B Pharmacy
Subject- Pharmaceutical Biotechnology
Sub Code- BP605T

UNIT- 3

Venkatesh Naik V
Assistant Professor
BALAJI COLLEGE OF PHARMACY,
Anantapur.

Objective of Course

Understanding the importance of enzymes in Pharmaceutical Industries.

Learning Outcomes

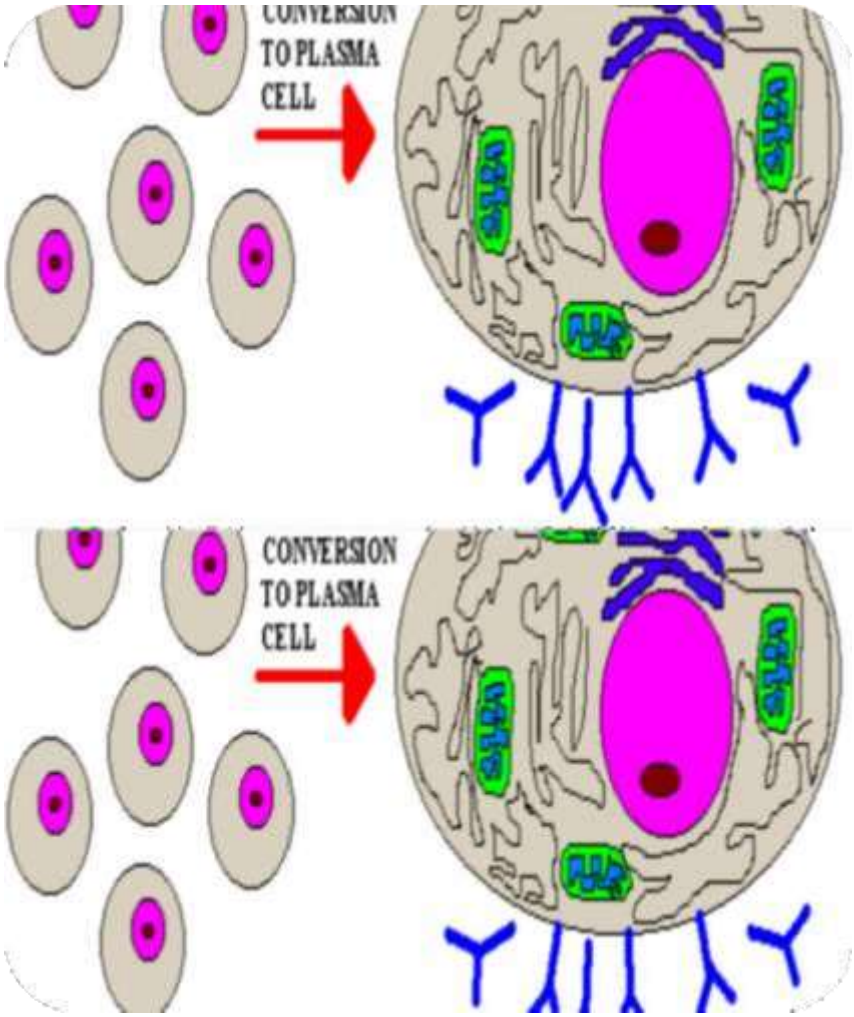
1) Students will learn about various types of immunity including Immunoglobulins, MHC and hypersensitivity reactions.
2) They will also learn about hybridoma technology, immunostimulation and immunosuppressions.
3) They will also able to know about stoarage and stability conditions of official vaccines.

Cellular Immunity

It is an adapative immune response that is principally delibrated by thymus- derived small lymphocytes, which are recognized as T cells. They do not terminate infected cells or pathogens, however they trigger and direct further immune cells to do so. Cellular immunity is a protective immune process that involves the activation of phagocytes, antigen-sensitized cytotoxic T cells and the release of cytokines and chemokines in response to antigen. Cellular immunity is most effective against cells infected with viruses, intracellular bacteria, fungi and protozoans, and cancerous cells. It also mediates transplant rejection.

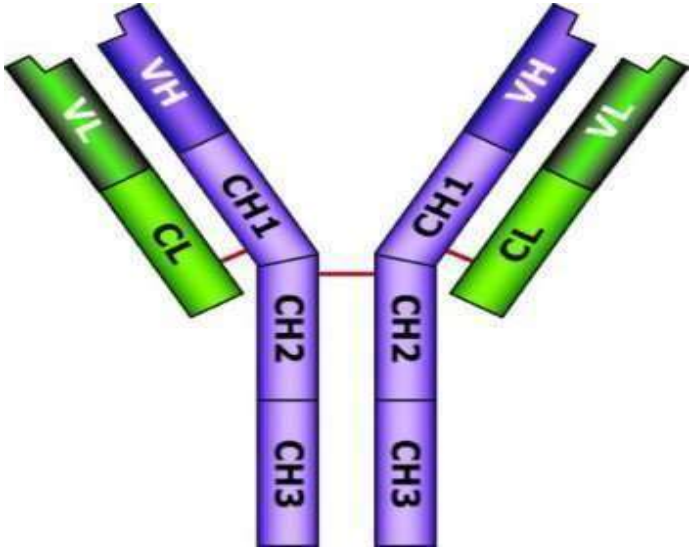
Humoral Immunity

- Results in production of proteins called “immunoglobulin's” or “antibodies”.
- Body exposed to “foreign” material termed “antigen” which may be harmful to body:
  virus, bacteria, etc.
- Antigen has bypassed other protective mechanisms, ie, first and second line of defense.



CLASSES (ISOTYPES) OF IMMUNOGLOBULINS

- Classes based on constant region of heavy chains
  - Immunoglobulin A (IgA)
  - Immunoglobulin D (IgD)
  - Immunoglobulin E (IgE)
  - Immunoglobulin G (IgG)
  - Immunoglobulin M (IgM)

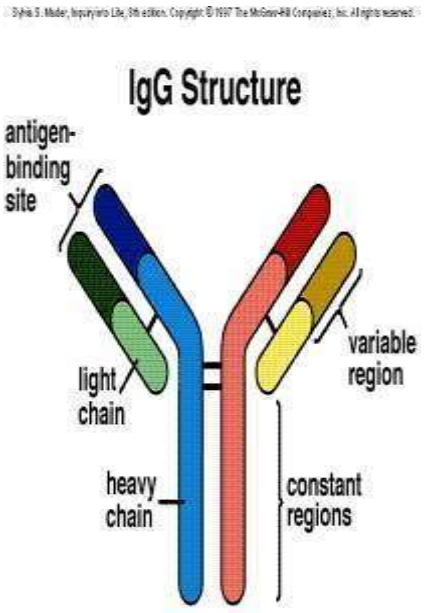


- Differentiation of heavy chains
  - Length of C region, location of disulfide bonds, hinge region, distribution of carbohydrate
- **Classes have different effector functions**

Immunoglobulin Classes

I. IgG

- **Structure: Monomer**
- **Percentage serum antibodies: 80%**
- **Location: Blood, lymph, intestine**
- **Half-life in serum: 23 days**
- **Complement Fixation: Yes**
- **Placental Transfer: Yes**
- **Known Functions: Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn.**



Immunoglobulin Classes

II. IgM

- **Structure: Pentamer**
- **Percentage serum antibodies: 5-10%**
- **Location: Blood, lymph, B cell surface (monomer)**
- **Half-life in serum: 5 days**
- **Complement Fixation: Yes**
- **Placental Transfer: No**
- **Known Functions: First antibodies produced during an infection. Effective against microbes and agglutinating antigens.**

Immunoglobulin Classes

III. IgA

- **Structure: Dimer**
- **Percentage serum antibodies: 10-15%**
- **Location: Secretions (tears, saliva, intestine, milk), blood and lymph.**
- **Half-life in serum: 6 days**
- **Complement Fixation: No**
- **Placental Transfer: No**
- **Known Functions: Localized protection of *mucosal* surfaces. Provides immunity to infant digestive tract.**

Immunoglobulin Classes

IV. IgD

- **Structure: Monomer**
- **Percentage serum antibodies: 0.2%**
- **Location: B-cell surface, blood, and lymph**
- **Half-life in serum: 3 days**
- **Complement Fixation: No**
- **Placental Transfer: No**
- **Known Functions: In serum function is unknown. On B cell surface, initiate immune response.**

Immunoglobulin Classes

V. IgE

- **Structure: Monomer**
- **Percentage serum antibodies: 0.002%**
- **Location: Bound to mast cells and basophils throughout body. Blood.**
- **Half-life in serum: 2 days**
- **Complement Fixation: No**
- **Placental Transfer: No**
- **Known Functions: Allergic reactions. Possibly lysis of worms.**

CLASSES (ISOTYPES) OF IMMUNOGLOBULINS

- Additional classification based on light chains
  - **Kappa**
  - **Lambda**
- Each IG has either kappa or lambda, not both
  - **IgG kappa**
  - **IgG lambda**
- No functional differences between light chains

IgE AND IgD ANTIBODIES OF THE IMMUNE RESPONSE

- **IgE**
  - Binds with high affinity to receptors on mast cells, basophils and activated Eosinophils
  - Longer half-life when cell bound
  - Initiates a strong inflammatory reaction to parasites
  - Involved in allergic reactions
- **IgD**
  - Antigen receptor on mature B-cells
  - No other known function

MHC (Major Histocompatibility Complex)

It is defined as set of genes that code for cell surface proteins essential for the acquired immune system to recognize foreign molecules in vertebrates, which in turn determines histocompatiblity. It is of four types.

1) Class 1 molecules
2) Class 2 molecules
3) Class 3 molecules

FUNCTIONS OF MHC

1) It binds to endogenous antigen and present to T helper cells.
2) They are found on surface of all nucleated cells.
3) They are secreyed protein possesing immune functions.
4) They are also involved in complement activation.
5) They involved in inflammation caused by cytokines and tumor necrosis factor.

Hypersensitivity

- **Hypersensitivity** refers to having an extreme sensitivity to stimulation of the senses, i.e., touch, sight, hearing, taste, and smell. Children who are hypersensitive may complain about sensory stimuli that seem minor to others. They also find it almost impossible to regulate their emotional and behavioral responses to high levels of sensory stimulation. For example, a hypersensitive child may lose his temper as a result of being hugged.

Causes of Hypersensitivity

- There are several known causes of hypersensitivity in children, including:
- Sensory processing disorders, including sensory integration disorder
- Pervasive developmental disorders, including autism
- Neurological disorders, including peripheral neuropathy
- Traumatic brain injury
- Fibromyalgia
- Structural abnormalities in any one of the sensory systems, e.g., having a deviated septum
- Damage to any one of the sensory systems, e.g., damage to your auditory system caused by overexposure to loud noises
- In addition, the following disorders have been linked to hypersensitivity in children:
- Anxiety disorders, such as obsessive-compulsive disorder
- ADHD
- Fragile X syndrome
- Down syndrome
- Cerebral palsy
- Epilepsy

Types of Hypersensitivity

- There are five types of hypersensitivity in children. Each type of hypersensitivity is very uncomfortable to the child that experiences it. They are:
- Hypersensitivity to touch and movement
- Visual hypersensitivity
- Auditory hypersensitivity
- Hypersensitivity to taste
- Hypersensitivity to smell

The 4 types of hypersensitivity

- Type I (Immediate)
  - Typical allergic response
- Type II (Cytotoxic - cell-bound antibody)
  - Antibody binding to target cells alters function (death, activate, inhibit)
- Type III (Soluble antibody immune complexes)
  - Antibody/antigen complexes float around and activate immune system
- Type IV (Cell-mediated)
  - T cell-mediated response

End effect is Antibody-mediated

(Note: Still involves T cells for antibody class-switching)

End effect is T cell-mediated

Immune suppression

It is a reduction of the activation or efficacy of the immune system. It is a situation in which the body's immune system is intentionally stopped from working or is made less effective, usually by drugs, especially in order to help the body to accept an organ that has been taken from another person's body.

It is of two types:

1) Deliberately induced immunosuppression: It occurs in case of medications and during any organ transplantations.
2) Non- Deliberate immunosuppression: It occurs in case of many complement deficiencies like many types of cancer, chronic infection and human immunodeficiency virus (HIV).

GENERAL METHODS USED FOR PREPARATION OF VACCINES

Cholera Vaccine (Bacterial vaccine)

Cholera is serious infection which is caused by spirillium vibrio cholera, cholera vaccine is homogenous suspension which is prepared from killed cholera vibrio's strain having antigenic efficacy and purity

Category: Active immunizing agent

Dose: Initial dose is 0.5ml and second dose is 1.0 ml

Storage: Store at temp between 2-8°C

BCG Vaccine

BCG is a suspension of living cells, which is obtained from Mycobacterium tuberculosis which is known as bacillus calmette guerin.BCG vaccine is a live freeze dried vaccine which is used for prevention of tuberculosis.

It is a freeze dried prepartion stored in dark 2-8°C.

Should be protected from light.

Its single dose 0.1ml.

Diptheria Vaccine

Diptheria is a communicable disease which is caused by Corneybacterium diptheria which colonizes and forms a pseudo membrane at the infection site. Diptheria toxin is synthesized and secreted as a single polypeptide chain which is having A Chain and B Chain.

It is an acti9ve immunizing agent.

Its initial dose is 0.5ml, second dose is 0.5ml after 4 weeks and third dose is 0.5ml after another 4 weeks.

It should not contaion more than 30LF of diptheria toxoid per dose of 0.5ml.

Polio Vaccine (viral vaccine)

Polio is a live oral aqueous suspension. It is obtained from attenuated strains of polio myelitis virus. There are three distinct antigenic types of polio myelitis types 1,2,3 and each has much greater antigenic stability.It should be stored in sealed tight resistant containers at a temprature between 2-8°C .

It has potency not les than 2.5 units for each dose.

It is freeze dried powder or pellets which in reconstituted with suitable solvent.

Tetanus (Antitoxin)

Tetanus antotoxin is a liquid preparation which is containing specific anto-toxin globulins which are obtained by hyper immune sysytem of horse and other suitable animals. These liquid preparations contains suitable antimicrobial preservative.

It is passive immunizing agent.

It should be givenm by subcutaneous and intramuscular injection.

Tetanus toxoid should be stored between 2-8°C.

It is freeze dried or pale yellow liquid or cream coloured powder and pellets.

HYBRIDOMATECHNOLOGY

- Hybridoma technology is a method for producing large number of identical antibodies called monoclonal antibodies.
- It was discovered by *G.kohler* and *C.milstein* in 1975. they were awarded nobel prize for physiology and medicine in 1975.
- The hybrid cells are produced by fusing B- lumphocyte with myeloma cells or tumour cells.
- The B-lymphocyte have the ability to produce large number of antibodies and tumour cells have indefinite growth.
- This is why two cells are used for the production of hybrid cell.

Procedure

1. The mouse is immunised by specific antigen injection against which monoclonal antibodies have to be produced.
2. After 72 hrs of immunisation spleen is collected from mouse.(antibody prodcing B cells).
3. The B cells are fused with immortalised myeloma cells by polyethylglycol or sendai virus.
4. The B cells are fused with immortalised myeloma cells.
5. The fused cells are incubated in the HAT medium.

HAT medium

- The hybridoma cells or fused cells are selected using selective media are called HAT medium.
- It contains Hypoxanthine, Aminopterin and Thymidine.
- The unfused B cells will die due to their short life span.
- The myeloma cells can synthesise DNA nucleotides using two pathway : Denovo pathway and salvage pathway.

- In HAT medium, the myeloma cells are unable to replicate because the denovo pathway is blocked by aminopterin in the medium.
- When denovo pathway is blocked, the cell will utilise salvage as an alternative pathway. But it cannot takesplace due to the lack of HGPRT (Hypoxanthine-guanine phosphoribosyl transferase).
- so it is contributed by **B cell** and is rich in HGPRT.
- The salvage pathway is also inhibited due to the mutation of Thymidine Kinase (TK), an enzyme that catalyses the phosphorylation reaction.
- The resulting clones of hybridoma cells secrets large quantities of monoclonal antibodies.

Applications

- It is used for the early detection of pregnancy.
- Detection and treatment of cancer.
- Diagnosis of leprosy.
- Treatment of autoimmune diseases.
- Radiolabelled monoclonal antibodies are used in vivo for detecting or locating tumour antigen.
- Used for making immunotoxins that inhibit protein synthesis.
- Eg: ricin, shigella toxin & diphteria toxin.

THANK YOU