Page No. Thursday
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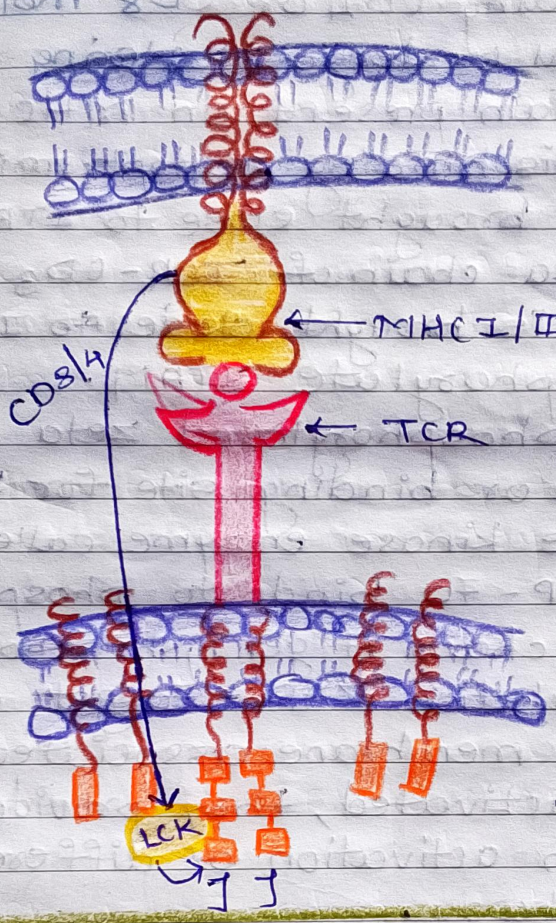
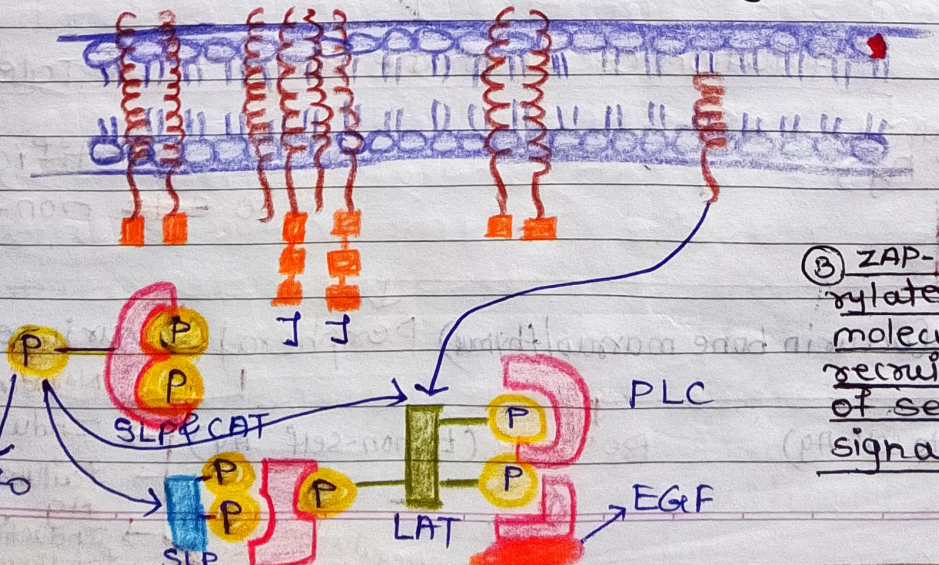
* TCR-CD_3 Activation Pathway :—

① Association of MHC complex with TCR —



② CD4/8 Associates LCK phosphorylates ITAMs of Zeta chain, create docking site for ZAP-70



③ ZAP-70 phosphorylates adaptor molecule that recruit components of several signaling pathways.

- first TCR recognize the process peptide molecule from either MHC class I or class II.
- After binding CD_4 or CD_8 molecule present on T cell undergo clustering & binds to invariant chain present on MHC molecule.
- After this binding, cytoplasmic associated LCK molecule is brought close to ITAM present on zeta chain of TCR-CD_3 complex.
- Once LCK is brought close to ITAM of zeta it phosphorylate ITAM.
- The phosphorylated zeta chain provide docking or binding site for binding site for tyrosine kinase enzyme called ZAP-70.
- Once ZAP-70 binds to phosphorylated ITAM of zeta chain it get phosphorylated, upon phosphorylation ZAP-70 get activated.
- once this membrane associated adaptor molecule are activated, they provide achoring site & helps in activation of different signaling pathway.
- Once these signaling pathways are initiated they induce expression of genes requires for T-cell activation & proliferation.

* **Regulation of Immune response (Tolerance)**

① Natural (to self Ag)

② Artificial (to non-self Ag)

Central (occur in bone marrow/thymus)

Negative (to self Ag)

positive (to non-self Ag)

Peripheral (occur in peripheral blood)

Negative selection

Induction of Anergy

Failure to encounter Ag

Inductional death by fas ligand pathway.

Regulation by Treg cells.

CREDIT – II

Regulation of Immune Response

Page No. Friday
Date 23/8/24

(A) Immunological Tolerance —

(1) Central & Peripheral Immune tolerance —

- Ability of body to tolerate self antigen & our immune system do not response against to the self antigen.

- Types of Immunological tolerance —

Immunological Tolerance

① Central Tolerance

② peripheral Tolerance.

① Central Tolerance —

- The cells which are against to the self antigen or the cell or the lymphocytes which ultimately recognize to the self antigen, they are just detected & remove in primary lymphoid organ this known as central tolerance.
- Central tolerance for the T cells which are reacting against self antigen, these are removed or deactivated in the thymus.
- While central tolerance of B cell this self reactive B cells this are degrading in bone marrow.

- Working of central Tolerance —

- Where the B cells are developed in bone marrow, self antigen after the maturation any B cell that is reactive that reacts with self antige, so it is ultimately degrade.

- The body will either kill B cell because this is self reacting recognize self Antigen so, it will either kill by the apoptosis. or it may be receptor editing.
- self antigen receptor editing change into another form of receptor, which not able to recognize self antigen. so, this B cell is cannot recognize by self antigen.

2) Peripheral Tolerance ——
- When the self reacting lymphocytes, they are detected & removed in the secondary lymphoid organ known as peripheral tolerance.

- Peripheral Tolerance also involves ——
  1) Apoptosis (programmed death of cell)
  2) Anergy (A state of unresponsiveness)
  3) Regulation of Immune system By T Regulatory cells.

- T cell will react with self antigen, so body will recognize the T-cell in the secondary lymphoid organ & ultimately body will make This T-cell responsiveness or either killed.
- Body can anergic state T-cell or self reactive T-lymphocytes as unresponsive & this T-cells also regulates by the T-lymphocytes, body tolerate self reactive cells which reactive against the self antigen.

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(2) Mechanisms of Tolerance Induction (Related Experimentation using transgenic animals) —

• Transgenic animal —

- Transgenic animal is an animal whose genome has been altered by the inclusion of foreign genetic material.
- an organisms genome is the set of genes that ensure the transmission of hereditary material.
- The foreign genetic material is introduced to the organism through rDNA technology.
- rDNA technology is a group of techniques used to cut apart & splice together pieces of DNA.
- The purpose of adding a new gene to an organism's genome is to have the organism produce a protein or set of protein that it did not produce before the gene was added.

• Transgenic mice —

- Development of techniques to introduce clone of genes into the mice embryo permitted immunologist to studying effect of immune system in vivo.
- Transgenes made it easier to study immunoglobulin, TCR, Class I & II MHC molecules, various foreign antigen & no. of cytokines.
- The various types of transgenic mice which is available in market & readily use in immunological research.
- If introduced genes integrated studying into germ line cell. it can be transmitted to progeny.

- The transgenic animals are produced by using following steps:

Pregnant female Mice

↓

Remove fertilized egg

↓

Injection of gene into pronucleus.

↓

Mice — Reimplant embryo into uterus of pseudopregnant female mice.

↓

10 – 30 % offsprings have transgene.

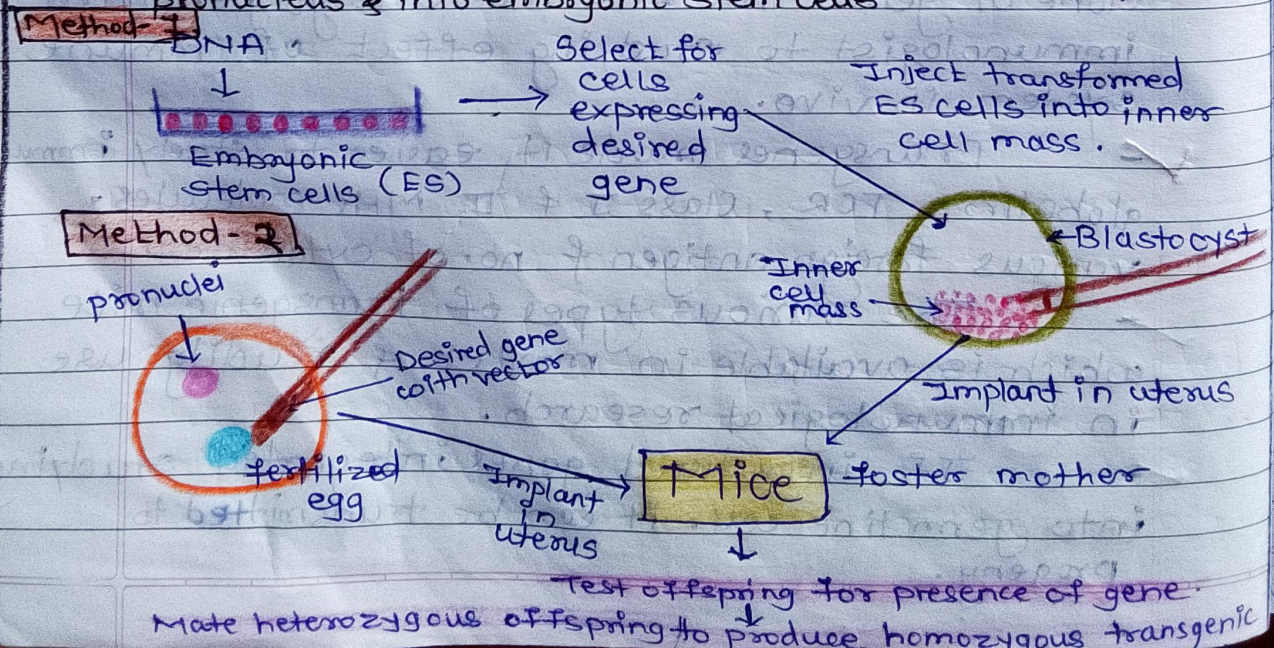
↓

Selection of Transgenic mice

↓

Transgenic mice can readily transfer transgenes into progeny.

fig. Diagram depicting the injection of DNA into the pronucleus & into embryonic stem cells



- Mechanism of tolerance Induction —

- Tolerance induction is a key area of research in immunology, particulary for understanding how immune system can be trained to accept rather than attack specific antigens.
- Transgenic animals, especially mice, are commonly used in these experiments to explore the mechanisms behind this process.
- Tolerance induction refers to the immune system's ability to remain unresponsive to specific antigens.
- This is crucial for preventing auto-immune diseases, where the immune system mistakenly targets the body's own cells, & for success of organ transplants.
- There are two primary types of tolerance induction : a) central tolerance
  b) peripheral tolerance.

Wednesday
28/8/24

3) T-cell mediated suppression of immune response (Treg cells) —

- Out of all the subsets of T cell (TH, TK, Ts and Td) Ts (suppresor) or T regulatory cell play important role in development of tolerance to self reactive antigen.
- Treg cells are used to treat auto-immune disease & it also help in organ transplant & cancer therapy.

Strain

- Treg cells are component of immune system which suppressed immune response or other cells & present excessive reaction.
- This cells are produce in bone marrow but mature into functional Treg cell into thymus.
- Exact process of Treg cell selection is still unknown but it is believed that it depend upon affinity of cell towards self antigen.
- T selection process of Treg cell is also called as antilock process.
- In negative selection the T cell which recognize self antigen with very high affinity will undergo apoptosis, whole the affinity survive and become Treg cell.
- If cell do not recognize self antigen it become effector T cell.

* - T cell mediated suppression of the immune response primarily involves a subset of T cells known as regulatory T cells (T reg.)

- functions of Treg cells —

1)- Treg cell activity suppressed over-activation of immune system & prevent self reactivity (auto-immunity).
- Treg cell activity has been reported to increase in case of infection like HIV, microbacterial infection like tuberculosis like while parasitic infection like malaria.

IFN γ → increase IL-12 dominance
IL-4 → stimulates B cell to produce IgE Ab.
IL-5 → activates Eosinophils & mast cells.
IL-13 → induce production of mucous in intestine.

- Immune response to particular pathogen must induce an appropriate set of effectors that can eliminate disease agent or its cytotoxic product from the host.
- Large body of effector cell simplified difference cytokine secretion pattern of Tcell.
- CD_4 + T cell (TH cell) exerts their function through cytokine secretion.
- There are two subsets of CD_4 + (TH cell) as TH_1 and TH_2, on the basis of cytokine.

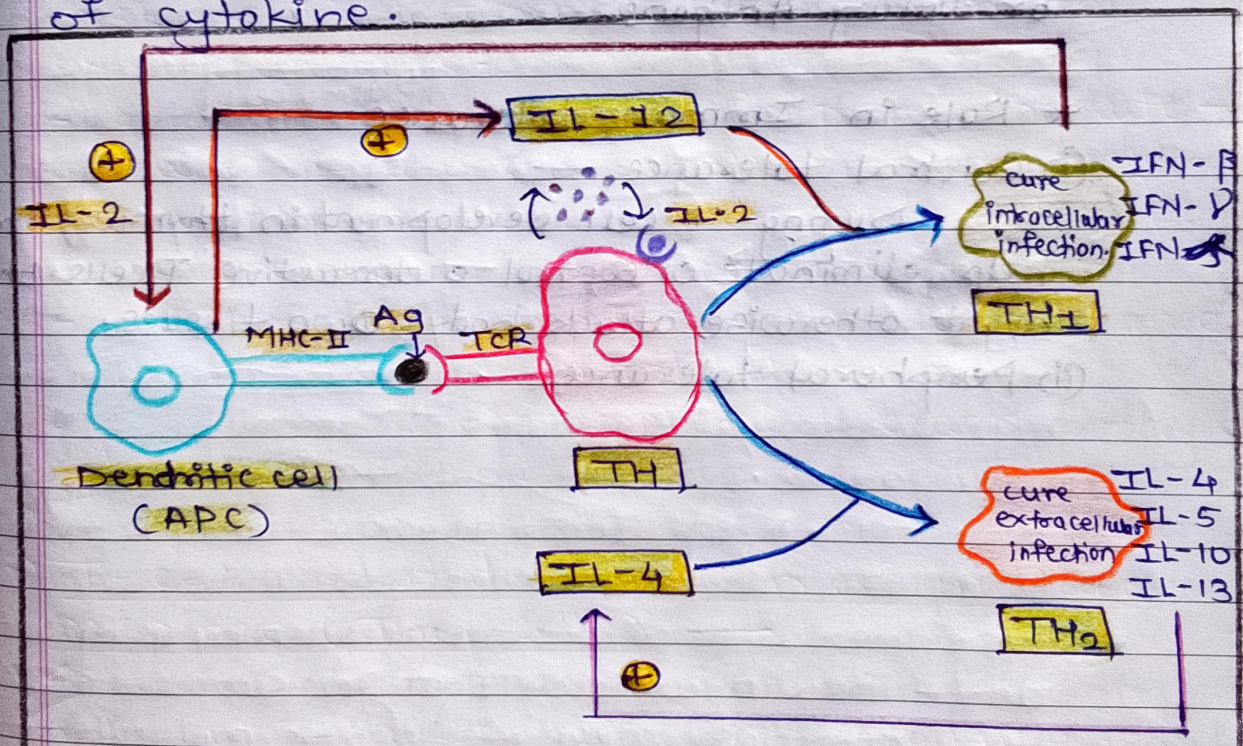


fig. Cytokine regulated cross regulation of TH subsets (TH1 and TH2)

*Development & Types of Treg cells —
1) Thymic (t Tregs) or Natural Tregs (n Tregs) —
- These Tregs develops in thymus & are naturally occurring.

- They recognize self Ag & are essential for maintaining self tolerance, preventing autoimmunity.

2) Peripheral (pTregs) or Induced Tregs (iTregs) —
- These Tregs are generated in peripheral tissues from conventional CD_4+ Tcells in response to specific Ag in presence of certain cytokines like TGf-β.
- They play role in maintaining tolerance to non-self Ag., such as those from commensal microbes or dietary Antigens.

* Role in Immune tolerance —
(i) Central tolerance —
- During T cell development in thymus, Tregs help eliminate or control autoreactive T cells that might otherwise attack body's own tissues.
(ii) Peripheral tolerance —

(B) Types of Immune Response Regulation —

(2) Niel's Jerne's Immune network theory and its experimental Evidences —

- Any discussion regarding of immune response will not come to the end without mentioning A Niel's Jern's ediotypic network theory.
- In 1973 Jern propose this theory so as to give possible solution for regulation of immune response.
- In 1985 he was honoured to with nobel prize for this work. This theory suggest that Ag enter inside the body & activate very specific clones of B cell secreting Ab against it.
- The Ago binding site present on antibody is composed of overlap between light & heavy chain domains called ediotopes.
- These regions are said to be complementary to antigenic epitope (epitopes are antibody binding site present on antigen.)
- Antibody molecules itself are protein so can behave as antigen.
- Antibody molecules against ediotope (anti-ediotopic antibody) which exhibit internal image of external antibody.
- Like wise anti-antiediotopic antibody is a external image of antiediotopic antibody.
- Theoretically these sequence can grow further consisting network of ediotopic antibody however experimentally it has been observed that it extend to just 3 steps.

- As a consequences of such antibody network antigen is rapidly remove from circulation.
- And it antibody show feedback regulation on formation of antibody once step ahead.
- Thus these network of antibody avoid over production of antibodies.

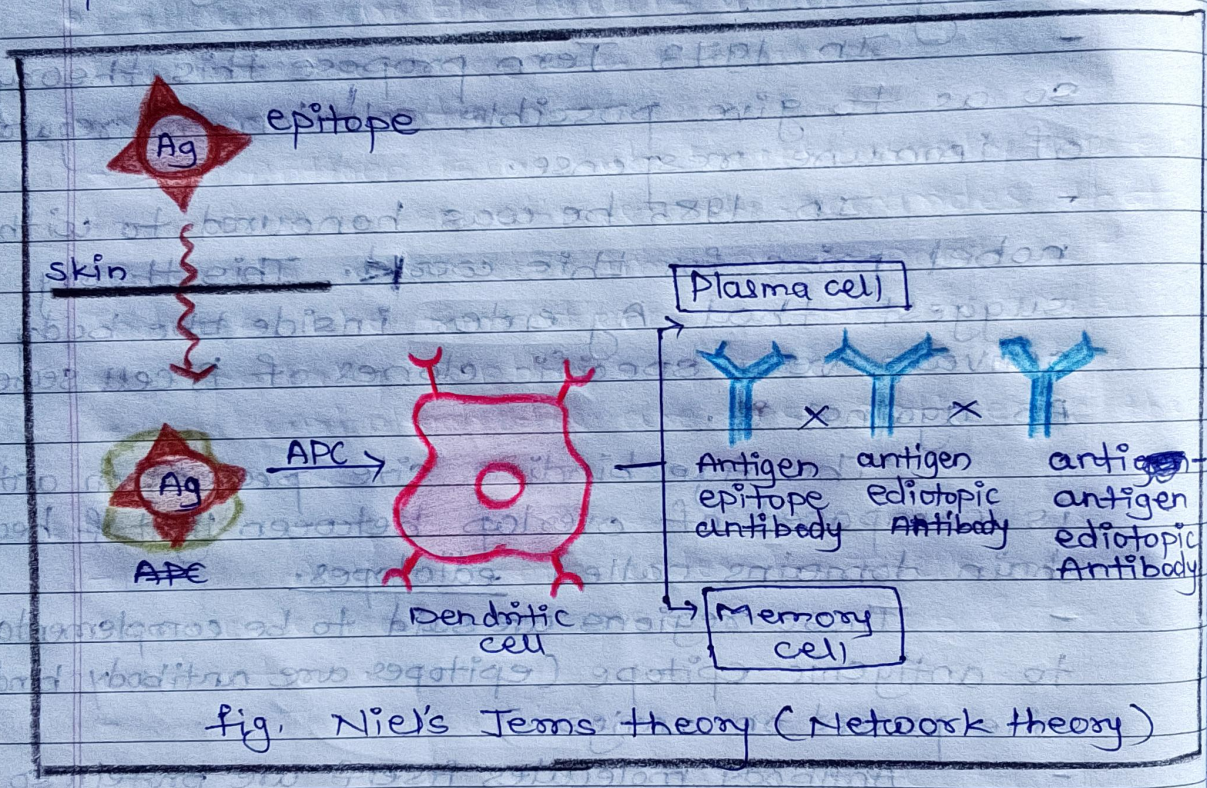
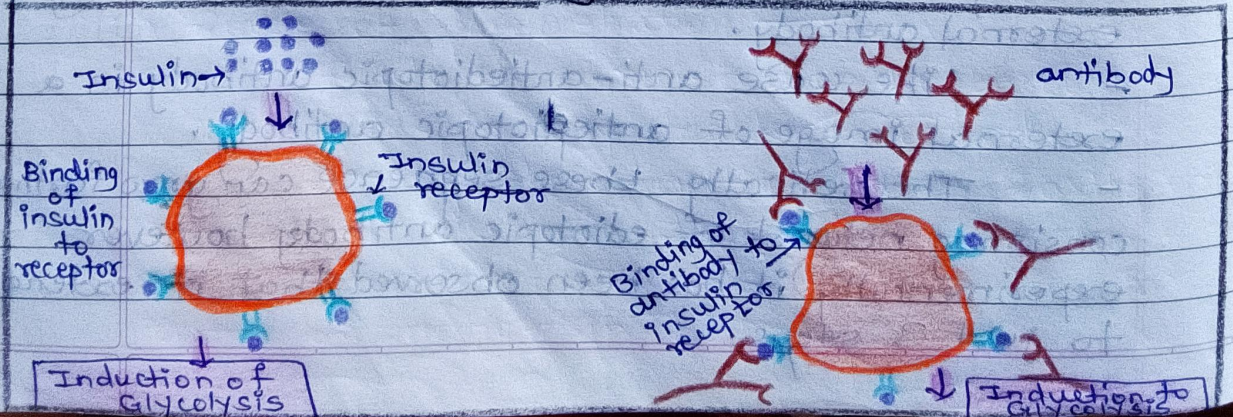


fig. Niel's Jerns theory (Network theory)

* Experimental proof of theory given by using anti-anti-insulin antibody that are supposed to be the internal image of insulin molecule —



- This antibody should bind to insulin receptor present on cell membrane, & should induce glycolysis in tissue culture cell in which insulin is not added.
- This experiment provides experimental proof of antigen-antibody complex network theory.
- So, it can be conclude that regulation of immune response is critical & done carefully in the body, because inappropriate response can have serious & life threatning consequences.

(1) Regulation by antigen, antigen-antibody complexes

- T & B cell are triggered by antigens, offer effective invegment of their antigens specific receptor with appropriate co-stimulation.
- In this case T cell engagement is not with the antigenic cell with the part of T.
- This part is bound to MHC-I or MHC-II on APC. (antigen presenting cell)
- Effective immune response will remove antigen from the system.
- Repeated antigen exposure and required to maintain T & B proliferative & active.
- Nature of Ag, its dose & route of administration have interference on development of immune response.
- At the end of immune response reduced antigen exposure result in reduce IL-2 expression of its receptor leading through apoptosis of antigen specific T cells.

- Majority of Antigen specific cell die at the end of immune response while minor population of long lived antigen specific T and B cell survive to give rise to memory cell.

- following are the factors which regulate immune response —

(i) Nature of antigen —

- Antigen is a substances usually protein in nature & sometimes poly saccharides that generate a specific immune response & induces the formation of a specific antibody or specially synthesized T cells or both.
- Carbohydrates are less antigenic than proteins.

(ii) Dose —

- Antigenic encounter by T cells induces immunological synapse formation & T-cell activation
- The insufficient dose or the higher dose of antigen may not stimulate the immune response.
- Because the amount of administered fails to activate enough lymphocytes.

(iii) Route of Administration —

- Route of administration are through food, water, respiratory track & wound.
- Among these antigen coas fastly enter into blood via wound.
- Therefore wound is fastest way for entry of antigen in body.

* Regulation of Immune response by antibody—
- Antibody shows feedback control on immune response.
- passive administration of IgM enhance the immune response to particular antigen, where as IgG suppressed the immunity.
- The ability of antibody to enhance or suppressed immune response has clinical applications of antibody.
- IgM & IgG can be readily use in therapeutic purpose.

(3) Cytokine mediated cross-regulation of TH subsets (TH1 — TH2) :—
- Immune response to particular pathogen must induce an appropriate set of that can eliminate disease agent or its cytotoxic product from the host.
- for neutralization of bacteria antibodies are required (humoral immune response), while for killing intracellular pathogen cell mediated immunity is important.
- Large body of effector cell implited different cytokine secretion pattern of T cell.
- CD_4^+ T cell (TH cell) exerts their function through cytokines secretion.
- There are 2 subsets of CD_4^+ (TH cell) i.e. TH1 and TH2 on the basis of cytokine secretion pattern. Both subsets secrets interlukins but differ in other cytokine production.

- TH1 & TH2 cells are characterized by the following functional differences —

• **TH1** →

- It is responsible for many cell mediated functions such as delayed types of hypersensitivity & activation of TK cell.
- This subset is also associated with inflammation & tissue injury.

• **TH2** →

- TH_2 subset stimulate eosinophil activation & differentiation.
- It also provide helps to B-cell and promote the production of IgE, IgG & IgM.
- The TH_2 type of subset also involved in allergic reactions.

• At the time of cross regulation, following event occurs —

(i) Initially antigen presenting cell (APC) or dendritic cell present the process antigen on MHC class II molecules.

(ii) This antigen presented to new CD_4 cell (TH) which bind to it with the help of TCR.

(iii) The Bound T-cell produce IL-2 cytokine which act on same cell & activate it.

(iv) If activated TH cell present in IL-12 dominating environment, it generates population of TH1 cell.

(v) The produced TH1 cell secrete large no. of different types of cytokines, Like Interferon β (IFN-β), Interferon γ (IFN-γ) and Interferon α (IFN-α), etc.

(vi) A positive feedback is developed when interferon-γ (IFN-γ) is secreted by TH1 subset, because, it stimulate APC to produce more Interlukin-12 (IL-12).

(vii) If environment is dominated by IL-4, TH gives rise to TH-2 cell.

(viii) The produced TH to secrete profile of cytokine like IL-4, IL-5, IL-10 and IL-13, etc.

(ix) Cytokine produced by TH_2 positively regulate its own production.

(x) Cytokine produce by TH1 and TH_2 also induce negative feedback on each other.

4) Regulation of the Complement System: Classical and Alternative pathway:—

- Complement system is a part of humoral defense or it is included in innate immunity.
- Complement system consists of over 30 proteins circulating in the blood & on the surface of cells, acting as a part of body's innate immune response.
- This complement system can present all time activated in the body.
- This network of complement proteins present in blood acts as "complement" the ability of antibodies & phagocytic cells to clear pathogens from the organisms.
- This proteins called as 'C' proteins or complement proteins and are present in human serum. (mainly synthesized by liver)

- These complement proteins are heat labile i.e. inactivated by heating serum at 56°C for 30 minutes.
- complement system helps the body fight infection by marking pathogens for destruction by phagocytes, and by enhancing body's - infection - fighting cells.
- Each complement protein from $C_1 - C_{30}$ cleaved into two fragment: such as for C_1 protein, it cleaved in C_1a & C_1b & same for remaining all.
- 'b' is a larger fragment involved in complement activation pathway & 'a' is a small fragment which diffused into the blood & get deactivated except for C_2 protein 'C_2a' is the larger fragment & 'C_2b' is the smaller.

• functions of complement system —

(i) Classical pathway —

(i) opsonization —

- Coats pathogens with proteins (like C3b) to enhance their recognition & ingestion by phagocytic cells (macrophages & neutrophils)

(ii) Cell Lysis —

- forms the membrane attack complex (MAC) which disrupts the membranes of pathogens, leading to cell death.

MAC (C5b 6,7,8,9)

↓

Ruptures bacterial cell wall & destroys it.

C3b activates → Neutrophils, macrophages

opsonization → Phagocytosis → kills foreign microorganisms.

(iii) Inflammation —

- Activates the release of pro-inflammatory molecules that increase blood vessel permeability & recruit immune cells.
- Inflammation → Release of inflammatory substances & destruction of pathogen.

(iv) Immune complex clearance —

- Helps remove antibody-antigen complexes from the circulation & tissues, preventing damage caused by their deposition.

- Complement system activation pathways —

- The complement system can be activated by the following pathways:
  1) Classical pathway (antibody-dependant)
  2) Alternative pathway (antibody-independant)
  3) Lectin pathway (activated by mannose-binding lectin)

(1) Classical pathway —

- complement activation by classical pathway commonly begins with formation of soluble Antigen-Antibody complexes, antibody like IgG, IgM.

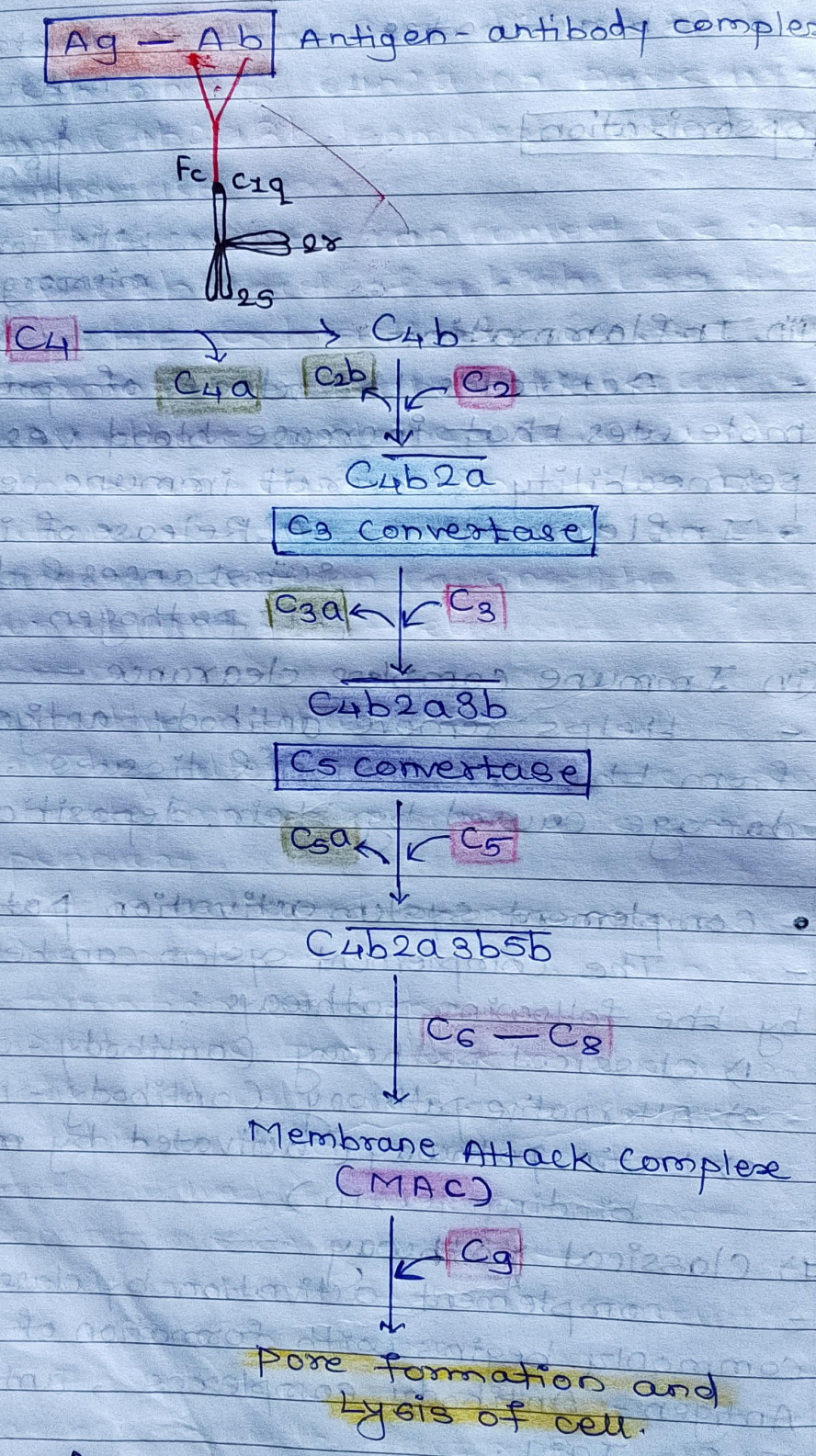


fig. ~~Complement system~~
Classical Pathway.

- The classical pathway is typically initiated by the binding of complement protein C1q to the Fc region of antibodies (IgG or IgM) that are attached to pathogens or foreign cells.
- Because the complement proteins are named in order of their discoveries, so their numbering in their name do not always reflect order in which they react.
- formation of Ag-Ab complex induce confirmational changes in Fc portion of Ab that forms binding for proteins of complement system which include C1 (C1q and 2 molecules of each C1r and C1s).
- Ag-Ab complex convert C1r to active serine protease enzyme which cleaves C1s & activates it.
- C1s has 2 substrates C4 and C2 out of which C4 is get activated when C1s hydrolysed it into 2 parts C4a & C4b.
- C4b binds to surface of Ag & C4a diffuse in blood & get deactivated.
- C1s also cleaved C2 into 2 parts C2a & C2b out of which C2b diffuse in blood & get deactivated while C2a bind ~~to C1s also cleaved C2 into~~ C4b on the surface of Ag to form C4b2a complex.
- This C4b2a complex called as C3 convertase, because it convert C3 into active form by cleaving it into C3a and C3b.

- out of this C3a diffuse in blood, while C3b bind to C4b2a on Ag surface to form C4b2a3b complex called as C5 convertase
- This complex can convert C5 into C5a & C5b, out of which C5a diffuse in blood, while C5b activate C6, C7, and C8 forming membrane attack complex (MAC)
- All these proteins (C6, C7, C8) together with help of C9 protein, after the formation of MAC forms pore on the surface of antigen causing the lysis of bacterial (antigen) cell.

(2) Alternative pathway —
- The alternative pathway is a part of the innate immune response & is antibody - independant.
- It is activated sponatenously by the hydrolysis of C3 in plasma, which leads to the formation of C3b.
- pathogens that lack regulatory mechanisms like bacterial cell walls, provide surfaces where this activation can continue unchecked.

• Steps involved in alternative pathway -

(i) Spontaneous C3 activation —
- small amounts of C3 are constantly cleaved into C3a & C3b in plasma.
- If C3b binds to pathogen surface, it can associate with factor B, which is then cleaved by factor D to form the C3 convertase (C3bBb).

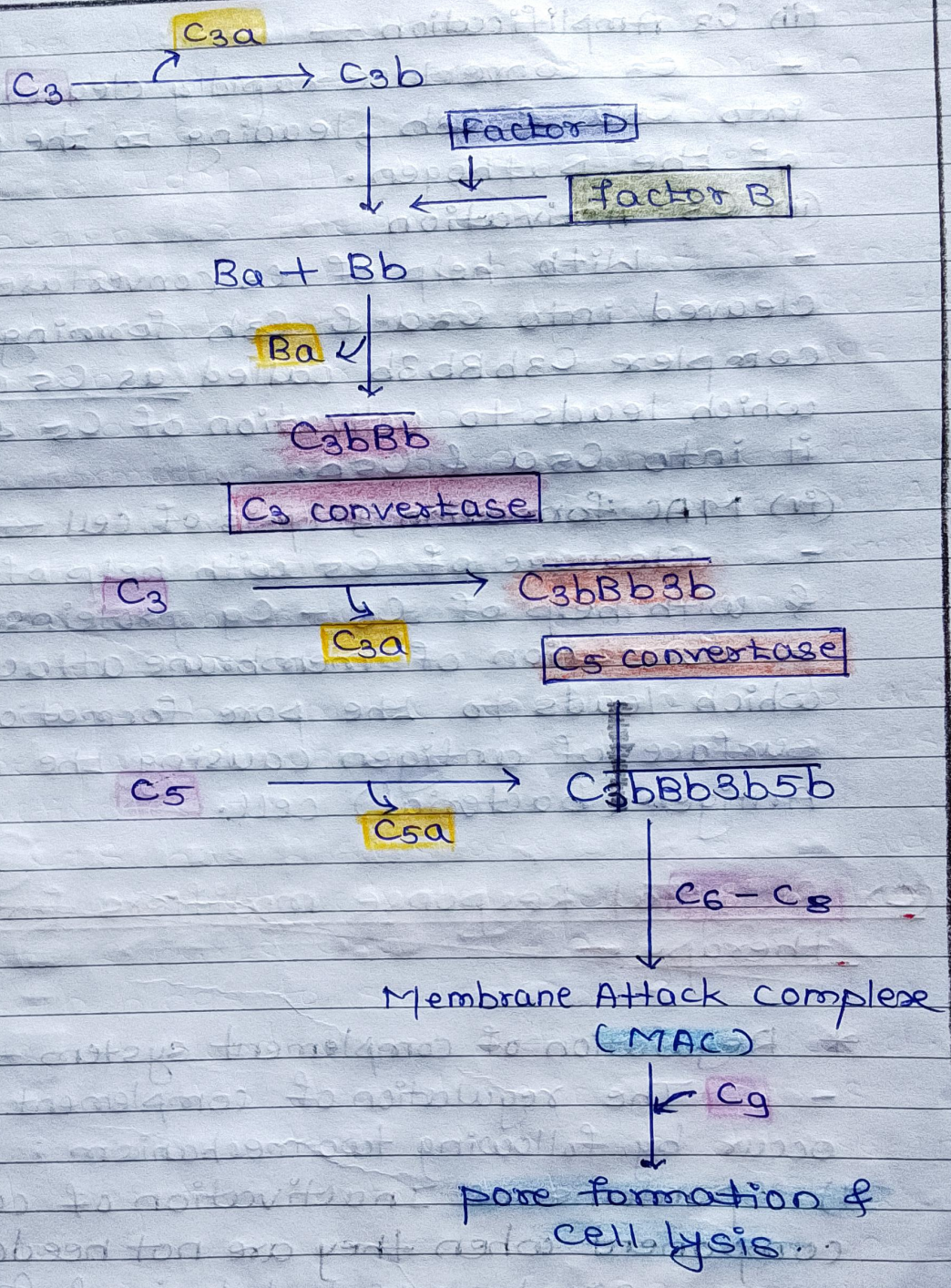


fig. Alternative pathway.

(ii) C3 Amplification —
- C3 convertase rapidly cleaves more C3 into C3a & C3b, leading to the opsonization of the pathogen.

(iii) C5 Activation
- With help of C3 convertase C3 gets cleaved into C3a & C3b forming the complex C3bBb3b called as C5 convertase which leads to activation of C5 & cleaved it into C5a & C5b.

(iv) MAC formation & lysis of cell —
- Cleavage of C5 with help of C5 convertase & with help of C6 - C9 proteins there is formation of membrane attack complex which leads to the pore formation on the surface of antigen causing the lysis of Antigen (bacterial) cell.

(c) Biological Response modifiers for cancer therapy —

* Regulation of complement system —
- The regulation of complement system occur by following two mechanism:

1) Spontaneous Inactivation of complement component when they are not needed —
- eg. spontaneous hydrolysis of C3b occurs when it diffuse 40 nm away from the C4b2a (classical pathway) or C3bBb (alternative pathway.

2) Regulation by Regulatory protein —

- This proteins inactivate complement components when not needed.
- This is the most important mechanism of complement pathway regulation.
- This regulatory protein act at many stages of complement pathway.

like, (a) Before assembly of convertase -
(b) After assembly of convertase -
(c) At MAC formation stage -

- This complement regulatory proteins are also called as RCA (Regulators of complement Activation)
- This proteins contains sequence of approximately 60 amino acids which called as short conences sequences.
- All these proteins are encoded by genes located on chromosome number 1.

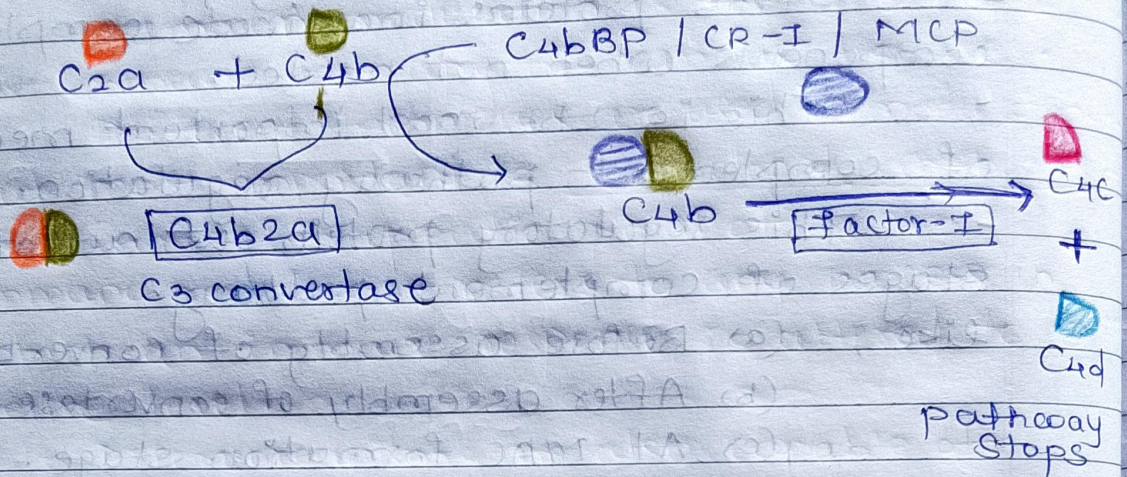
(a) Before Assembly of convertase (C3 and C5 convertase) —

- In complement pathway following proteins plays important role:

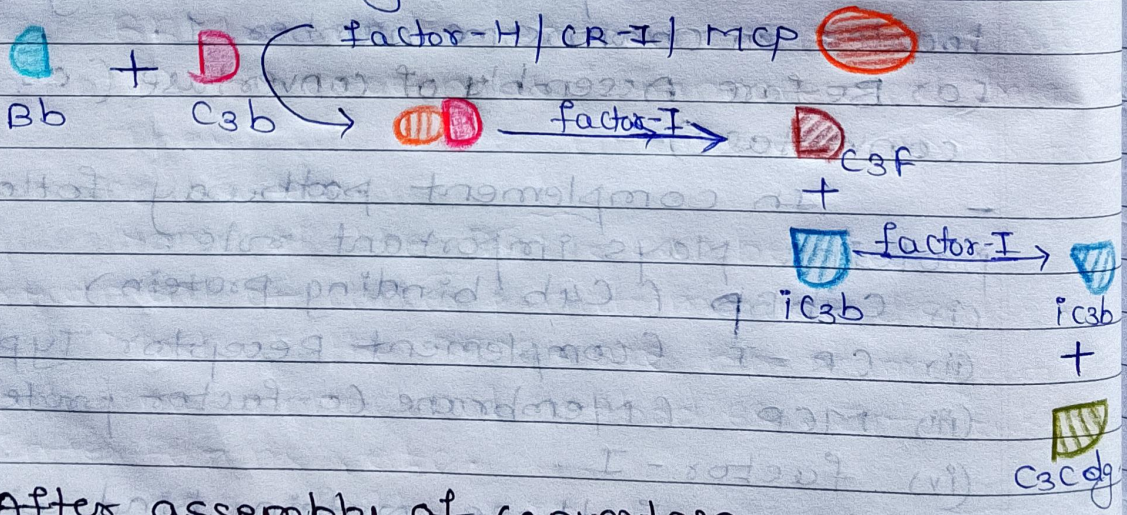
(i) C4bBP (C4b binding protein)
(ii) CR-I (Complement Receptor Type-I)
(iii) MCP (Membrane Co-factor protein)
(iv) factor-I

- One of these proteins bind with C4b & prevent its association with C2a.
- once regulatory proteins bound to C4b, another protein called factor-I act on C4b & cleave it into C4c & C4d (classical pathway.

- In classical pathway:



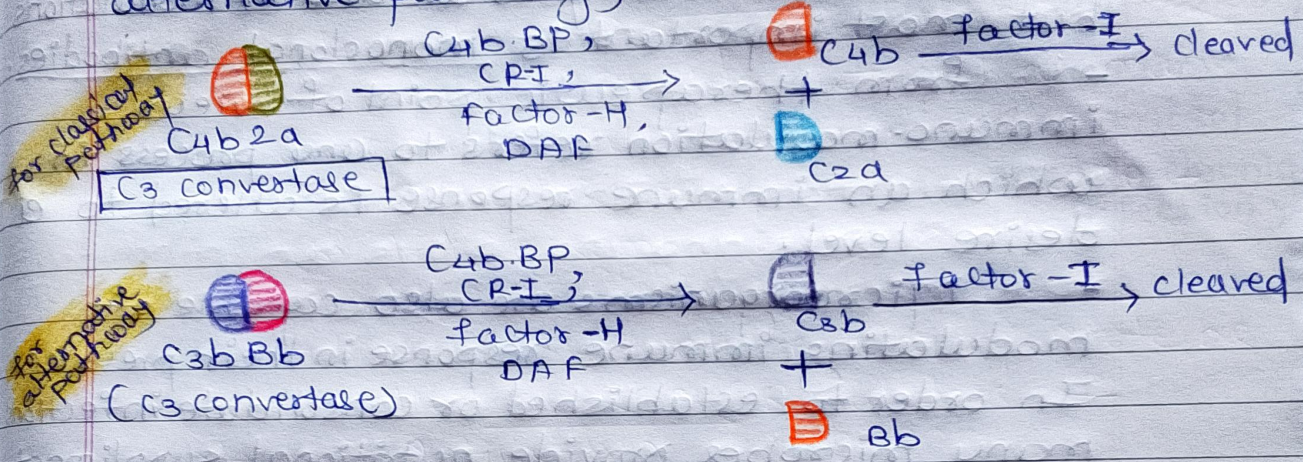
- Similar mechanism prevent assembly of C3 convertase in alternative pathway.
- In this mechanism CRI / MCP / factor-H bind to C3b & then factor-I cleave it into C3F and iC3b
- This iC3b again cleaved by factor-I C3C and C3Cdg.



(b) After assembly of convertase —

- Regulatory proteins like C4b BP, CR-I, factor-H, DAF (Decay Accelerating factor) have ability to dissociate C3 convertase by enzymatic reaction.
- They act on C2a in classical pathway & Bb in alternative pathway.

- once these component of C3 convertase are dissociated, factor-I cleared remaining component of C3 convertase (C4b in classical & C3b in alternative pathway).



(c) Regulation at MAC complex formation stage -

- A protein called 'S' bind to C5b-C6-C7 & induce hydrophilic transition in it which prevent its entry into cell membranes & inbibit membrane attack complex (MAC) formation.

C5b-C6-C7 + 'S' protein

↓

Hydrophilic transition

↓

It prevent entry in cell membrane

↓

Inhibit MAC formation.

(C) Biological Response Modifiers for Cancer Therapy (BRMs)

- Immunomodulation is the emerging approach for immune therapy of various diseases.
- Different techniques are used in modulations but most important is monoclonal antibodies.
- from therapeutic point of view immuno-modulation refers to any process in which an immune response is altered to a desire level.
- Microorganisms are also capable of modulating immune responce in their presence. In order to established or consolidate infections many microbes provide nutritional supplement which also enhances immune system performance so as to protect body from infection.
- BRMs are substances which have modified immune response.
- BRMs can be endogenous (produce naturally in body) or exogenous (pharmaceutical drugs).
- They can either enhance or supress the immune system.
- Some of the BRMs can be used in treatment of cancer & autoimmune disease.
- Most of the BRMs are bio-pharmaceuticals including monoclonal antibodies, Interleukins, Interferons, etc.
- Some of the effects of BRMs include Nausea, vomiting, Diarrhoea, loss of appetite, fever, chills, muscle ache, weakness, skin rash, etc.
- Some extract of medicinal mushrooms are also used as natural BRMs.

Classification of BRMs

① Derived from bacteria
- CPA
- Micobacterium antigen
- LPS of grame -ve organism
- BCG

② Derived from Eukaryotes
- Thymic hormones
- Cytokines
- Monoclonal antibodies.

③ Derived from Biochemical compounds
- Insulin
- Levamisol (used in chronic infection)

* functions of BCR —

① Antigen Recognition —
- BCR recognize antigen on surface of bacteria & viruses which is first step of immune response.

② Signal transduction —
- When BCR binds to antigen it sends signals into the cell.

③ Antigen processing —
- BCRs deliver antigen to cell's interior where they are broken down & returned to B cell surface as peptides.

④ T cell activation —
- This peptides are represented to TH cells, which then produe proteins that B cell to proliferate & differentiate into antibody-secreting cells.

⑤ Memory Cell formation —
- After an immune response, some activated B cells differentiate into memory B cells which remember antigen & provides quick action against re-exposure of antigen.

⑥ Regulation of Immune Response —
- BCR signalling plays an important role in regulating immune responses.