

## Unit 1

### Innate and adaptive immunity

#### History

Emil von Behring / Kitasato

- Serum from vaccinated animals was protective

Metchinkoff (1880)

- Cell based immunity

Merrill chase (1940)

- Transfer of WBC (immunity to tuberculosis)

There have been 19 nobel prizes in immunology since 1901

#### Immunity

- From immunis – latin: state of protection from infectious diseases
- Body's ability to resist and eliminate harmful foreign materials / abnormal cells
- Does following activities
  - Defense: viruses / bacteria
  - Removal: worn out cells / tissue debris
  - Identification and destruction: abnormal cells / mutant cells
  - Rejection: foreign cells
  - Inappropriate responses: allergies / autoimmune diseases
- Has 2 types
  - Innate
    - External defenses
      - Anatomical barriers
        - Mechanical factors
          - Skin
          - Mucociliary escalator
          - Flushing action of saliva, tears and urine
        - Chemical factors
          - Antimicrobial peptides in sweat
          - HCl in stomach

- Lysozyme in tears / saliva
- Biological factors
  - Normal flora: microbes in body → 1000 species of bacteria → competes with pathogens for nutrients and space
- Internal defenses
  - Cellular
    - Neutrophils
      - Most abundant WBC (50-60%) important for innate immune system
      - Efficient phagocytes
    - Phagocytosis
      - Phago: eat | cyte: cell
      - WBCs ingest and kill invading pathogens
      - Process:
        - Recognition and adherence
          - Pathogen-associated molecular pattern (PAMP) binds with Toll-like receptor on phagocyte
        - Engulfment
          - Extensions of cytoplasm enclose the particle inside phagosome
        - Intracellular killing
          - Phagosome fuses with lysosome (phagolysosome)
          - Lysosome digests (kills) phagosome
          - Lysosome include: hydrogen peroxide / nitric oxide
        - Exocytosis
          - Indigestible materials released
  - Monocytes
    - Migrate into tissues and become macrophages
    - Macrophages
      - Big eaters

- For phagocytosis of microbes in tissue
  - Antigen presentation
- Natural killer cells
  - They are not B lymphocytes or T lymphocytes
  - Important part of innate immunity
  - Kills infected cells
  - Kills cancer cells
  - Process:
    - Recognizes MHC I on healthy cells
    - If MHC I is present it does not kill the cells
    - Infected cell that does not present MHC I is killed
- Toll-like receptors (TLRs)
  - Transmembrane proteins
  - Present on macrophages
  - Present in vertebrates
  - Important part of innate immune system
  - Process
    - Look out for microbes
    - Bind to microbes
    - Trigger a cascade of events that kill pathogens
    - Innate immune sensors
    - Phagocytosis or apoptosis of infected cells: killing of infected cells
    - Secretion of cytokines/interferon or inflammation: enhanced immune response
- Extracellular
  - Cytokines
    - Small proteins secreted by cells of immune system
    - Affect behavior of other cells
    - Signaling molecules
    - Key players in innate and acquired immunity

- Released by: neutrophils / macrophages / TLRs / NK cells / Lymphocytes
- Eg: Interferons, interleukins, Tumour necrosis factor (TNF)
- Interferons (IFN)
  - Signaling proteins produced by virus infected monocytes and lymphocytes
  - Interfere with virus replication
  - Warns neighbouring cells about virus
- Interleukins (1-37)
  - Not stored inside cells
  - Synthesized and secreted in response to infection
  - Modulate behaviour of immune cells
  - Secreted by T-lymphocytes and macrophages
  - What do they do:
    - Activation of immune cells
    - Increase antibody production
    - Proliferate immune cell
    - Cause inflammations
- Tumour necrosis factor (TNF)
  - Kills cancer
  - Induces fever and inflammation
- Complement (C<sup>`</sup>)
  - Distinct plasma proteins that react with one another (C1-C9)
  - Binds to microbes
  - Essential part of immune response
  - Enhances adaptive immune response
  - Role
    - Facilitates phagocytosis
      - Neutrophils have C<sup>`</sup> receptors which bind to bacteria having C<sup>`</sup>
      - This initiates phagocytosis
    - Direct lysis of pathogens

- Activated C<sup>3</sup> form complexes that create holes in bacterial cell wall
    - Water and salts diffuse through these holes
    - Bacterium swells and bursts
  - Inflammation
- Coagulation proteins
  - Mechanism to stop bleeding after injury to blood vessels
  - Pathways involve
    - Platelets
    - Coagulation factors
    - Vitamin K
  - Platelets attach to endothelium
  - Platelets start to release fibrin and seal endothelium
  - Fibrin network stops RBC and clots
  - Coagulation balance
    - Coagulation proteins
      - Clotting
      - Inflammation
      - Apoptosis
    - Anticoagulants
      - Prevent blood clotting
      - Inhibit inflammation
      - Inhibit apoptosis
- Inflammation
  - Response to pathogens and irritants
  - Swelling of tissue
  - Key player in innate immune response
  - Everything in innate immunity leads to inflammation
  - Inflammation leads to: vasodilation and increased capillary permeability
  - Vasodilation
    - Heat / redness
    - Fever

- Capillary permeability
  - Swelling
  - Pain
  - Temporary loss of function
- Role
  - Initiation of phagocytosis
  - Limit the speed of infection
  - Stimulate adaptive immune response
  - Initiate tissue repair
- Adaptive immunity
  - Refer to sir's notes

### Important questions

- Types of vaccine
  - A biological preparation that improves immunity to a specific disease
  - Contains an agent resembling disease causing microorganism in a weakened or killed form
  - Agent stimulates body's immune system to destroy it and keep a record of it
  - Immune system can then more easily recognize and destroy these in later encounters
  - Vaccine comes from "Variolae vaccinae" termed by Edward Jenner
  - There are 7 types of vaccines
    - Live attenuated vaccines
      - Contain version of living microbe that has been weakened
      - So that it doesn't cause disease but provoke transient growth within host
      - Good teacher for the immune system since they are the closest to a natural infection
      - Achieved by growing pathogenic bacterium under abnormal culture conditions
      - Attenuated strain of Mycobacterium bovis (BCF) was developed in medium containing increasing concentrations of bile

- After 13 years
- Eg. Measles, mumps, chickenpox
- Inactivated vaccines
  - Contains microbe killed via chemicals, heat or radiation,
  - They are more stable and safer than live attenuated vaccines because dead microbes cannot mutate to disease causing state
  - Eg. Influenza, polio, hepatitis A, rabies
- Subunits Vaccines
  - Include only the antigens that best stimulate the immune system instead of the entire microbe
  - Some cases use epitopes – part of the antigen that antibodies and T cells recognize
  - Contain only essential antigens and not all molecules that make up microbes
  - Eg. Plague immunization
- Toxoid vaccines
  - It is for bacteria that secrete toxins, or harmful chemicals
  - When bacterial toxin is main cause of illness
  - Toxins can be inactivated by treating with formalin, these detoxified toxins are called toxoids
  - Eg. *Crotalus atrox* used to vaccinate dogs against rattlesnake bite
- Conjugate vaccines
  - Conjugation is done to treat outer coating sugar molecules (polysaccharides)
  - Polysaccharide coatings disguise bacterium's antigens in immature immune systems (Infants and younger children)
  - Eg. *Haemophilus influenzae* type B vaccine
- DNA vaccines
  - Experimental vaccines
  - Dispose off all the parts of a microbe except its genetic material

- Eg. Influenza vaccine
- Recombinant vector vaccines
  - Experimental
  - Use attenuated virus or bacterium to introduce microbial DNA to cells
  - Vector refers to virus or bacterium used as carrier
  - Eg. DPT
- Difference between innate and adaptive immunity

Innate Immunity	Adaptive immunity
General and non specific. It is the first line of defence against pathogens	It is built up as we are exposed to diseases or get vaccinated
Response is quick short / intermediate	Response is slower than innate
Low potency against pathogens	High potency against pathogens
Can be inherited	Cannot be inherited
Cannot remember pathogens	Can remember past pathogens that have been encountered
Present at birth	Develops over lifespan of the individual
Found in both vertebrates and invertebrates	Only found in vertebrates
Comprises of skin, mucous, epithelial cells, phagocytes, etc	Comprises of lymphoid organs that produce specialized cells called t cells and b cell
Eg. A cut on the skin, if results in swelling and inflammation, is an example of innate immunity at work	Eg. Disease like chicken pox enable the adaptive immune system to remember. Hence likelihood of contracting the illness is greatly minimized

- Explain endogenous and exogenous pathway
  - These are a part of antigen processing and presentation
  - Antigen processing is cleavage of proteins by enzymes into small fragments and their association with MHC molecules by the antigen presenting cells



- Antigen presentation is the presentation of processed peptides in association with MHC molecules on the surface of processing cells
- Endogenous pathway
  - Also known as cytosolic pathway or MHC class I pathway
  - Endogenous proteins are processed in cytosol or in secretory vesicles
  - Presented on class I MHC molecules to CD8+ T cells
  - Endogenous proteins are derived from proteins inside the cells
    - Include altered self protein antigens (tumor antigens) or non self proteins (viral antigens)
  - Endogenous antigens can be processed and presented by any nucleated cell
  - Pathway
    - Step 1a: ubiquitination
      - Pathogenic proteins are bound with ubiquitin due to high error rate in translations
      - The tagged proteins are then taken to the proteasome
    - Step 1b: proteasome mediated processing
      - Proteasome cleaves these proteins into peptides and amino acids by the use of proteases
    - Step 2: transfer of peptides by tap proteins
      - TAP (transfer associated with antigen processing)
      - TAP 1 and TAP 2 form a heterodimer in membrane of endoplasmic reticulum to facilitate selective transport of peptides from cytoplasm to endoplasmic reticulum
      - TAP can transport 8-15 amino acids long peptides
    - Step 3a: generation of class I MHC
      - Calnexin (chaperone) binds to newly synthesized alpha-chain of MHC I and prevent degradation until beta-2-microglobulin binds
      - Tapasin and calreticulin bind to newly formed MHC I complexes.
      - Tapasin form bridge between tap proteins and MHC I
      - Calreticulin prevents any other peptide from binding
    - Step 3b: association of peptides with Class I MHC

- Peptides replace tapasin and calreticulin and bind to antigenic peptide binding groove of Class I MHC molecule to form pMHC
  - Peptide binding gives stability. This allows transfer to surface
- Step 4: transport of pMHC to cell surface
  - pMHC-I complex is transported from ER via golgi bodies in a membrane bound vesicle to the surface
- Step 5: presentation
  - Membrane of transport vesicle fuses to cell membrane and pMHC complex binds to cell membrane,
  - This presents the peptides on antigenic peptide binding groove towards the exterior to be recognized by T cell
- Majority of peptides are ready after leaving proteasome to be transported to ER but some still need trimming
- Cytosolic proteases identify and trim NH2 terminal after proteasome cleavage
- Sometimes peptides are trimmed inside the ER to fit in class I MHC antigenic peptide binding groove
- Exogenous pathway
  - Also known as endocytic or MHC class II pathway
  - Proteins processed in endosomes and presented on class II MHC molecules to CD4+ T cells
  - Derived from proteins produced outside the cells
    - Include bacterial, viral, protozoal, fungal and parasitic antigens
  - Can be processed and presented by APCs
  - Pathway
    - Step 1: generation of peptides
      - Peptides are derived from engulfed pathogens
      - APCs internalize antigens by
        - Phagocytosis and endocytosis
          - Macrophages
        - Only endocytosis
          - Dendritic cells
          - B cells

- Exogenous antigens are degraded into peptides within endocytic vesicles under highly acidic conditions by the influence of more than 40 hydrolases that cut the antigen in 13-18 amino acid long peptide
- These peptides are loaded onto MHC II
- Step 2: generation of MHC II molecule
  - 2 chains alpha and beta present of MHC II present in ER
  - One molecule of Ii in groove
  - This complex transported to golgi apparatus into special vesicles
  - Vesicles deliver MHC II to endocytic compartments where peptide loading is done
  - Ii
    - Binds to MHC II to prevent other peptide binding
    - Transports MHC II from golgi to endocytic compartments
    - Signals in tail of Ii lead to proper sorting of MHC II
    - In compartment Ii is cleaved to leave CLIP in groove (Class II associated Invariant chain Peptide)
- Step 3: peptide loading
  - Molecule with Ii transported to endosome where peptides are present for loading
  - In compartment Ii is cleaved by proteases into a small fragment called CLIP
  - CLIP prevents premature binding of peptides
  - MHC-DM removes CLIP and helps load antigenic peptide into groove to form pMHC II
  - Acidic pH required for exchange of peptides
- Step 4: pMHC transport
  - pMHC transported to plasma membrane in a vesicle
  - transport vesicle fuses with cell membrane and pMHC is displayed on cell surface
  - presented to T cells with CD4+

- Difference between endogenous and exogenous

Exogenous	Endogenous
Class II MHC used	Class I MHC used
Antigen source is Exogenous	Antigen source is endogenous
APCs are DC, MO and B cells	APCs are all nucleated cells
Responsive T cells are CD4+ tcells	Responsive T cells are CD8+ tcells
Cellular compartment is endosome	Cellular compartment is cytosol
Peptide degradation by endosomal and lysosomal proteases	Peptide degradation by cytosolic proteasome
Ii and HLA-DM is involved in transport of peptides for loading	TAP is responsible for transport of peptide for loading

- Short note on autoimmune diseases

- Autoimmune diseases refer to illness or disorder that occurs when healthy cells get destroyed by the body's own immune system
- Autoimmune diseases are not contagious. They are sometimes genetic and other time triggered by viruses, certain chemicals and other things in the environment
- Treatment for autoimmune diseases generally focuses on reducing immune system activity
- Examples:
  - Rheumatoid arthritis
  - Lupus
  - Inflammatory bowel disease
  - Multiple sclerosis
  - Guillian Barre syndrome
  - Psoriasis
  - Graves' disease

### Unit 3

- Explain reverse vaccinology

- Technique of identifying proteins that are exposed on the surface by using genome instead of the microorganism
- Flowchart for reverse vaccinology goes as
  - Genome sequence analysis
  - Prediction of novel antigens
  - Recombinant protein expression
  - Immunogenicity testing to animal model
  - Development of vaccine
  - Vaccines
- Epitope prediction means the discovery of peptides that could mimic protein epitopes and possess the same immunogenicity as the whole protein
- Reverse vaccinology deals with computational analysis of genome that can be used for the prediction of the epitopes. Hence epitopes play an important role in development of a vaccine candidate
- B cells are important in recognizing the epitopes of the antigens that can be identified by the paratopes of the antibody
- T cells play a role in cell mediated immunity, processed antigenic peptides interact with t cell when they are presented
  
- Difference between reverse and rational vaccinology
  - Cant fucking find it mate
  
- Prediction of b cell and t cell epitopes
  - Epitopes
    - Antigenic determinant or epitopes are the portions of the antigen molecules which are responsible for specificity of the antigens in antigen-antibody (Ag-Ab) reactions and that combine with the antigen binding site of Ab, to which they are complementary
    - Occur on surface of the protein and are more flexible than rest of the protein
  - Paratope

- Recognized by both t and b cells.
- B cell epitope prediction
  - Structural analysis of antigen antibody complexes
    - Ray crystallography
    - Nucleic magnetic resonance
    - Electron microscopy
  - Functional analysis
    - Mass spectrometry
    - Elisa
    - Elisprot
    - Western blot
- T cell epitope prediction
  - It is done by almost the same methods that are used for b cell epitope prediction
- Pros of epitope prediction
  - Insilico approach is always preferable
  - Fast and efficient
  - Can be used for detection of antibodies produced as a result of infections, allergies, autoimmune diseases, cancers, etc
- Cons
  - Theoretical prediction is highly challenging task
  - Only proteins can be targeted using this process, their biomolecules can't
- Tools for b cell epitope prediction
  - Discotope
    - This method incorporates solvent-accessible surface area calculations
    - Contact distances are calculation
  - ElliPro
    - Predicts epitope based on solve-accessibility and flexibility
- Tools for T cell epitope prediction
  - TepiTool
    - Provides prediction of peptides binding to MHC class I and class II molecules
    - It is wizard with 6 steps

- Each field is filled with default recommended settings for prediction and selection of optimum peptides
-