Cells and Structure of the immune system and Innate immunity (W1)

Cells and structure of immune system

We know the immune system is important because…
- SCID: severe combined immunodeficiency Disease
- Acquired Immunodeficiency Syndrome (AIDS)

Layers of Protection in Immunity

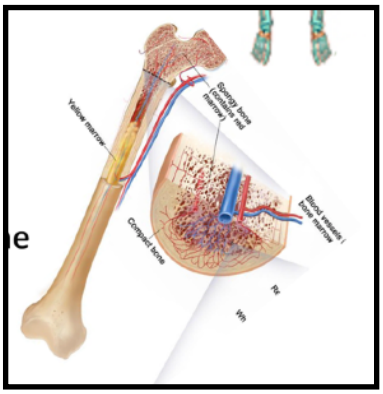
Physical barriers
- Keep it out
- Performed structures and environment
- Inhospitable to pathogens

Innate immunity
- Deal with it now
- Quick acting
- Soluble and cellular agents
  - Detect and destroy

Adaptive immunity
- And remember in case it comes back
- Specific and tailored responses
- And provides long term protection for future encounters

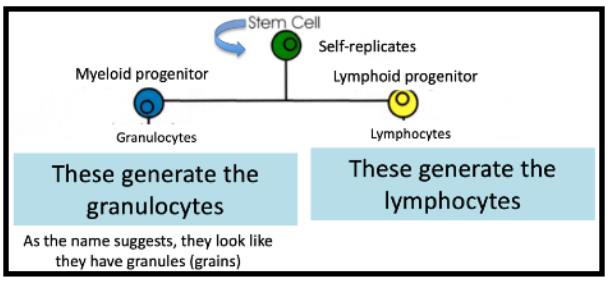
The immune system is like our own personal defence force
- The remarkable thing is that this system is able to deal with many **different** and **diverse** types of threats
- Bacteria, viruses, parasitic worms
- At any one time, the immune system doesn't know where the next attack may come from
- As a protective system, it must be ready to deal with anything, anytime

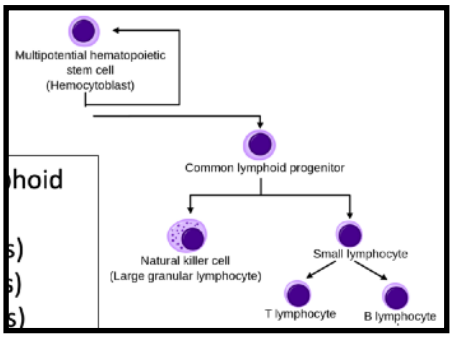
The immune system
- As a basic principle, it is designed to recognise and respond to differences
  - For pathogens it is easy to appreciate but this is also the reason why tissue transplants are not straight forward
  - Humans have subtle molecular differences
- As a mechanism of protection (of the species), it must be able to respond to something it has not yet encountered
- In each person, the immune system has the ability to remember past infections to deal with repeat infections - adaptive immunity
  - This is seen in the long-term protection achieved with vaccinations
  - This can be natural (infection) or man-made (vaccines) vaccination
  - It is also the reason why preformed long-term protection cannot be genetically passed onto the next generation - it is unique to each person



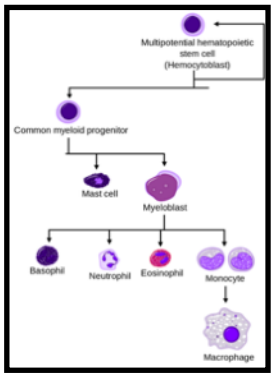
Cells of the immune system
- All **blood cells** are generated in the **bone marrow** within your bones
- The **haematopoietic stem cell** (**HSC**) found in the bone marrow is the source of these- about 1 in 100,000 bone marrow cells
- HSC have capacity to renew themselves
- And can differentiate into other cell types
- HSCs are the critical cells for successful bone marrow transplants

Cell lineages from the HSC
- Three key lineages that come from the HSC are:
  - **Lymphoid** - Cells of immune system
  - **Myeloid** - Cells of immune system
  - **Erythroid** - Red blood cells and platelets





Lymphoid compartment of immune cells
- From the common lymphoid progenitor we get:
  - Natural killer (**NK** cells)
  - T lymphocytes (**T** cells)
  - B lymphocytes (**B** cells)

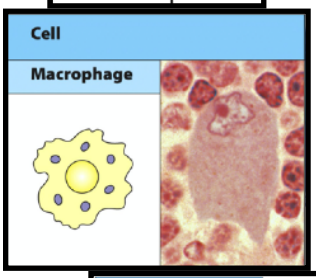


Myeloid compartment of immune cells
- From the common myeloid progenitor we get the:
  - Megakaryocytes (platelets)
  - Erythrocytes (Red blood cells)
  - Mast cells
  - Myeloblasts (precursor to more cells)
- From the myeoblasts we get the major **granulocytes**
  - Basophils
  - Neutrophils
  - Eosinophils
  - Monocytes (develop into macrophages in tissues)
  - Macrophages
- Dendritic cell
  - Key cell that has innate properties but critical for developing adaptive immunity
  - Evidence that DCs can develop from both lineages

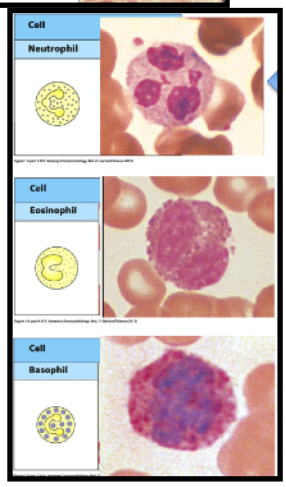
Some of these cells in more detail
- **Myeloid** cells are generally released directly into the **blood** as circulating immune cells
- **Maturation** of T and B lymphocytes involve some additional steps
- **T** cells mature in the **thymus**
- **B** cells mature in the **bone marrow**
- Both found in blood but also in lymphoid organs

Analysis of human blood	
Cell	size
erythrocyte (RBC)	6.5-8 um
Leukocytes (WBC)	
Lymphocyte	6-18 um
monocyte	12-20 um
Neutrophils	12-15 um
Eosinophils	12-15 um
basophils	12-15 um
platelets	2-4 um

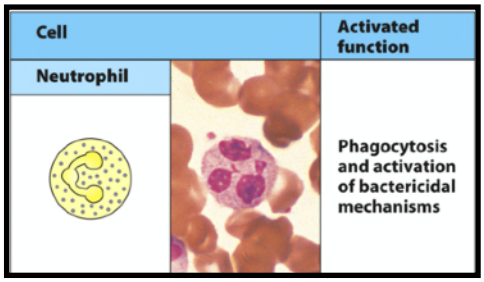
Size of lymphocytes
- Broad range in sizes
- Vary with state of activation and activity (enlarge when activated)
- Larger cells tend to be active cells



Monocyte/Macrophage
- Macrophages are the mature form of circulating monocytes
- Monocytes moved into tissue and differentiates into macrophages
  - This is a continuous process
- Macrophages present/reside in all tissues where they can respond to pathogens
- They are phagocytic cell type
  - Capable of engulfing and killing microorganisms
  - Scavenger cells clearing dead cells and debris
  - Can “process and present” antigens - more on this later

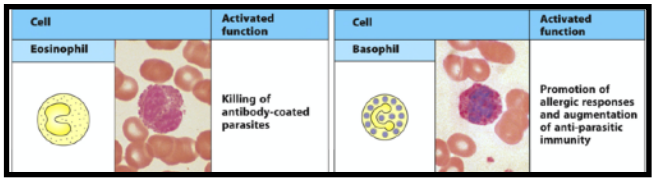


The Granulocytes - Neutrophils, Eosinophils, Basophils
- Look very carefully at these images
- Have intracellular granules that contain toxic material that can help kill microorganisms
- Neutrophils - one nucleus, multilobed (2-5 lobes)

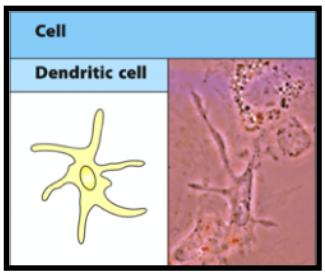


The Neutrophil
- Most numerous of the innate immune cells
- Has characteristic multi lobular nucleus when stained
- Key phagocytic cell of the innate immune response
- People with hereditary deficiencies in neutrophil function associated with overwhelming bacterial infections
- Only migrates into tissues from blood when required
  - Mainly found in blood
- Neutrophils = phagocytes only in circulation. Macrophages = phagocytes inside the tissues

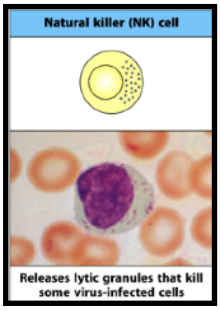
The Eosinophil and Basophil
- Much less abundant than neutrophils
- As granulocyte, granules contain enzymes and toxins to combat microbes
- Important function in combating parasites too large to ingeset-suicide killers
- Key cells associated with allergic responses
  - Histamine
  - Enzymes
  - Soluble immune signals

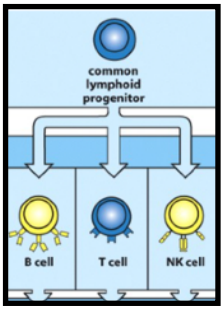


The dendritic cell
- Characterised by long dendrites
  - Increases surface area
- The key cell of the immune system that can ‘capture and present’ antigen
  - For generating adaptive immune responses
- A true phagocyte that can engulf and degrade microbes
  - Using phagocytosis
  - Also constant sampling of extracellular fluid and content via process called macropinocytosis
- They are generated in bone marrow, circulate in blood and enter tissues
- A number of different types of dendritic cells



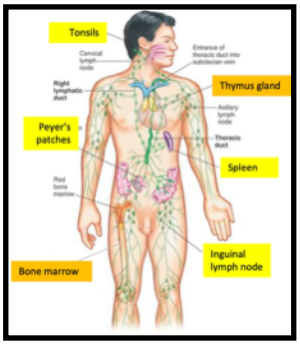
The lymphocytes
- Natural killer cells
  - important in killing of abnormal cells such as tumor cells or cells that may be infected with virus - i.e. stressed cell
- T and B lymphocytes
  - Key cells of the adaptive immune response
  - Have specificity for target which is defined by specific cell surface receptors
  - Their development, activation and effector function will be a main topic later

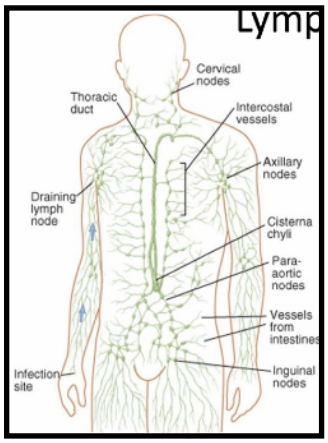




There is 5*10^10 white blood cells in 5 litres of blood

Structure of the immune system - the network of the immune system
Circulation
- Cells don’t randomly move through the body’s tissue
- With any destination, there needs to be a way of getting there or close
  - eg. Road system for vehicles
- Body’s two main circulation systems:
- Blood circulation
  - Movement of oxygen, nutrients and cells to tissues
- Lymphatics
  - Movement of fluid and cells **from** tissues
  - Key feature of lymphatics is the presence of lymphoid organs



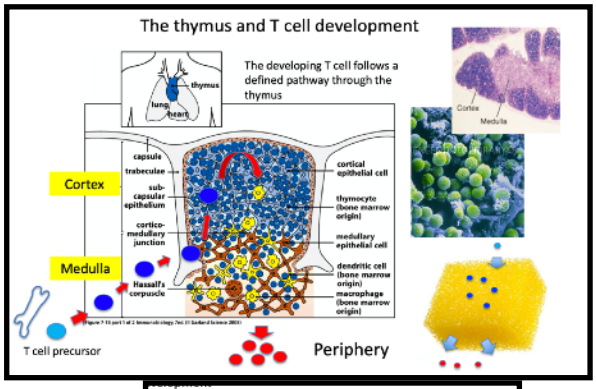


Lymphatic system
- Necessary for maintaining homeostasis of fluid in the tissues
  - Absorbs and drains fluid between cells that has leaked out from the blood vessel
- Lymph fluid is clear(ish) and doesn't contain red blood cells
- Starts as small capillary structures that converge into larger vessels that become the **afferent lymph vessels** that drain **into** the lymph node
  - These are separate to the vessels in which blood flows
  - Lymphatic vessels also have valves that prevent back flow
  - Muscle and thoracic movement provides the pressure to move the fluid in the absence of a pump
- **Efferent vessels** ultimately become the large lymphatic vessel called the **thoracic duct** which drains **into** the **superior vena cava** and into the **blood**

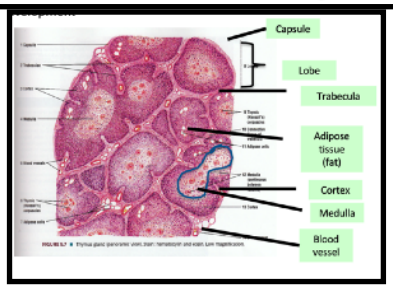
Lymphatics to lymph nodes
- In the context of immunity, lymphatics and lymph nodes are important in the generation of an adaptive immune response because they enable the transport of pathogens from distant sites to lymph nodes to enable T and B cells to be selected

Lymphoid organs
- **Primary** lymphoid organs
  - Where immune cells are **made** and/or **mature**: Bone Marrow & Thymus
- **Secondary** lymphoid organs
  - Where immune responses are **generated**:
  - Spleen
  - Peripheral lymph nodes: Tonsils, Peyer's patch (prevent growth of bacteria in intestines), Inguinal lymph node, all other lymph nodes

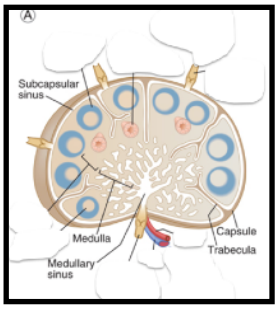
A quick tour of some key immune organs
- A thing to remember about the immune organs is that they are not just bags full of cells
- They have defined structures to provide a physical environment
- They will often have specialised cell types within these that will perform a specific function
  - eg. The thymus is there to make/mature T cells
- The different types of cells that are often found in these organs can be localised to specific areas
  - i.e. they are not randomly distributed within the organs or compartments



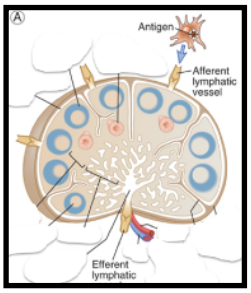
The thymus and T cell development:
- Massive proliferation from T cell precursor
- Within the thymus T cell precursor mature to be released
- This is highly organised process with a defined process- later lecture/lesson on T cell

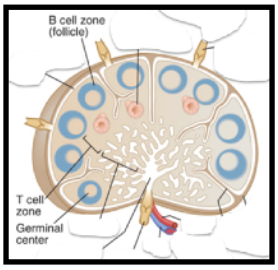


The lymph node:
Structural components
- Outer Capsule
- Trabecula (give structure/rigidity)
- Subcapsular sinus (sinus = cavity)
  - Cavity that is under capsule which allows cells to dissipate through the lymph node (increase efficiency as higher surface area)
- Medulla (inner region)
- Medullary sinus

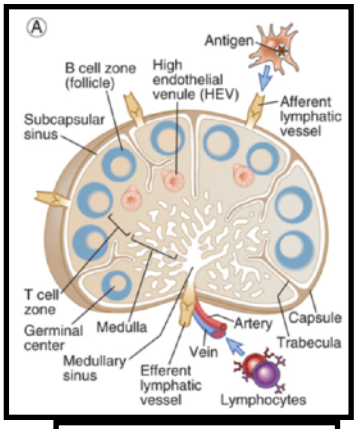


Lymph- Antigen Pathway
- **A**fferent lymphatic vessel
  - **Into** the lymph node
- **E**fferent lymphatic vessel (only 1)
  - **Out** of the lymph node (**E**xit)
- **A** before **E**



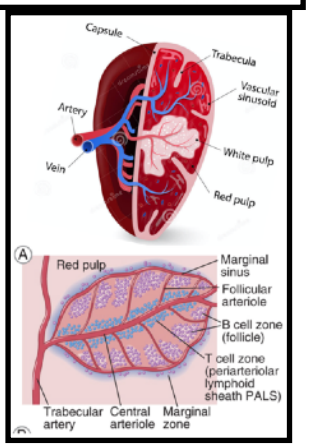


Cellular regions
- T cell zone
- B cell zone
- Germinal centre
  - Where further maturation of the B cell response occurs once the B cells are activated to make antibody

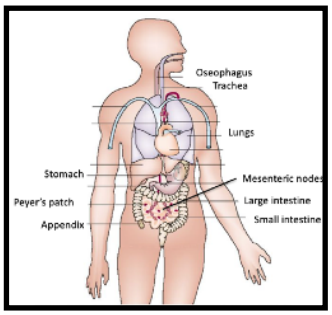


The lymph node structure summary:
- High endothelial venules (HEVs) are blood vessels especially adapted for lymphocyte trafficking

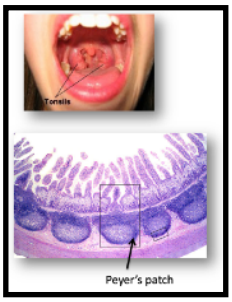
The spleen
- Consider the spleen as the lymph node that deal with antigens in the blood
- Not associated with lymphatics - just blood
- As with tissue lymph node it is where antigen presenting cells (dendritic cells), T and B cells get together to initiate immune response
- There are regions enriched in specific cell types
  - White pulp - lymphocytes
  - Red pulp - red blood cells
- Note clustering of T and B cells into specific regions to sample blood born material



Mucosal immune system
- Pathogens/toxins often enter our bodies across mucosal surfaces
- Surface areas:
  - Skin - 2 m^2
  - Lung - 140 m^2
  - G.I. -200 m^2
- Thus, a very large commitment of lymphocytes is needed to protect these surfaces



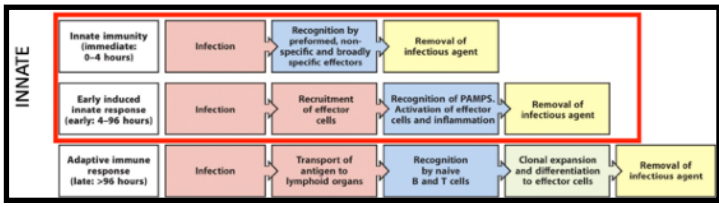
The mucosal immune system
- Immune system that is associated with the respiratory and gastrointestinal tracts
- These routes are open to the environment and so also need to be able to participate in immunity to pathogens through these entry points
- Set up is similar to other lymphoid tissues with collection of lymphoid cells and serves same purpose
- Two examples are tonsils (neck) and Peyer's patches (gut)



Innate Immunity I

Innate

- Key features and mechanisms associated with innate immune response
- Physical mechanisms
- Cellular mechanisms
- Soluble mechanisms



Cytokines

- Introduction to the key method of communication used by the immune system
- Reference and examples of different cytokines will be peppered throughout the semesters

Migration

- Movement of cells across vessels into tissues

Physical/anatomical or natural barriers:

Skin, gastrointestinal tract and respiratory tract

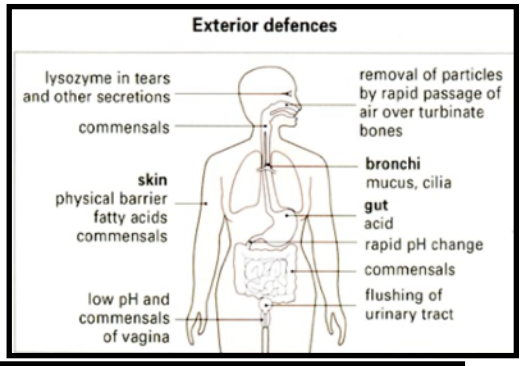
- Importance of these layers becomes obvious once they are breached
- Burns, puncture wounds

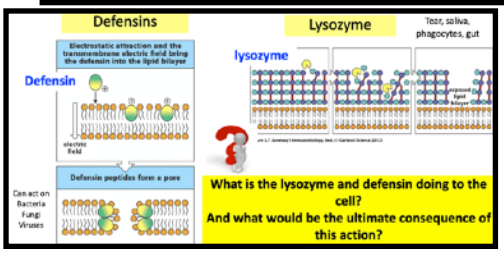
Protection can be a simple physical barrier

- Skin, mucosal walls, secreted mucus, air flow (cough, sneeze) or flushing

Also production of antimicrobial agents or environments have evolved

- Acid and enzymes in gut
- Enzymes in tears
- Lysozyme and phospholipase A2
- Antimicrobial peptides
- Defensins





Actions of **Defensins** and **Lysozymes**:

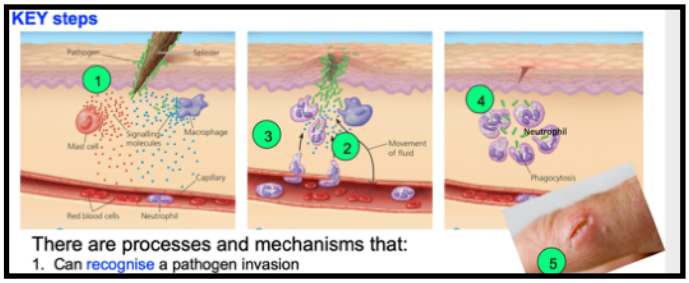
Both pathogen or cell damage can trigger an innate cellular response

- Pathogen derived
  - Highly conserved structures from pathogens
- Non-pathogen associated
  - Cell derived molecules related from damaged/dying cells
- Just a cut can trigger inflammation which is utilising these innate immune cells

Pathogens - What if they do get in?

There are processes and mechanisms that:

1. Can **recognise** a pathogen invasion
2. **Respond** to that invasion
3. Send out **signals** to **recruit assistance** (other immune cells) to help deal with the invasion
4. **Deal** with invasion to eliminate pathogen through various mechanisms
5. This scenario would also be an example of **INFLAMMATION**



Pathogen **A**ssociated **M**olecular **P**atterns (PAMPS) - structures on pathogens that innate cell recognise

- A key to innate immunity is recognising structures on pathogens that are only found in/on pathogens
- These are called Pathogen Associated Molecular Patterns (PAMPS) - such as
  - Flagellin

- Lipopolysaccaride
- dsRNA
- Many more

Pathogen Associated Molecular Patterns (PAMPS)
- Innate immune cells can recognise structures that may be shared by many different microbes

Nucleic acid
- **RNA**: double stranded RNA and 5'triphosphate RNA - feature of many virus and bacteria but either not found, or not cytosolic, in mammalian cells
- **DNA**: unmethylated CpG nucleotides - feature of microbial DNA but not mammalian DNA

Microbial surface structures
- Surface **glycoproteins** (**GP**) and **lipoproteins** (**LP**) - viruses and gram positive bacteria have it
- Membrane components
  - All bacteria: **peptidoglycans** (**PG**) (amino acid and sugar polymer network)
  - Gram positive bacteria: **lipoteichoic acid** (**LTA**)
  - Gram negative bacteria: **lipopolysaccharide** (**LPS**) and **flagellin**
  - Fungi: β-glycans, mannans, zymosan. (different forms of sugars)
- Individual classes of pathogens may have a range of different PAMPS
- Some PAMPS may be specific to a class of pathogen
- Some specific PAMPS may be found in more than one type of pathogen

And then how do WE recognise THEM?
- What is the interaction that allows recognition to occur?
- **P**attern **R**ecognition **R**eceptors (**PRRs**)

Families of Pattern Recognition Receptors (PRRs)

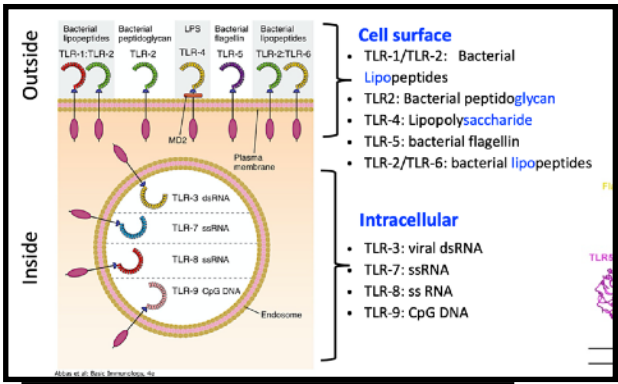
Toll like receptors (**TLRs**)
- Large family of proteins found on surface and intracellularly
- Recognise PAMPS
- Location of individual TLR give specificity to what is recognised
- Leads to activation of cell

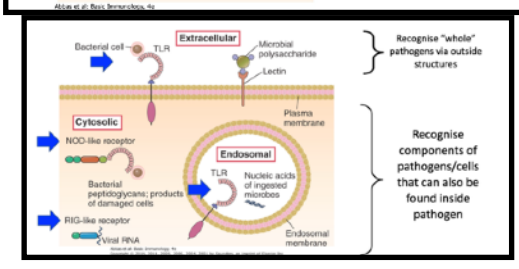
NOD-like receptors (**NLRs**)
- Family of proteins found intracellularly
- Recognise PAMPS and DAMPS (damage associated molecular patterns)
- Leads to activation of cell and specific inflammatory response

RIG-like receptors (**RLRs**)
- RIG = Retinoic acid-Inducible Gene
- Found intracellularly
- Viral RNA
- Leads to activation of cell and specific anti-viral responses

Other PRRs
- C-type lectin receptors: fungi
  - Lectin is a form of sugar molecule
- Intracellular DNA sensors: wide variety of PAMPs. AIM2 (inflammasome component) most widely studied

The Toll Like Receptors
- 10 TLR members in humans
- First identified in fruit fly as molecule that was important in protection against fungi
- While they all are similar in general structure they have different specificity in what they bind to
  - eg. TLR 3 binds to dsRNA
  - TLR 4 binds to LPS
  - TLR 5 binds to flagellin





- Normally found as dimers (two molecules)

Why these 3 main PAMPS?

- An important feature of PAMPS is that they are often **important** for the **survival** of the pathogen as a **species**
- Pathogens cannot easily evolve away from these PAMPS and thus provide a target for our cells to evolve a receptor to recognise them

Danger **A**ssociate **M**olecular **P**atterns (DAMPs)

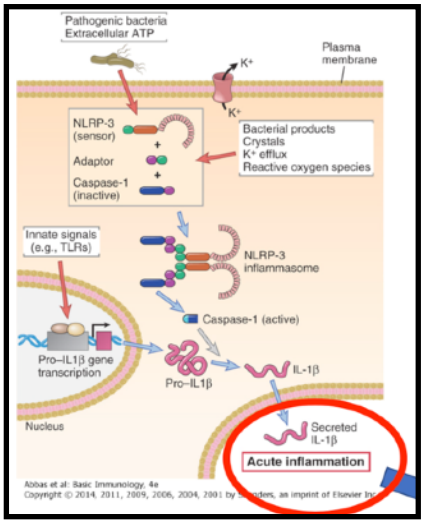
- Non-pathogen associated
- Cell derived molecules released from damaged/dying cells

NOD-like Receptors and the Inflammasome

- The concept of clearing pathogens and dead cellular material is similar
- Similar cells can be involved
- This needs to be triggered by signals rather than be active all the time
- NLRs respond to microbial products (PAMPs) **and also** products generated by cell damage/ death (DAMPs)
- Important molecules DAMPs that activate NLRs are:
  - ATP
  - Uric acid crystals (associated with gout)
  - Intracellular ion concentrations (eg. K)
  - Various cellular substances (cholesterol, free fatty acids
- NLR activation leads to the formation of an **inflammasome**

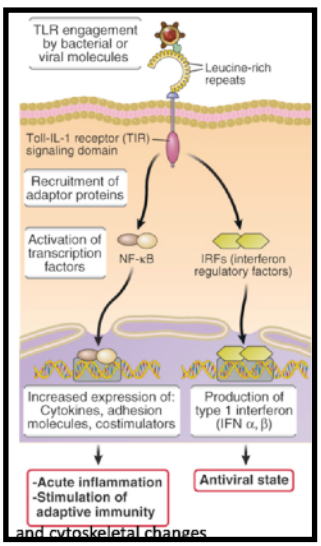
What is the inflammasome?

- Large **intracellular multiprotein complex** made up of **receptors** (eg. NLRs), **adaptor proteins** (eg ASC proteins) and **pro-caspase 1** (enzyme that catalyses cytokine creation reaction)
- Potent activator of **inflammation**
  - Cytokines **IL-1β and IL-18** production



The NLRP-3 inflammasome (to promote inflammation)

- The process of clearing pathogens and dead cellular material is similar and involves immune cells
- Recruitment of cells to site is the basis of inflammation
- This needs to be triggered by signals rather than being active all the time
- **Triggered** by:
  - Microbe products
  - Agents associated with cellular damage
  - Reactive oxygen species
- Result in activation of **inflammatory response** or **cell death**
- Generates an inflammatory signal that draws more immune cells to the site to help clear up whatever is causing the issue - pathogen or damage tissue
- The cytokine IL-1 is good at promoting inflammation



The surface triggering of receptors (eg PRR) will lead to a range of intracellular actions

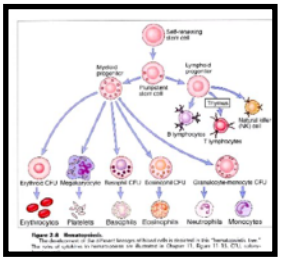
- Intracellular pathways that involve a range of “adaptor” proteins that get signal from surface to **transcription factors**
- Specific transcription factors can bind to genes to **turn** them **on** (or **off**)
- Range of cellular responses
  - Structural
  - Soluble

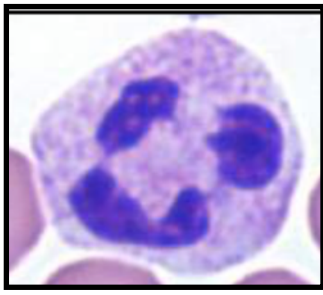
Innate Immunity II

Some effector mechanisms of innate cells

- **Phagocytosis**
  - Macrophages
  - Neutrophils
- **Degranulation**
  - Eosinophils
  - Basophils
  - Mast cells
- **Direct action** on infected host cells (our cells)
  - Natural killer cells

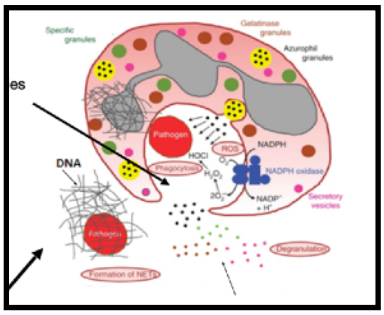
Neutrophil





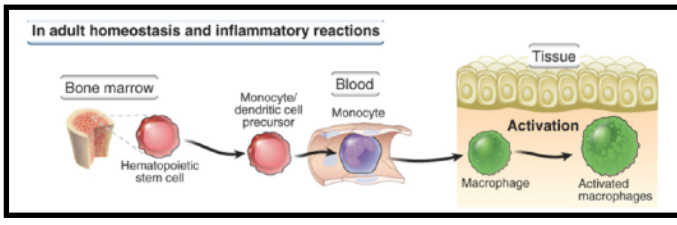
- Polymorphonuclear: multi-lobed nucleus
- Most abundant leukocyte (white blood cell) in the blood
  - 4000-10,000 per microlitre
  - Not normally found in tissues
  - Migrate to tissues in response to signal of microbial invasion
- One of the first cells to respond (Acute)
- Highly mobile
- Respond rapidly to chemoattractant signals at sight of infection/damage —> chemotaxis
- Production of neutrophils increases in response to infection
  - Up to 20,000 per microlitre —> increase in neutrophil count is sign of infection, as the body responds to the infection
- Action is rapid and neutrophils die after few hours

Neutrophils: effector functions

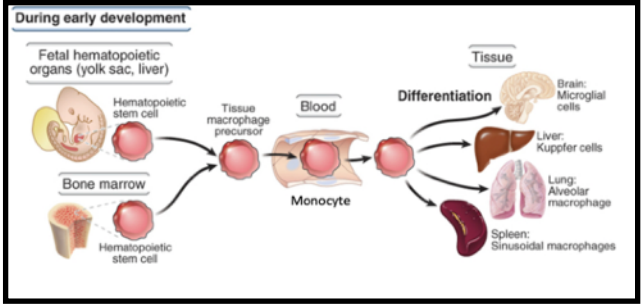


1. **Phagolysosome**
   - Reactive oxygen species
2. **Degranulation**
   - Release of granules with cytotoxic, anti-microbial molecules
   - Reactive oxygen species
   - Nitric oxide
   - Lysosomal enzymes
3. **Neutrophil extracellular traps** (NETs)
   - Web of chromatin and serine proteases (break down proteins)
   - Concentrates anti-microbial molecules on pathogen
   - Physically traps pathogen

Monocyte/Macrophage



- Less abundant than neutrophils
  - 500-1000 per micro litre
- Can normally be found in tissues as resident macrophages
  - Will often be known with different name in some tissues
- During infection, immature monocytes migrate from the blood stream to the site of infection (**chemotaxis**) where they can differentiate into macrophages following activation
- Unlike neutrophils, macrophages are long lived (weeks)
  - First encounter with macrophage will set off cascade of events designed to eliminate microbe —> phagocytosis and… send signals (cytokines) to immune system that breach has occurred
  - To recruit more immune cells to help - inflammation

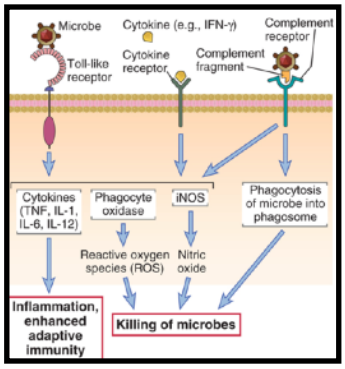


Mononuclear phagocytes in other tissues

- Within different tissues these cells will reside and have been given specific name to identify their location

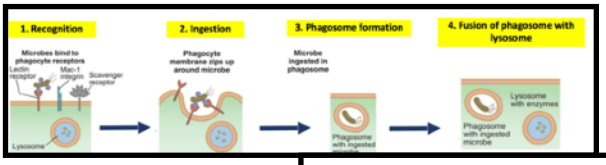
Macrophages: effector functions

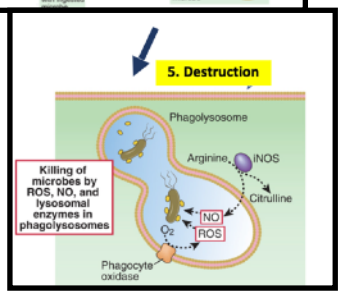
1. Major **cytokine** and **chemokine** producers
   - At sight of infection eg:
   - Tumour necrosis factor alpha (TNF-alpha)
   - IL-1
   - CXCL8 (important chemokine)
   - Activates both innate and adaptive immune responses
   - Signals to local endothelial cells: increased expression of adhesion molecules and increase immune cell migration
2. **Phagocytosis**



Phagocytosis

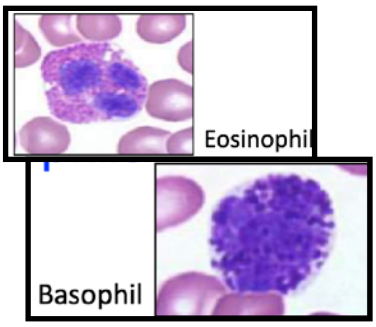
1. Recognition
2. Ingestion
3. Phagosome formation
4. Fusion of phagosome with lysosome
5. Destruction





- Microbe becomes enclosed in a membrane component called a **phagosome**
- Phagosome fuse with lysosomes to produced **phagolysosome**
  -where all the action takes place
- **Phagolysosome** is **activated** which switches on a number of **enzymes** to make **destructive molecules**
- Phagocyte oxidase
  - Converets oxygen to superoxide and oxygen radicals
- Inducible nitric oxide synthase - produces nitric oxide
- Lysosomal proteases - attacks microbial proteins

Eosinophils and Basophils



- Much less abundant than other cells in the blood
- Roles are mainly associated with dealing with parasites and worms - things that are too big to be phagocytosed
- Eosinophils are also key cell associated with allergic reactions where their activation can cause tissue damage
- Part of the granulocytes, like neutrophils
- Effector function: **Degranulation**
  - Release of granules with cytotoxic, anti-microbial molecules
  - Includes range of molecules including histamine, heparin, lysosomal enzymes
- Eosinophils release their cytotoxic granules which destroy very large parasites

Mast cells

- Located in the skin and mucosal epithelium (lungs, gut)
- Can respond to microbes via TLRs
- Important for protection against helminths (parasites)
- Effector function:
- Mast cells are granulocytes: **degranulation**
  - Histamine - vasoactive
  - Proteolytic enzymes - disrupt proteins
- Also secret pro-inflammatory mediators to attract leukocytes
  - Prostaglandins
  - Cytokines