1. Basic Concepts in Immunology

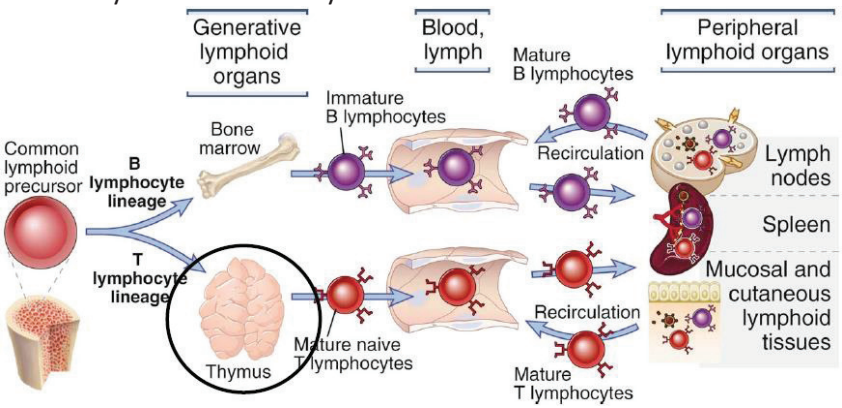
What is the Immune System?

•The immune system = A collection of cells, molecules and tissues that mediate resistance to infections and eliminate tumours
-Prevents infections, eradicate established infections, detect and eliminate tumours, tolerate self
-The immune response = coordinated and tightly controlled reaction of the immune system to infectious microbes

•Immunity = Resistance to disease
-Infectious diseases caused by bacterial, viral, fungal and parasitic infections
-Disease also includes tumour

•Where is the immune system located?
-The immune system is integrated with other systems: GIT, cardiovascular, respiratory, nervous, endocrine, epithelial
-Epithelial cells of skin, gut, respiratory tract, secretions (sweat, tears), mucus (nose, trachea, gut), urine, proteolytic enzymes, low stomach pH, normal gut flora
-Immune cells are scattered in all parts of the body:
-many of them actively travel or 'migrate' via blood and lymphatic vessels
-others sit in the one place for a long period of time: resident cells

•Anatomy of the immune system



-Leukocytes originate from bone marrow stem cells
-Hematopoietic progenitors in bone marrow: multipotent
1) Myeloid progenitor → innate immune system (Eosinophil, basophil, neutrophil, monocyte)
2) Lymphoid progenitor → adaptive immune system (B and T lymphocytes)
-Central sites = Lymphoid tissues or organs
1) Primary lymphoid organs: bone marrow and thymus
2) Secondary lymphoid organs: spleen, lymph nodes, mucosal and cutaneous associated lymphoid tissues
-Peripheral sites = All the other tissues and systems (skin, liver, gut, heart, CNS, muscle, lungs)

-Naïve lymphocytes have not encountered an antigen before while activated lymphocytes have
-Immature lymphocytes do not yet have antigen-specific receptors and cannot respond to foreign antigens
-Immature lymphocytes undergo maturation in primary lymphoid organs. They develop functional antigen-specific receptors and can respond to foreign antigens. They travel to secondary lymphoid tissues and peripheral sites.

▪Lymphocytes
1) B lymphocytes
-Maturation in the bone marrow
-Form part of the humoral immune response
-Main function: secrete antibodies (proteins that bind to extracellular antigens)
-Block infections and eliminate extracellular microbes

2) T lymphocytes

-Maturation in thymus (positive selection in cortex, negative selection in medulla)
-Form part of the cell-mediated immune response
-Eliminate phagocytosed microbes
-Kill infected cells and eliminate reservoirs of infection

a) Helper T cells (CD4+ T cells)
-Surface expression of CD4 molecule
-Help other cells of the immune response
    -Release cytokines
    -Via membrane bound molecules
-Some CD4 T cells suppress or regulate the immune response rather than activate the immune response

b) Cytotoxic T cells (CD8+ cells)
-Surface expression of CD8 molecule
-Kill their target cells (viral infections and tumours)
-Get help from CD4+ T cells

•Antigens = substances that induce an immune response
-Types of antigens: proteins, carbohydrates, lipids, chemical haptens
-Antigen-specific receptors on lymphocytes bind to epitopes (antigenic determinants formed from a few amino acids or sugars only)

•Cytokines = Proteins secreted by different immune cells
-Initiate inflammation and immune response
-Modulate inflammatory and immune reactions (activate, potentiate, suppress or inhibit)
-Principal mediator of communication between immune cells
-Target cells in:
    -Autocrine manner: acting on the cell that produced the molecule
    -Paracrine manner: acting on neighbouring cells
    -Endocrine manner: acting on distant cells or systemically
-Cells will only respond to a cytokine if they express the specific receptor

Principles of Innate Immunity

1. How does the innate immune system recognise microbes?
2. How do the components of innate immunity function to combat different kinds of microbes?
3. How do innate immune reactions stimulate adaptive immune responses?

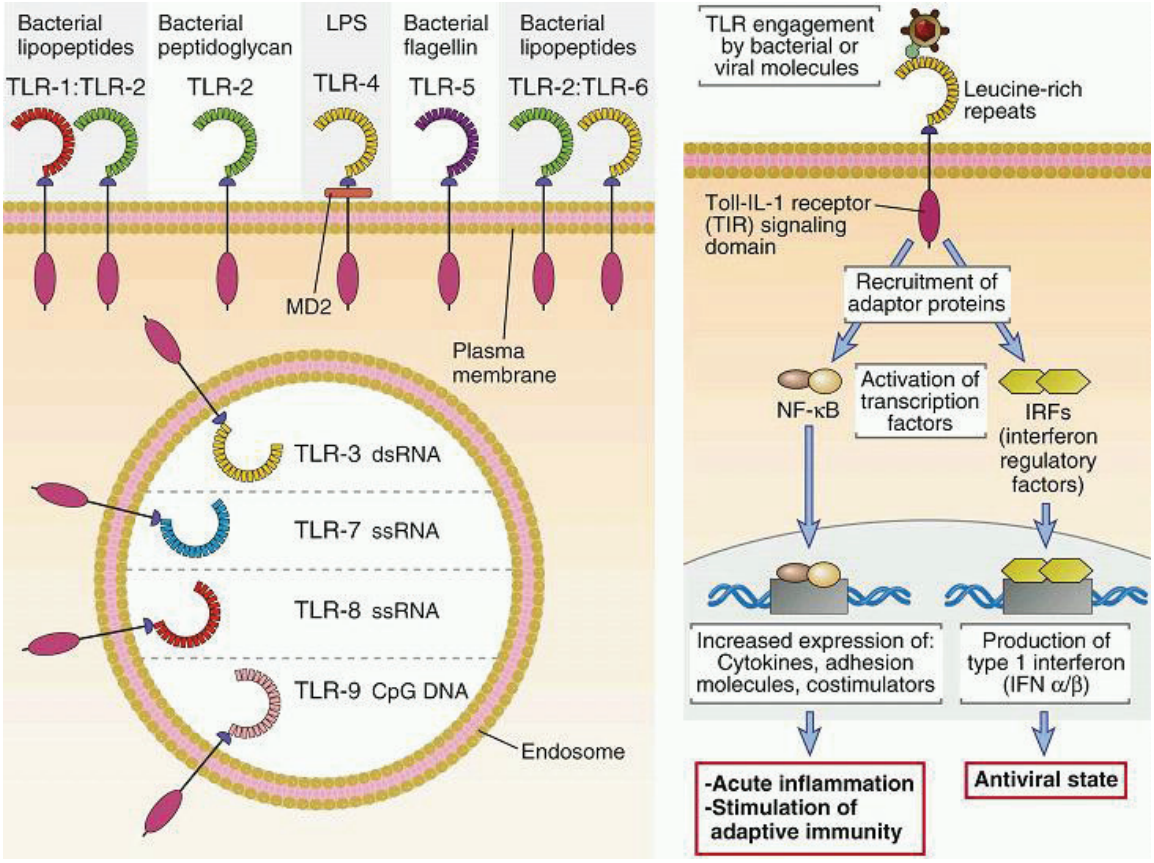
•Hallmarks of innate immune system:
1) Speed: early and rapid
2) Duration: short-lived (chronic inflammation)
3) Repetitive: responds the same way each time a microbe is encountered
4) Interactive: with other cells of the innate and adaptive immune system
5) Non-reactive to the host: innate immune cells detect dangerous pathogens via pattern recognition receptors

•Innate immune system is made of many different types of cells and molecules:
1) Epithelial barriers
2) Cells in circulation & tissues
-Phagocytes: neutrophils and macrophages that ingest microbes
-Exocytes: eosinophils, mast cells, basophils that release active mediators from granules
-Antigen presenting cells: dendritic cells, macrophages (and B cells)
3) Molecules
-Cytokines: tumour necrosis factor (TNF), interleukin-1 (IL-1), interferon gamma (IFN-δ)
-Plasma proteins: complement proteins, mannose-binding lectin, C-reactive protein

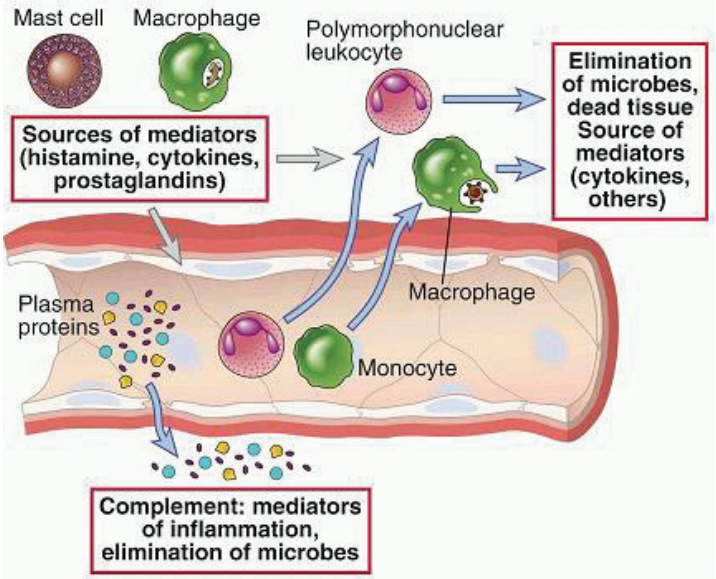
•Innate immune system recognises and responds to patterns expressed by pathogens

-Epithelial, endothelial and resident immune cells express Pattern Recognition Receptors (PRR) (E.g. keratinocytes, fibroblasts, endothelial cells, neutrophils, mast cells and macrophages)
-These PRRs do not react against the host but only recognise and respond to:
-Microbes express different microbial patterns Pathogen-Associated Molecular Patterns (PAMP)
-Damaged or necrotic cells release Damage-Associated Molecular Patterns (DAMP) (e.g. extracellular DNA)

▪Toll-like receptors (TLRs)
-An example of PRR
-Different TLRs have different cellular locations and are specific for different components of microbes
-TLRs present on the cell surface: recognise products of extracellular microbes
-TLRs in endosomes: recognise ingested/ phagocytosed microbes
-Signals generated by TLR engagement of PAMPs activates transcription factors that stimulate expression of genes encoding cytokines, enzymes, other proteins of antimicrobial functions and degranulation of mast cells



•Binding of PAMPs to PRRs triggers a cascade of events



1) Release of histamine and inflammatory cytokines (TNF and IL-1)

2) Histamine induces vasodilation
-Dilated blood vessels allow for more blood flow to the area (redness) and fluid to come in (swelling)
-Blood carries innate immune cells and plasma proteins (complement and antibodies)

3) TNF and IL-1 induce the expression of adhesion molecules on endothelial cells lining the blood vessels (veins)
-Attracts neutrophils and monocytes
-Perform phagocytosis
-Secrete more inflammatory cytokines
-Extend web-like extracellular traps for extracellular bacteria

4) Attracted cells adhere to endothelial cells only at sites of inflammation and enter the inflamed tissue

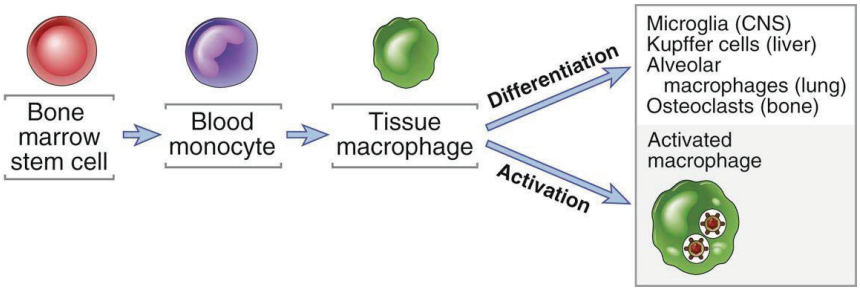
•Cells involved in innate immune system
1) Mast cells
-Circulate blood as immature mast cell progenitors
-Enter tissue and mature (function depends on the location)
-Mature mast cells are resident cells in peripheral tissues exposed to the environment (skin, lung, gut)
-Strategically located next to and along blood vessels for fast response
    -Probe the lumen to pick up antibodies (IgE)
-Long-lived
-Express PRRs
-Contain preformed cytokines (translation and transcription complete)

▪Functions
-Secrete or degranulate to release soluble mediators
    -Cytokines
    -Histamine and other inflammatory mediators (cause allergies)
-Regulate or suppress the immune response – contributing to tumour growth
-Antibacterial functions (E-coli infections)
    -Recruitment of inflammatory cells to sites of infection or “danger”
        -Histamine
        -TNF (increase vascular permeability and recruit neutrophils)
-Kill bacteria by entrapping them in extracellular structures called “traps”
-Allergies and asthma
-Autoimmune diseases
-Cancer

2) Neutrophil (Polymorphonuclear leukocytes)
-Most abundant leukocyte in peripherally circulating blood (not normally found in tissues)
-During inflammatory events signals are sent to the bone marrow to rapidly increase neutrophil production
-Fastest, coordinated and directed migration to inflamed tissue
-Infiltrate inflamed peripheral sites
-Short-lived (usually no more than a few hours)

▪Functions
-Secrete cytokines
    -Further promotes inflammation and recruitment of others cells (innate and adaptive immune system)
    -Promotes phagocytosis by macrophages
-Kill bacteria by entrapping them in extracellular structures called Neutrophil Extracellular Traps (NET)
-Potent antibacterial functions
-Can perform phagocytosis
-Help tumours grow

3) Monocytes/ Macrophages



-Monocytes in blood migrate into inflamed tissues to become macrophages
-Macrophages are resident cells in tissues
-Often long-lived (different from neutrophils) – may contribute to disease symptoms (e.g. TB – avoid fusion with lysosome and live within phagosome by adaption)
-Express PRRs for many different microbial constituents

▪Functions
-Potent antibacterial functions
-Phagocytosis (PAMPs binding to PRRs)
-Production of soluble mediators (PAMPs binding to PRRs)
-Secrete cytokines (inflammation and recruitment)
-As Antigen Presenting Cells, macrophages can present antigens and "talk" to lymphocytes
-Like neutrophils, some macrophages may help tumours to evade the immune system and grow (Tumour Associated Macrophages or TAMs)

4) Eosinophils
-Like mast cells, they contain numerous (pre-formed) cytoplasmic granules filled with various preformed inflammatory and antimicrobial mediators
    -Eosinophilic cytoplasmic granules are special because they contain enzymes that are harmful to the cell walls of parasites and helminths (parasiticworms)
    -The contents of these granules can also damage host tissues
-Normally in blood but some eosinophils are found in peripheral tissues (mucosal linings of the respiratory, gastrointestinal, & genitourinary tracts)
-During inflammation eosinophil numbers increase by recruitment from the blood
-Participate as effector cells in adaptive immune responses (particularly humoral or antibody responses)

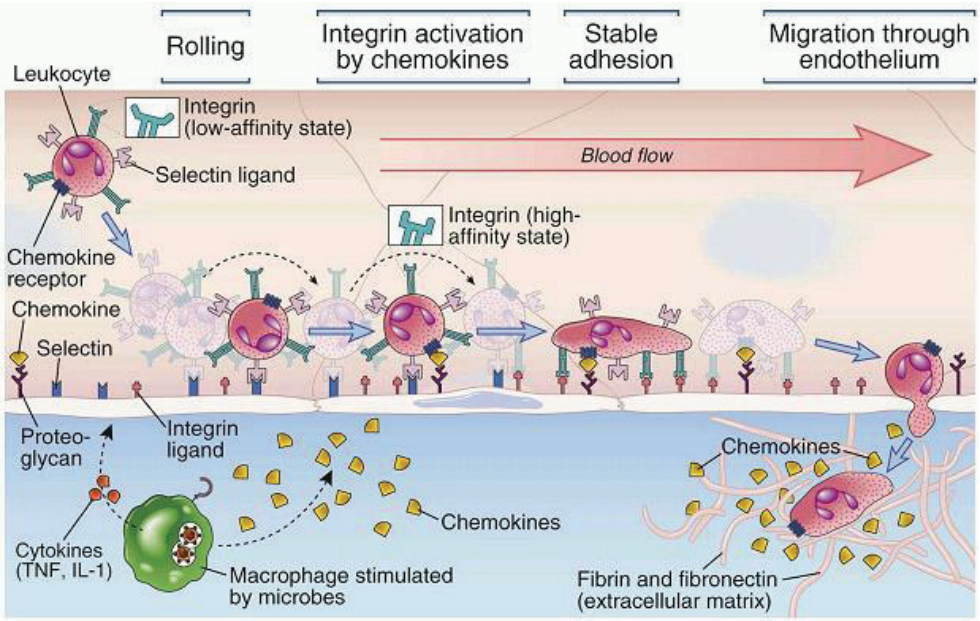
5) Basophils
-Constitute less than 1% of blood leukocytes
-Blood granulocytes with many structural and functional similarities to mast cells
-Contain numerous (pre-formed) cytoplasmic granules filled with various inflammatory and antimicrobial mediators
-Normally in blood, not found in peripheral tissues
-During inflammation basophil numbers increase by recruitment from the blood
-Important supporting role in the development of adaptive immune responses (particularly humoral / antibody responses)

6) Natural Killer (NK) cells
If microbes breach epithelia and enter the tissues or circulation they are attacked by
    -Phagocytes (neutrophils, macrophages)
    -Plasma proteins, including the proteins of the complement system
    -Natural killer cells
-Natural killer (NK) cells are a class of "innate" lymphocytes that recognise infected and stressed cells
    -Kill
    -Secrete the cytokine IFN-γ
-Important role in anti-viral immunity

•Chemotaxis – How do leukocytes know where to go?
-Chemokines = Chemo-tactic cytokines (released by bacteria)
-Different cells express different chemokine receptors → allows the cell to respond to different chemokines

-At source of infection, the concentration of chemokines are at a maximum and further away, the concentration decreases to form a chemical gradient
-Leukocytes move by following the gradient

•Leukocyte rolling – How do leukocytes travel?

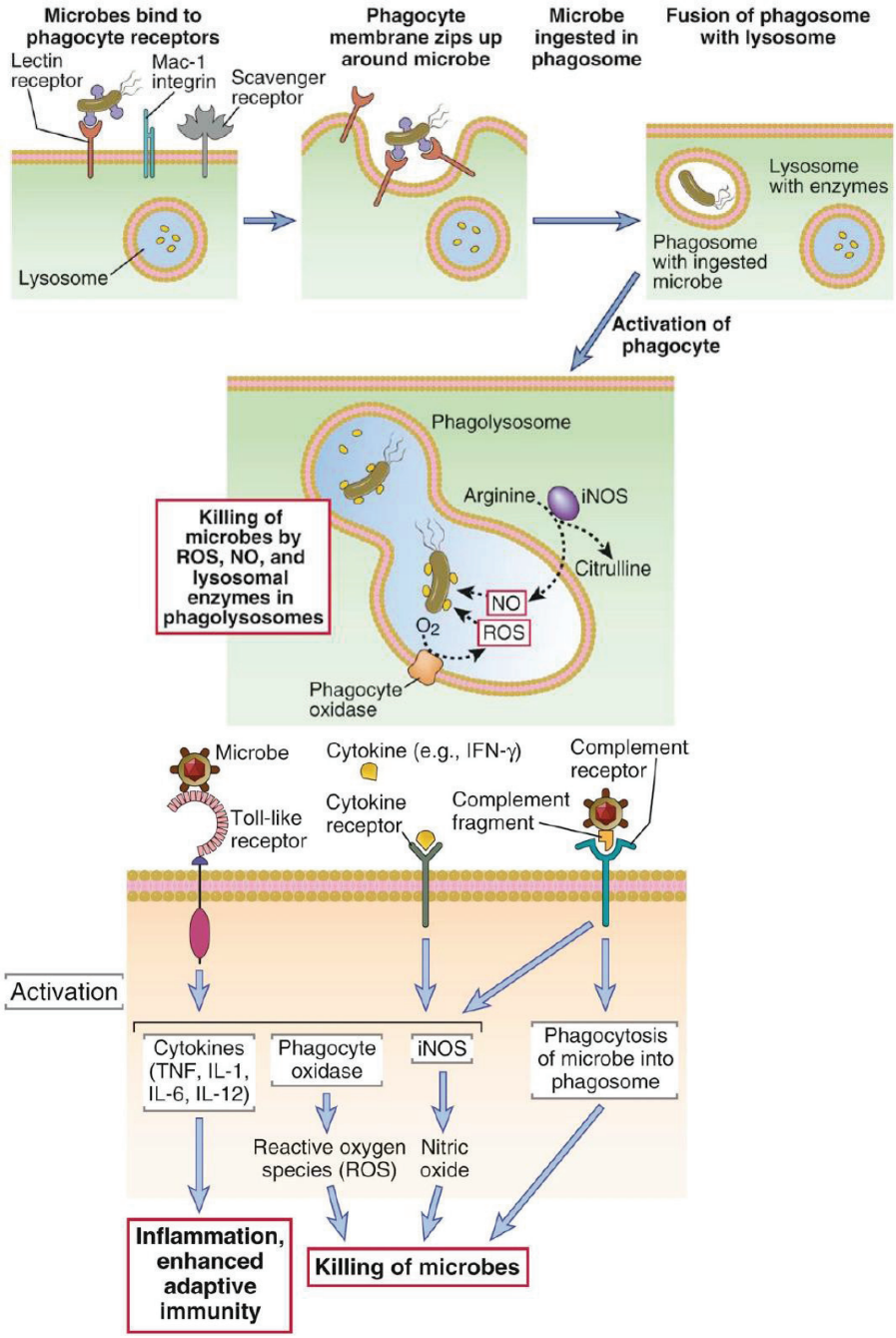


-Leukocytes only <u>adhere to the surface of veins</u>, they do not crawl out of arteries

-At the peripheral sites of injury or infection, tissue resident cells PRRs are bound by PAMPs of microbes
-The resident cells promote inflammation and secrete cytokines
-TNF and IL-1 are released and stimulate endothelial cells to rapidly express two adhesion molecules/ surface proteins: <u>E-selectin</u> and <u>P-selectin</u>
-Circulating leukocytes express surface carbohydrates and display on the membrane
-Selectins bind <u>weakly</u> to carbohydrates (low affinity), causing them to stick and “roll” along the endothelium

-At the peripheral sites of injury or infection, tissue resident cells produce chemokines
-Leukocytes express another set of adhesion molecules called <u>integrins</u> (the “integrate” extrinsic signals into cytoskeletal alterations)
-The chemokines stimulate a rapid increase in the affinity of the leukocyte integrins for their ligands on the endothelium and direct the migration of cells
-The firm binding of integrins to their ligands arrests leukocyte rolling
-The cytoskeleton of the leukocyte is reorganised
-Leukocyte spreads out on the endothelial surface
-Firm adhesion is quickly followed by extravasation into the inflamed tissue (connective tissue)
-Chemokine directed migration of leukocytes to the microbes

•Phagocytosis
-Macrophages and neutrophils ingest microbe by phagocytosis
-May be triggered or enhanced by microbes binding to phagocyte receptors and PRRs
-Phagocyte membrane zips up around the microbe and forms a phagosome
-Phagosome fuses with lysosome (low pH, lysosomal enzymes) to form a phagolysosome
-Enzymes & toxic substances in phagolysosome kills microbes
    -Nitric Oxide (NO)
    -Reactive oxygen species (ROS) – preformed OR production may be triggered/ enhanced by PRR binding
-Production of Cytokines (inflammation, enhanced adaptive immunity)



•Innate immunity does not act alone or in isolation and developing innate immune response is critically important for the initiation and development of adaptive immune responses
-Intracellular bacteria have evolved to live and replicate in intracellular vesicles - usually in macrophages
-Innate mechanisms of phagocytosis are not sufficient to kill these organisms
-To effectively eliminate phagocytosed pathogens phagocytes (e.g. macrophages & neutrophils) must be activated by T cells
    -through cell-to-cell contact
    -T cell-derived cytokines
-Activation induces additional killing mechanisms

•Antigen Presenting Cells (APC)
-Specialised at capturing microbes and other antigens
-Upon internalisation APC "present" the antigens to T lymphocytes
-Tissue resident APCs (location dependent functions)
    -Langerhans Cells in the Epidermis of skin
    -Dermal Dendritic Cells (DC)
    -Alveolar Macrophages in the Lung
    -Kupffer cells in the Liver
-APCs recruited during inflammatory events

-Inflammatory dendritic cells (DC)
-Monocytes / macrophages

-APCs in the secondary lymphoid tissues
-B cells
-Follicular Dendritic Cells (FDC)

▪Dendritic cells
-Resident cells
-Strategically located to maximise chance of 1st encounter
-At peripheral sites, express a range of PRRs
-Detect PAMPs and trigger the production of inflammatory cytokines
-In dermis, DC extend their dendrites to search for pathogens
-"Professional antigen presenting cells"
-Responsible for initiating adaptive immune responses

1) Antigen processing
-Captured proteins are broken down into peptides
-The peptide antigens are loaded onto special surface molecules, Major Histocompatability Complex (MHC), in order for T cells to "see" them

2) Dendritic cell maturation
-Activation of DC by TNF and IL-1
-Upregulation of molecules that allow DC to fully activate naive T cells

3) Dendritic cell migration
-Downregulaiton of adhesion molecules, lose dendrites and exit the periphery
-The DC follow the chemokines to the naive T cells circulating in the T cell zone of lymph node via lymphatic vessels

4) Activate T cells