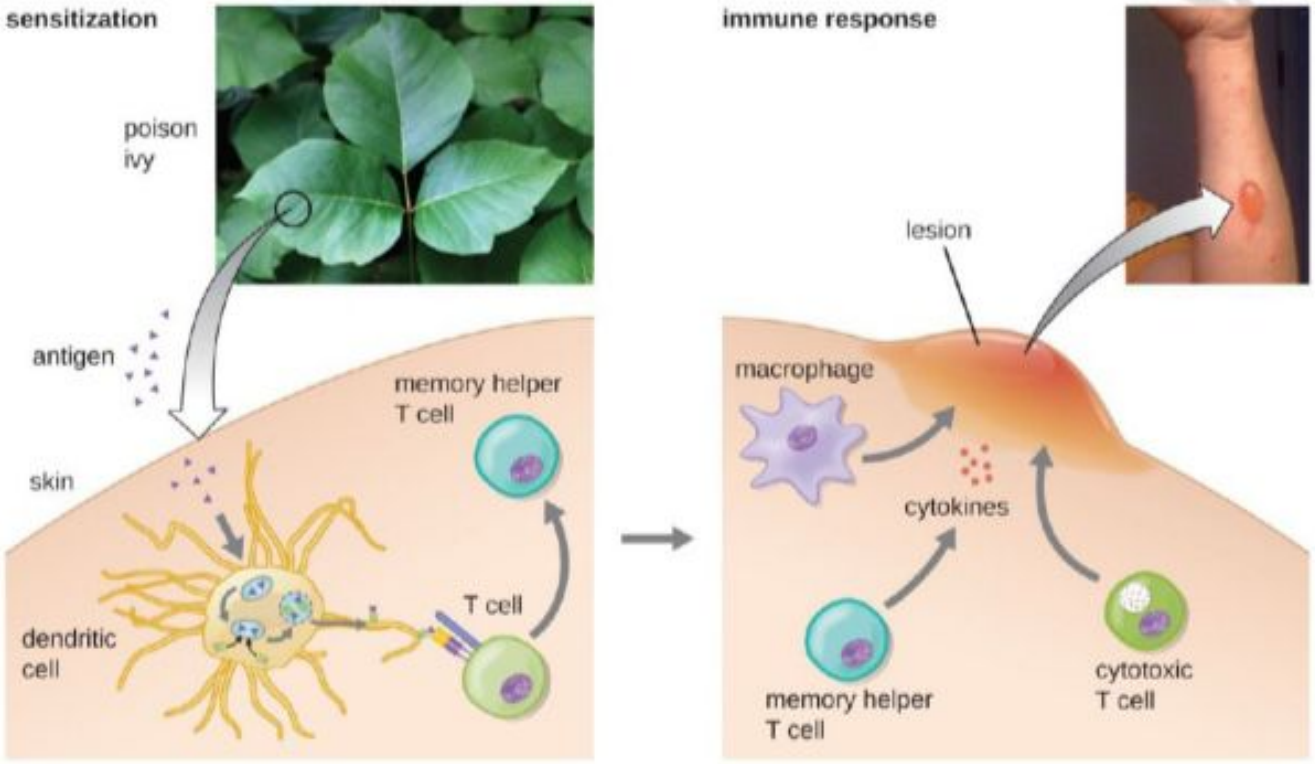
Contact dermatitis- parthenium and poison ivy plant has allergen **Uroshiol** on their surface when these allergens deposit at the epidermis the antigen presenting cells as dendritic cells, langerhans cells interact with allergen or antigen.
Dendritic cells present these allergens with MHC- II molecule.
CD4 cell attach with this version and antipgen-presenting cell complex and releases cytokines. T cells proliferate into two subsets TH -1 and TH- 17.
TH-1 cell releases many cytokines but mainly interferon-gamma which are responsible for different manifestation and produce delayed type hypersensitivity. They act on macrophages so their

a) Phagocytic ability increased
b) Their expression of MHC II molecules increased and
c) They increase the secretion of interleukin 1 and tissue necrosis factor alpha(TNF-alpha 5).

This causes increased inflammation
Another side TH-17 secretes interleukin -17 and interleukin- 22. They bring more inflammatory cells like neutrophil and monocyte to the site of allergy so dermis and epidermis got more macrophages neutrophil and monocytes. These cells try to destroy these allergens by phagocytosis or by releasing enzyme so by mistake they start to damage own skin cells of epidermis and dermis and also this leads to

-Local cell damage
- Inflammation

- Redness
-Itching
-Vesical eruption
- Raised temperature.



Source: https://open.oregonstate.education/microbiology/chapter/19-1hypersensitivities/

Hybridoma Technology (Monoclonal Antibodies)

Hybridoma means the hybridization of activated B lymphocytes and myeloma cells in vitro. The production of monoclonal antibodies by the hybrid cells is referred to as hybridoma technology.

Monoclonal antibody is a single type of antibody that is directed against a specific antigen is determinant or epitope.

Each antigen has specific antigen determinants (epitopes) located on it the antibodies have complementary determining region (CDRs) which are mainly responsible for the antibody specificity.

Antibodies are produced by specialized cells called B lymphocytes cells. B lymphocyte cell isolated and cultivated and they would be able to produce the desired antibodies in nearly unlimited quantities without contamination by other antibodies but unfortunately a B cell reproduce only a few things under the useful cell culture condition. This problem was solved by hybridoma technology.

This technology was discovered by Neil's Herne, George Koehler and Cesar Milstein in 1975, for this they got Nobel Prize in 1984.

Production of monoclonal antibodies for steps in hybridoma technology.

1) Immunization
2) Cell fusion
3) Selection of hybridomas
4) Screening the product
5) Cloning and propagation
6) Characterization and storage

1) **Immunization**- The first step in hybridoma technology is to immunize an animal usually a mouse with appropriate antigen the antigen is injected subcutaneously. Injections at multiple sites are repeated several times this enables increased stimulation of B lymphocyte which is responding to the antigen. Three days prior to killing of the animal a final dose of antigen is intravenously administered. The concentration of the desired antibody is analysed in the serum of the animal at frequent intervals during the course of immunization. When the serum concentration of the antibody is optimal the animal is sacrificed and spleen is removed

aseptically. Spleen is disrupted by mechanical or enzymatical method to release the cells. The lymphocytes of the spleen are separated from the rest of the spleen cells by density gradient centrifugation.

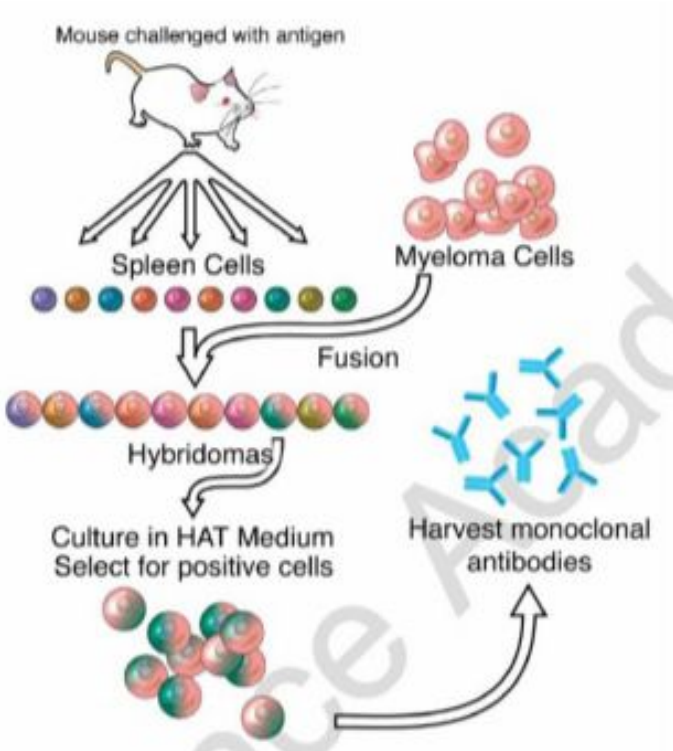
2) **Cell fusion**- myeloma cells are mutated: These mutated myeloma cells have non-functional HGPRT gene. The thoroughly washed lymphocytes are mixed with defective myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes). Since it is toxic. Polyethylene glycol is removed by washing and the cells are kept in a fresh medium. These cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.
3) **Selection of hybridomas** -When the cells are cultured in HAT medium only hybridoma cells grow while rest will slowly disappear this happens in 7 to 10 days of culture.

   Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridoma cell is so diluted that the individual well (aliquots) contain on an average one cell each. These cells when grown in a regular culture medium produce the desired antibody.
4) **Screening the product**- the hybridomas must be a screened for the secretion of the antibody of desired specificity. The culture medium from each hybridoma culture is periodically tested for the desired antibody specificity. The two techniques namely ELISA and RIA are commonly used for this purpose. The antibody secreted by the hybrid cell is referred to as monoclonal antibodies.
5) **Cloning and propagation**- The single hybrid cell producing the desired antibody are isolated and cloned. two techniques are commonly employed for cloning hybrid cell a) limited dilution method and b) soft agar method

   Limiting dilution method - In this procedure, the suspension of hybridoma cells is serially diluted and the aliquots of each dilution are put into micro culture wells. The dilutions are so made that each aliquots in a well containing only a single hybrid cell. This ensures that the antibody produced is monoclonal.

   Soft agar method: in this technique the hybridoma cells are cultured in soft agar it is possible to simultaneously grow many cells in a semi solid medium to form colonies these colonies will be monoclonal in nature.

6) **Characterization and storage:** The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity.



Application of monoclonal antibodies

Monoclonal antibodies with specificity and high-purity have a wide range of applications

1) Diagnostic application
2) Therapeutic application
3) Protein purification and
4) Miscellaneous application

1) **Diagnostic applications:** Monoclonal antibodies may be employed as diagnostic reagent for biochemical analysis or as a tool for diagnostic imaging of diseases.

A. Monoclonal antibodies in biochemical analysis- Diagnostic test based on the use of monoclonal antibodies as reagents are routinely used in radio immunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) in the laboratory. These assays measure the circulating concentration of hormones insulin, human chorionic gonadotropin hormone , growth hormone , progesterone , thyroxine, tri iodothyronine, thyroid stimulating hormone, gastrin , renin and several other tissues and cell products as blood group, antigen , blood clotting factor , interferon interleukin , histocompatibility antigen tumor markers etc.

Pregnancy - by detecting the urinary level of human chorionic gonadotropin.

Cancer estimation of plasma carcino embryogenic antigen in colorectal Cancer and prostate specific antigen for prostate cancer.

Infectious diseases by detecting the circulatory levels of antigen specific to the infectious agent for example antigen of Neisseria Gonorrhoea and herpes simplex virus for the diagnosis of sexually transmitted diseases.

B. Monoclonal antibodies in diagnostic imaging this technique are referred as immuno scintigraphy. The radiant isotopes commonly used for labelling monoclonal antibodies are iodine 131 technetium 99. Is a better diagnostic tool than the other imaging techniques such as CT scan ultrasound scan and magnetic resonance.

a) Myocardial infarction- the cardiac protein miocene is analysed by anti miocene monoclonal antibodies with radioisotope indium chloride.

b) Deep vein thrombosis - monoclonal antibodies directed against fibrin or platelets can be used.

c) Atherosclerosis- monoclonal antibody is tagged with radial able directed against activated platelets can be used to localise the atherosclerotic lessons by imaging technique.

d) Cancer- different tumor markers are are detected with the help of monoclonal antibodies.

alpha fetoprotein marker - cancer of liver and germ cell of testis

epidermal growth factor receptor marker- for melanoma tumor-associated cell surface antigen marker- for various cancers.

2) **Therapeutic applications**- monoclonal antibodies used in

a) Destroying disease causing organism- monoclonal antibody is promoting efficient opsonization of the pathogenic organism and enhances the phagocytosis. In fact monoclonal

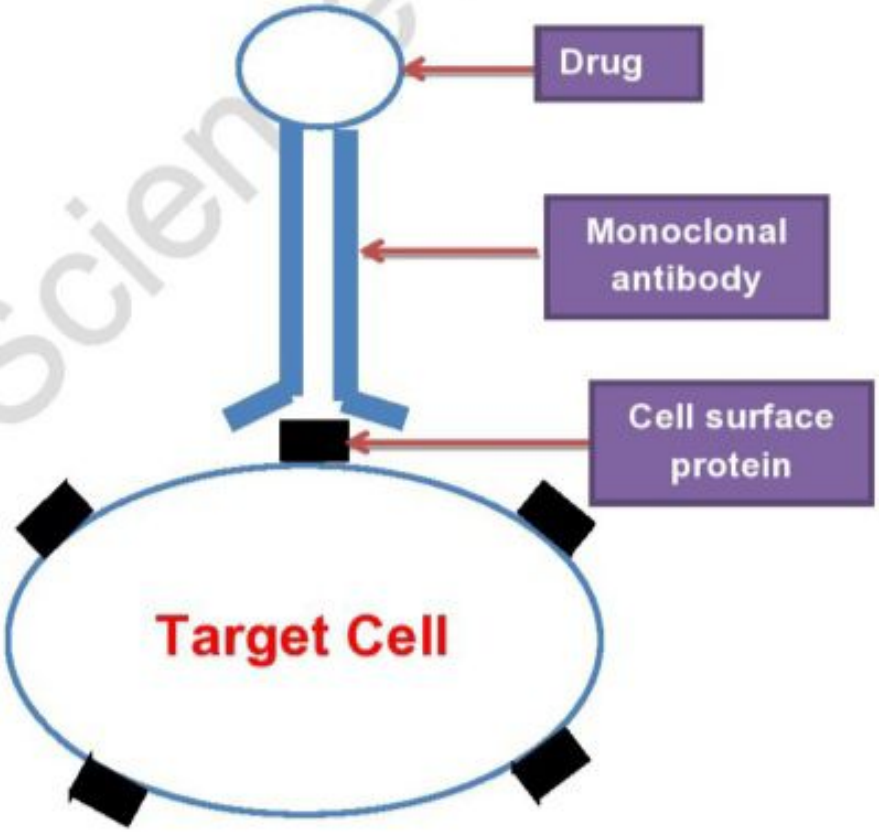
antibodies were found to protect chimpanzees against certain viral hepatitis B virus and bacterial as e coli haemophilus influenzae streptococcus and pseudomonas infection.

b) In the treatment of cancer- monoclonal antibodies against the antigen on the surface of cancerous cell are useful for the treatment of cancer the antibodies bind to the cancer cells and destroyed them.

c) In the immunosuppression of organ transplantation- in normal medical practice immunosuppressive drugs such as cyclosporine and prednisolone are administered to overcome the rejection of organ transplantation but nowadays the monoclonal antibodies OKT3 are used as immunosuppressive agent after organ transplantation in human.

d) In the treatment of AIDS: monoclonal antibodies are very much effective in the AIDS treatment also.

e) In drug delivery- monoclonal antibodies are used in drug delivery to the direct tissue in general the drugs are less effective in Vivo. This is mainly due to that sufficient quantity of the drug does not reach the target tissue. This problem can be solved by using tissue specific monoclonal antibodies.



3) **Protein purification** the immobilized monoclonal antibodies are very useful for the purification of protein by immuno-affinity method.
4) **Miscellaneous application** - different monoclonal antibody are also used as abzymes which has catalytic activity. They are also known as catalytic antibodies

Blood Products and Plasma Substitutes

Blood Products

Blood is a connective tissue composed of a liquid extracellular matrix called plasma that dissolve and suspend various cells and cell fragments.

Blood has **two components**:-

1) **Blood plasma-** A watery liquid extracellular matrix that contain dissolves substances. Blood plasma is about 91.5 % water and 8.5 % solute. Most of solutes are protein are called plasma proteins like albumin, globulin, fibrinogen.

2) **Formed elements-** are cells and cells fragments. the formed elements of the blood include three principal components:

- RBC (red blood cells), white blood cells(WBC) and platelets.
- RBCs and WBC are whole cells where as platelets are cell fragments.
- Blood is about 45% formed element and 55% blood plasma.
- The percentage of total blood volume occupied by RBC is called haematocrit.
- For example a haematocrit of 45 indicate that 45% of the volume of the blood is composed of RBC; the normal range of haematocrit for adult female is 38 to 46% and for adult male is 42 to 54%.

Official products of blood:

- Whole human blood
- Concentrated human RBC
- Dried human RBC
- Dried human serum
- Human fibrinogen
- Human thrombin
- Human normal immunoglobulin injection
- Human fibrin foam

Whole Human Blood

Blood is fluid connective tissue, contains cells and plasma. The basic principle of blood grouping is to prevent antigen antibody reaction while transfusion of blood.

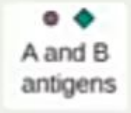
RBCs of human being contain some antigens called **agglutination** reacted with corresponding antibodies called **agglutinins.**

In 1900 **landsteiner** discover the **ABO system** a blood group and found two types of antigen present antigen A and antigen B.

Blood transfusion

Donor- those who donate the blood

Recipient -those who receive the blood

	Blood Type			
	A	**B**	**AB**	**O**
Red Blood Cell Type				
Antibodies	 Anti-B	 Anti-A	**None**	 Anti-A and Anti-B
Antigen on Red Blood Cell	 A antigen	 B antigen	 A and B antigens	**None**

During transfusion of blood from one person to another the AB group must be tested according their ability to donate.

Rh system- In addition to **ABO system,** the human erythrocytes contain another group of antigen called Rh antigen. This was discovered by **landsteiner and wiener** in 1940 in the RBC is of rhesus monkey so it is known as rhesus factor also. The commonest Rh antigen is called **antigen D.**

It is highly immunogenic and its antibody is called anti D. Based on the presence or absence of the D antigen, the human blood can be group as Rh positive and Rh negative.

The blood is transferred from one person to another during acute hemorrhage, chronic anaemia, haemophilia, pre-operative time and in burns.

Collection of human blood- human blood is collected from the median cubital vein in front of the elbow into a sterile plastic container containing anticoagulant solution.

- During collection the bottle is gently shaken in order to mix it there for formation of small clot is prevented.
- Maximum of 420 ml is taken at one time.
- Immediately after the collection the container sealed and cool 4-6°C. in this temperature the contamination is not possible.

Precaution before collecting-

The donor must not be suffering from syphilis, malaria, anaemia, jaundice, eosinophilia, HIV and other communicable diseases.

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The donor must not be suffering from syphilis, malaria, anaemia, jaundice, eosinophilia, HIV and other communicable diseases.

The haemoglobin content of the blood must not be less than 13.3 % and 12.6% for male and female donor respectively.

Storage- human blood can be stored for 25 days at 4-6°C.

Label the label must contain:

- ABO system.
- Rh group
- Nature of antiserum
- Date of donation
- Storage condition and expiry date.

Acid citrate dextrose (ACD)- Acid citrate dextrose solution is used to preserve whole human blood.

Composition:-

Disodium hydrogen citrate	:- 2.5 gram
Dextrose	:- 3.9 gram
Water for injection	:- 120 ml
pH	:- 5

This quantity of ingredient is used to preserve 420 ml of blood.

Disodium edetate:- It is disodium salt of Ethylene diamine Tetra acetic acid (EDTA). It is chelate of calcium and can be used in the emergency control of hypercalcemia.

Dextrose (EDTA) is useful in lead poisoning.

In the case of lead poisoning one gram is diluted to 200 to 300 ml in saline or glucose solution is given as intravenous infusion twice daily for 3 to 5 days.

Anticoagulants

Anticoagulants are the substances used therapeutically to modify the process of coagulation. To understand the effect of the anticoagulant it is necessary to understand the exact mechanism of blood anticoagulants.

Clotting of blood or coagulation of blood-

It is a defense mechanism of the body. In this process blood is converted into a jelly like substance so excessive loss of blood is prevented.

Mechanism-

Stage 1- formation of active thromboplastin-

When injury occurs, tissue and platelets destroyed so platelet thromboplastin is released. This platelet thromboplastin is converted into active thromboplastin in the presence of calcium ion.

Stage 2-Formation of thrombin:-

Thromboplastin convert prothrombin into thrombin in the presence of calcium ion.

Stage 3-Formation of fibrin:-

Thrombin convert the fibrinogen that is a plasma protein convert into fibrin. Fibrin helps the clotting of the blood because fibrins are insoluble thread like structure.

Heparin

Heparin is naturally occurring compound found in secretory granules of mastcell **composed with alternating glucuronic acid and N-acetyl glucosamine units**.

Commercial Heparin is obtained from lungs and intestinal mucosa of pigs and cattle, since these are containing mast cells. Heparin acts indirectly by activating plasma antithrombin II to get a complex then this complex bind with clotting factor and inactivates them.

Uses- it is mainly **used in the prevention and treatment of thrombosis**, which prevent the clotting of blood within the blood vessel. Therefore it is very much used in post operative conditions where there is a risk of thrombosis.

Preparation and doses heparin is available in the form of sodium and calcium

Neutralization- protamine sulphate is to be administered to neutral heparin action.

Administration- Heparin must not be used intramuscularly. It is used as intravenous infusion and subcutaneous injection.

Low dose of 5000 IU is injected subcutaneously given every 8 to 12 hours starting one hour before surgery and continued for 7 to 10 days or till the patient discharged.

Precautions-Heparin must not be used in bleeding disorder thrombocytopenia severe hypertension, threatened abortion gastrointestinal bleeding, neurosurgery, tuberculosis, renal failure, chronic alcoholics cirrhosis.

Advantage- Heparin has distinct advantage over other anticoagulant since it is normal anticoagulant and therefore no toxic.

Concentrated Human Red Blood Corpuscles (Packed Red Cells)

It is prepared from human blood. It can be transferred to increase the oxygen capacity of blood. A packed RBC preparation is obtained by removal of 40% of plasma. The concentrated red cells have to be infused and strict aseptic precautions must be followed. Since there is a risk of bacterial contamination the product must be used within 12 hours.

Storage: 4-6 °C.

- Infusion for children -145 ml per kg body and for premature infants 10 ml per kg body weight.

Use:- It is used in the treatment of diseases such as chronic anaemia hemorrhage.

Human Plasma

Plasma liquid part of an clotted blood. It is used in oligemic shock in severe burns. it is available as citrate liquid plasma or powder plasma in a dry state. strict aseptic precautions are followed during the process of plasma protein.

a) Citrate liquid plasma:- Plasma is prepared by mixing equal parts of citrated whole blood from different persons, and then the plasma is separated by centrifugation and pooled.

In the preparation of citrate liquid plasma unstable proteins such as fibrinogen can be removed by adsorption on kaolin. Therefore the final products contain only stable proteins such as albumin and globulin. The total protein content is 4.5 % and it can be used for period of 2 years.

b) Dried plasma:- It is prepared by freeze drying. The supernatant fluid is separated and the plasma is dried. The plasma is stored at 4-6°C and tested for sterility. The total protein content of dried plasma is 4.5 %. It contains significant amount of fibrinogen.
In the dry state it remains stable for a period of 5 years.

Storage:- Dried plasma is kept below 20°C and is protected from light moisture and oxygen.

Reconstitution:- Before using the dried plasma must be reconstituted in a volume of water for injection, sodium chloride injection or a solution containing 2.5 % dextrose and 0.45% sodium chloride equivalent to equal volume of plasma. After reconstitution it must be used immediately.

Human Fibrinogen

- Fibrinogen is a soluble constituent of plasma. It is used with thrombin to form a clot of fibrin, use in skin grafting, nerve suturing and the treatment of burns.
- Here the plasma is separated by fractionation and centrifuged to collect the precipitate.
- The precipitate is dissolved in citrate saline and freeze dried. This is stored in a suitable container and the air is displaced by nitrogen.
- The citrate prevents spontaneous clotting when the material reconstituted. The solution must be used before 3 hours after reconstitution.

Human Thrombin

Thrombin is an enzyme used to convert fibrinogen to fibrin. This is used with fibrinogen to form clots and skin grafting, nerve suturing and in treatment of burns.

The plasma is separated, washed with distilled water and dissolved in citrate salt. It is converted to thrombin by adjusting the pH 7 and adding thromboplastin and calcium ions. This solution is filtered, stored in a suitable container and freeze dried. The air in the container is replaced with nitrogen.

Reconstitution- it is reconstituted in saline whenever required.

Standards for all blood products-

lood products must be standardized since it saves life and many of them are dangerous

1) **Identification test-** all the blood products must contain proteins this can be found out by precipitation test with specific antisera. proteins can also be identified by their sedimentation rate by using ultracentrifuge. ABO of plasma and Rh factor must be identified for whole human blood.

2) **Sterility and pyrogen test**-All the blood products must comply the stability and pyrogen test.

3) **Solubility test-** Solubility test is necessary while reconstituting. Therefore complete solubility in a solvent shows the protein nature of blood products have not deteriorated.

Plasma Substitutes

They are non human origin molecules. Plasma substitutes are high molecular weight substances which exert colloid osmotic pressure when infused into bloodstream.

Ideal properties of plasma substitute

- It must have an osmotic pressure comparable to plasma.
- It must contain the same viscosity.
- It must remain in circulation for an adequate period to perform its function.
- It must be free from toxicity antigenicity and pyrogenicity.
- It must not interfere with blood grouping for cross matching and erythrocyte sedimentation rate.
- The isotonicity must be equal to the blood plasma.
- It must eliminate completely from the body.
- It must be cheap easily available.
- It must be stable in liquid form at normal and sterilizing temperature.

Following plasma substitutes are

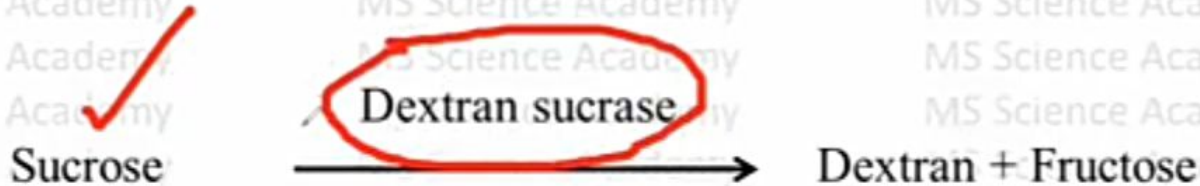
1) Dextran
2) Polyvinyl pyrrolidone
3) Gum saline

Dextran -It is polysaccharide.

- It is most commonly used plasma substitute.
- It is a polymer of glucose.
- It isolated from beet sugar which is formed by the action of bacteria called leuconostoc mesenteroides.
- It is available in two forms namely dextran- 70 and dextran- 40
- Osmotic pressure of dextran is similar to that of plasma;

- It expands the volume for 24 hours and it slowly excreted by glomerular filtration.
- It may interfere with coagulation and platelet function and thus prolong the bleeding time. Therefore, it must not be used in hyperfibrinogenemia, thrombocytopenia.
- Dextrans are potent antigen when administered in small doses by subcutaneous route, however administration of massive doses intravenously induces immunological paralysis.

Production:



Advantages

Dextrans are ideal plasma expander can be easily sterilized by autoclaving of filtration. It can easily stored for 10 years.

Adverse effect- hypersensitivity reactions like urticaria, itching, broncospasm, fall in blood pressure.

Precautions while administrating dextran- maximum volume of infusion is 1 litre.

It must be given when the urine output is less than 1500 ml per day and the blood urea is 60 mg or even higher.

Do not give more than 5 days.

Polyvinyl pyrrolidone PVP-

It is synthetic water soluble hydrophilic polymer. It interferes with blood grouping and cross-matching. It is histamine releaser. PVP has capacity to bind with drugs like penicillin insulin and produce a agglutination of erythrocytes. It is rapidly excreted through urine. It is discovered during second world war by Germans.

Due to carcinogenic property it is not preferred now a days.

Gum saline- It is composed with sodium chloride NaCl (0.9%) and gum acacia solution 6%. It was discovered in first world war by baylis.

General Method of Preparation of Antitoxin

- **Toxins** are the poisonous substances produced by pathogenic microorganisms and lead to infection or disease in men or animals. Toxins are of two type exotoxin and endotoxin.
- **Exotoxin**- these are the toxins which can diffuse freely through the bacterial cell wall into the blood or the medium in which the microorganisms are growing.
- **Endotoxins**- are the toxins which cannot diffuse through the bacterial cell wall and are retained within the bacteria. They are released only when the cells die and start disintegrating.

- **Antitoxins** are the substances containing antibodies produced by the blood which spatially neutralize the toxins produced by particular microorganism.

- So antitoxins are the antibody produced in response to a specific biological toxin for example diphtheria antitoxin tetanus antitoxin.

Preparation of Diphtheria antitoxin

Introduction-Diphtheria is an acute infectious disease caused by exotoxin of *corynebacterium diphtheriae*, clinically characterized by an inflammation at the site of infection, fever headache, sore throat and myocardial damage. Severe infection can be treated with diphtheria antitoxin.

Diphtheria antitoxin is prepared from the horse serum and is used for immediate protection; it is a kind of passive immunisation.

Culturing of strain- Pure diphtheria is isolated and the pure culture is grown in culture media at 37 °C for 4-7 days. 0. 5% phenol is added and the crude toxin is received through bacteria proof filtration.

Selection of animal - Generally horses are preferred because they are large so that more volume of blood can be taken.
Sometimes goats are also preferred to produce antitoxin for individual sensitive to horse proteins. The healthy horses are grouped and isolated for 7 days and they are examined for infectious disease such as glanders.

Glanders is a contagious infection in horse donkey and sometimes in human beings. This is caused by *pseudomonas mallei*.

Immunization- Horses are immunized actively with diphtheria toxoid intramuscularly. First 5 ml of diphtheria toxoid is injected into the horse to get primary immune response. After 2 days the dose is increased by 10 ml and gradually increased up to 600 ml to get a satisfactory antitoxin level.

Collection of blood- After 10 days blood is tested for adequate antitoxin. Then generally 8 litre of blood is collected 3 times in 8 days aseptically from the jugular vein. The bottle in which blood is collected should contain anticoagulant.

Resting period- After collection the blood, the horse is given 10 days rest. In meantime the diphtheria toxoid is injected to stimulate for the further antibody production.

Collection of blood- After resting period the bleeding is done again. The bleeding is continued until the animal stops to produce satisfactory antitoxin level.

Storage- Collected blood is stored in refrigerator.

Separation of serum- The serum is separated by adding calcium chloride to the blood and then it is filtered.

Purification- The serum is purified since it contain horse protein as albumin, β-globulin and γ-globulin which leads to anaphylactic shock and serum sickness. The serum is purified by fractional precipitation of protein by using Ammonium Sulphate and proteolytic digestion by adding pepsin.

Use- as a prophylactic dose given subcutaneously or intramuscularly.

General Method of Preparation of Toxoid

Definition:

- Toxoids are modified toxins that have lost toxigenicity but retained the antigenicity. Their toxigenicity is removed by the application of moderate heat and some chemical treatments such as formalin for this 0.3% formalin are used.

- For example- tetanus toxoid, diphtheria toxoid etc.

Toxins are of following types-

- ❖ Formal toxoid (FT)
- ❖ Toxoid antitoxin floccules(TAF)
- ❖ Alum precipitated toxoid (APT)
- ❖ Purified toxoid alum aluminium phosphate(PTAP)
- ❖ Purified toxoid aluminium hydroxide(PTAH)

Formal toxoid-This type of toxoid prepared by 0. 5% formaldehyde (HCHO) into toxin and incubated at 37°C for 3-4 weeks. It is an excellent antigen but it is used as unpurified form so it often caused severe reactions.

Toxoid antitoxin floccules- In this preparation 80 units of antitoxin mixed with 100 units of toxoid. After 3 weeks the floccules will settle down and the supernatant liquid is decanted.

Filtration will not be done because of the protein nature of the precipitate. The floccules are then washed with saline solution by decantation until it becomes colourless and resuspended in saline containing bactericide.

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Alum precipitated toxoid- The purified formal toxoid is adsorbed onto a mineral carrier such as aluminium hydroxide or aluminium phosphate. This adsorbed toxoid form deport after injection and serve as better antigen i.e. the antigenicity of the toxoid can be increased by adsorbing on mineral carrier. Alum precipitated toxoid produces much higher antibody level than formal toxoid or toxoid antitoxin floccules. Sometimes adults and children may show local hypersensitivity reaction to the carrier.

Purified toxoid aluminium phosphate- It is more pure as compared to alum precipitated toxoid. This can be achieved by strains are grown in semi synthetic medium. Magnesium hydroxide, ammonium sulphate and cadmium chloride are used to purify the toxoids by precipitating proteins, phosphates and remove the colour of suspension. Here aluminium phosphate gel is used as mineral carrier.

Purified toxoid aluminium hydroxide-The preparation is similar as like purified toxoid aluminium phosphate in which the aluminium phosphate is replaced by aluminium hydroxide gel as a mineral carrier.

Preparation of Tetanus Toxoid

Tetanus is an acute disease induced by the exotoxin of *clostridium tetani* and clinically characterized by muscular rigidity, painful paroxysmal spasm of voluntary muscles, specially the facial muscles, the muscles of back and neck and lower limb.

Selection of organism- Suitable good yielding strain of *clostridium tetani* is selected.

Preparation of toxin- This is prepared from the exotoxin of the anaerobe *clostridium tetani* which was grown in suitable medium. The strain produces two types of toxins namely the tetanolysin and tetanospasmin. Incubate it under optimal condition until toxin production has reached a satisfactory level.

Conversion to toxoid- Filtrate of broth culture is treated with 0.3 % formalin and incubated at 37°C for 2-3 weeks until the toxicity has disappear. This is called formal toxoid. This impure formal toxoid can be purified the latest purification technique. This is called purified formal toxoid. This purified formal toxoid is used in the preparation of toxoid vaccine.

This purified tetanus toxoid is used in three different types of vaccine namely

a) Preparation of Alum precipitated toxoid

b) Preparation of purified toxoid aluminium phosphate.

c) Purified toxoid aluminium hydroxide

a) **Preparation of Alum precipitated toxoid-** The purified formal toxoid is adsorbed on a mineral carrier such as aluminium hydroxide or aluminium phosphate. To that the antigenicity of the toxoid can be increased by absorbing on the mineral carrier.

b) **Preparation of purified toxoid aluminium phosphate-**This can be achieved by strains are grown in semisynthetic medium in the preparation of toxoid. Magnesium hydroxide and Ammonium sulphate and Cadmium chloride are used to purify the toxoid by precipitating proteins, phosphates and remove the colour of suspension. Here aluminium phosphate gel is used as mineral carrier.

c) **Purified toxoid aluminium hydroxide-** The preparation is similar as like purified toxoid aluminium phosphate in which the aluminium phosphate is replaced by aluminium hydroxide gel used as mineral carrier

Storage Condition and Stability of Vaccines

If the Storage Condition and Stability measurement are not applied then vaccines get expired very soon.

Storage Condition :-

Storage Condition involve to maintain potency of the product for potency.

→ To maintain the viability of living cell.

→ Prevention the denaturation of protein

* The reduce potency is due to chemical change so to maintain potency prefer Storage Condition -

→ All immunological store below the room temp. (2-8°C)

→ Products are very light and heat sensitive so such product keep away from light heat and figure scense light, radiations.

→ Container - glass - not react.

→ Some viral vaccines such as small

box and OPV are more stable at or below their freezing point (-20°C).

Storage Condition for some Vaccines

1. BCG → In dark freeze dried, 2-8°C effectiveness up to 2 years.
2. Cholera → 2-8°C not be frozen
3. Tetanus → " " " "
4. Polio → Best if stored at low as -20°C
   - Can be stored for 6 month at 5±3°C.
5. AIDS → 2-10°C should not be frozen.

Stability → It is the ability of a vaccine to retain it's chemical, physical microbiological and biological properties with in specified limit throughout its shelf life.

- Vaccines are prepared at right, freeze dried (reconstitution immediately prior to the time of administered).

→ Preservatives and sometime adjuvants are added.

- Preservatives maintain quality and potency through preventing Contamination.

* Adjuvants → boost the body's response to the vaccine.
→ They do not act as antigen.
→ for e.g → Aluminium salt, Mercury salt.

→ Residual cell culture media - prevent Contamination during the manufacturing process.

ex → Hepatitis A → 2 phenoxyethanol preservatives
Aluminium Hydroxide - Adjuvant

Serum immune blood derivatives and other products relative to immunity

Serum - fluid with solute components of blood which do not clot
Plasma - Clotting factor (fibrinogen) : Serum.

Classification of serum prepn

(i) Homologous serum
(ii) Heterologous serum.

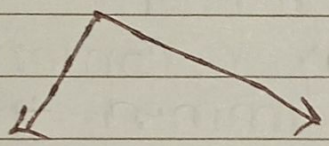
(i) Homologous Serum → Serum obtained from blood donors volunteers have been immunized.

→ Provide immunity in 4-6 weeks.

e.g. - Immune Sera (antisera)
Human Immunoglobulin.

(ii) Heterologous Serum → Serum obtained from blood of animals (horse) through hyperimmunized
→ Provide immunity 2-3 weeks.
e.g → Tetanus antitoxin
Gas Gangrene toxin
Diphtheria antitoxin.

Serum Immune derivatives



Immune Sera | Human Immunoglobulin

Immune Sera → / Antisera → Serum that contain antibodies for specific antigen called antisera. There are 2 types of Antisera and ie:-

(I) Monovalent → Serum containing antibodies specific for one antigen

(2) Polyvalent - Serum Containing antibodies ~~Specific~~ for more than one antigen.

Immune Sera Prepn

(1) Large vol^m of blood is collected from volunteers or may be hyper immunized horse by aseptic^ve

(2) Vessel Contain Citrate soln.

(3) Blood Cells are allowed to settle down and supernatant plasma is drawn off

(4) Filtrate on - to remove others

(5) Fractional precipitation is done by adding ammonium sulphate.

(6) Globulin Protein is recovered with pepsin to yield refined immune product Containing lab fragment.

(7) The antibody Content in refined product is determined the product is diluted to the required Conc^n and transferred into ampules.

(8) Two or more monovalent size may be blended together to provide a multivalent immune serum.

(9) The quality of globulin fraction and Contaminating proteins - by electrophoresis.

→ Uncleaved Ig and aggregates detect by size exclusion HPLC.

(2) Human Immunoglobulin Prepn.

Igs are the prepn of Igs, those are present in human blood (principally Igg class)

These are derived from the plasma of donate. blood and from plasma obtained by plasmapheresis.

↓

→ Specific Igs are Collected from the individual who has suffered recent infection or who has undergone recent immunization

↓

→ Plasma is detected for other infection also for eg- HIV 1, 2, Hepatitis C, Hepatitis B surface antigen are detected.

↓

Ethanol precipitation in cold used to Control protein Concentration pH and ionic strength also maintained → (i) freeze dried (ii) liquid prepn

→ Glycerine - Stabilizers
Thiomersal - Preservative

→ The protein composition determined by
(a) Sodium dodecyl sulphate polyacrimide gel electrophoresis.
(b) Nitrogen estimation
Molecular size - determined by HPLC.

The End