

Innate immunity

Course	 Immunology
Date	@April 7, 2024
Type	Lecture
Status	Completed
Reading	<input type="checkbox"/>

Innate immunity

- Rapid initial response (within hours)
 - Not necessary to cell proliferation
- Non-specific - broad
 - Recognise proteins that are shared between multiple organisms
- Do not vary in intensity depending on how many times the same pathogen is encountered
- Antimicrobial peptides
- Phagocytes
- NK cells
- Interferons
- Complement system
- Acute inflammatory response

Adaptive immunity

- Develops through exposure to pathogen
- Slow primary response (days)
 - Require time to proliferate
- Rapid memory response
 - Adaptive (acquired) responses are more vigorous upon re-encounter with the pathogen
- Specific
 - Only recognise one protein
- B and T cells drive effector responses to pathogens

Innate and immunity and adaptive immunity - Communication and cooperation

Host defences

Anatomical and chemical barriers (skin, mucus, epithelium) - Intrinsic - innate immunity - acquired immunity

- Sequential barrier

External defences - Anatomical and chemical barriers

- To enter the body, an infectious organism such as a bacterium initially has to pass the external defences which include the physical barrier of the **skin** and **internal epithelial layers** lining the major tracts of the body.
 - Protection at these surfaces is mediated by a variety of secretions.
 - Anywhere where exposed to outside environment is a barrier tissue

Site	Source	Specific substance secreted
Eyes	Lacrimal glands (tears)	Lysozyme
Ears	Sebaceous glands	Waxy secretion - cerumen 耳垢
Mouth	Salivary glands (saliva)	Lysozyme, proteases
Skin	Sweat glands (sweat) and sebaceous glands 皮脂腺 (sebum)	Lysozyme, high NaCl, lactic acid, fatty acids
Stomach	Gastric juices	Digestive enzymes, acid - low pH~2

Soluble mechanisms of innate immunity - preformed and react immediately

- Skin, mucus, ears consist of preformed soluble molecules (antimicrobials)
 - Antimicrobial enzymes (Lysozyme)
 - Antimicrobial proteins (defensins, cathelicidins, histatins)
 - Complement
 - Cytokines
 - Acute phase proteins

	Skin	Gut	Lungs	Eyes/noses/oral cavity
Cell types	Stratified epithelium	Single cell layer of columnar	Upper airway: pseudo	Pseudostratified columnar

	Skin	Gut	Lungs	Eyes/noses/oral cavity
		epithelium	stratified columnar epithelium Lower airway: single cell layer of columnar epithelium	epithelium
Mechanical - Epithelial cells joined by tight junctions	Longitudinal flow of air or fluid	Longitudinal flow of air or fluid	Movement of mucus by cilia	Tears/Nasal cilia
Chemical	Fatty acid; Beta-defensins, lamellar bodies, Cathelicidin	alpha-defensins (cryptidins), RegIII, (lecticidins), Cathelicidin	alpha-defensins, Cathelicidin	Histatins, beta-defensins

- If these defences are breached the cells and molecules of the immune system are encountered leading to recognition of the foreign organism and their disposal.

Defensins

- Nature's antibiotics: Kill bacteria, fungi and some viruses
 - Insert into the membrane of pathogen and cause loss of small-molecule gradients
- Small cationic antimicrobial peptides
 - Because of the positive charges, it sticks to the bacteria membrane (which is negative)
- Widely expressed (leukocytes and epithelium)
- Intracellular & secreted
 - E.g. Vesicles in neutrophils, and also epithelial cells in the gut
- Two main structural families
 - alpha-defensins: Constitutively expressed - can act immediately, preformed

- beta-defensins: Some constitutively expressed, others induced e.g. by LPS, i.e. more expressed upon infections

Through the secretion of soluble mediators innate immune responses typically leads to local changes that promote recruitment of immune cells to that site leading to amplification of the immune response and to **inflammation**.

Certain cytokines, such as IL-1, IL-6 and TNF, released by innate cells in response to infection can act on the liver to increase the synthesis and secretion of a number of plasma proteins collectively termed **acute phase proteins**.

- These include **C-reactive protein** (CRP, the plasma concentration of which may increase 1000-fold during infection), fibrinogen and various **complement** components including C3.
- Overall, the acute phase response achieves a beneficial effect through enhancing host resistance, minimising tissue injury and promoting the resolution and repair of the inflammatory lesion.

Cellular responses in innate immunity

- Inputs: "danger"
 - E.g. sentinel cells in the skin recognise the pattern of pathogen
- Outputs:
 - Response to threat, e.g. release soluble mediators
 - Communication to other immune cells

Recognition of pathogens by the immune system

Innate	Adaptive
<ul style="list-style-type: none"> • Structure shared by classes of microbes • About 1000 molecules can be recognised • Receptors germ-line encoded (limited diversity) <ul style="list-style-type: none"> ◦ No generation of new receptors, same as born 	<ul style="list-style-type: none"> • Specific epitopes • $>10E7$ molecules can be recognised • Receptor genes undergo somatic recombination (huge diversity) • Type of receptors: 2 (TCR & antibody)

- Types of receptors: <100
 - A small number of receptors recognises all different pathogens

Pathogen recognition

- Immune system must distinguish pathogenic infectious agents from commensals, self antigens, and environmental antigens
- Intracellular, cell surface and secreted (soluble) **pattern recognition receptors** (PRRs)
- PRR recognise **pathogen-associated molecular patterns** (PAMPs) and host **damage-associated molecular patterns** (DAMPs)
 - PAMPs are typically structural components of microbial organisms, such as a component of the cell wall of bacteria or the envelope of viruses, or they are derived from the microbial nucleic acid.
 - These motifs are typically conserved molecular structures that are shared by many different microbial organisms but not expressed by the host
 - Thus very few receptors can be used to 'recognise' diverse microbial organisms
 - DAMPs might be due to cell death or pathogen induced, e.g. mitochondrial DNA outside of cells
- Engagement of the PRR generates a signal which alerts the cell to the presence of an infection and initiates cellular activation

PAMPs

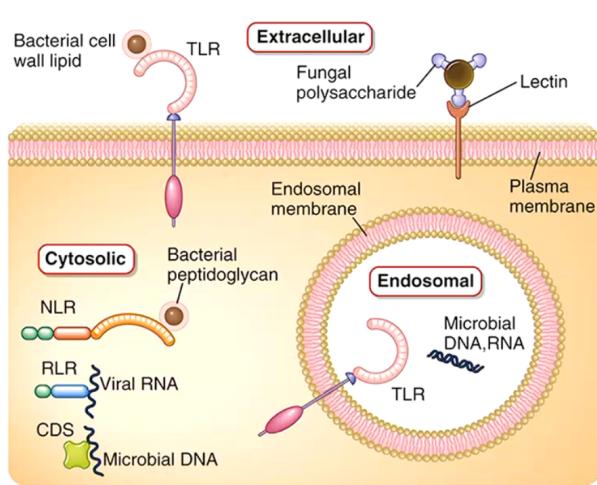
- LPS (lipopolysaccharides): outer membrane of Gram negative bacteria
- Peptidoglycan: major structural polymer of bacteria
- Lipoteichoic acid: surface-associated adhesion amphiphile, Gram+

DAMPs

- Uric acid crystals
- HSP70: released from stressed / damaged cells
- RNA/DNA
 - Nucleic acid not in nucleus → cell rupture
- Serum Amyloid A (SAA)

- dsRNA: double stranded RNA - virus
- ssRNA: virus
- Flagellin: structural protein of flagellum
- beta-Glycan
- Nuclear proteins (High Mobility Group Box-1 (HMGB)

PRR

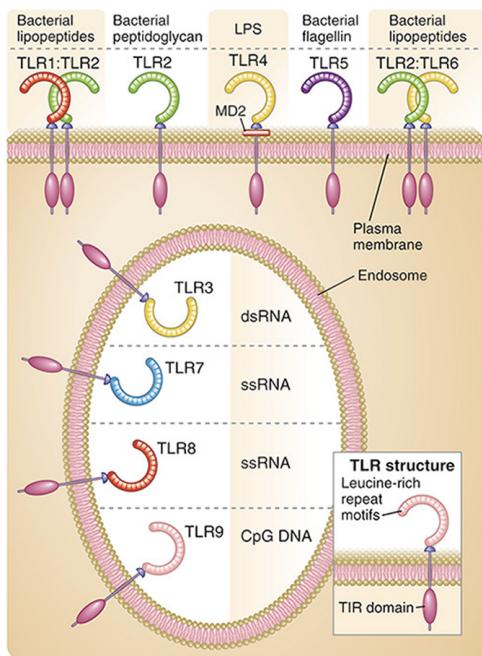


TLR: recognise LPS & peptydoglycan (membrane), viral nucleic acid (endosomal)

Lectin: bind multivalently to exposed microbial surface sugars

NLR

- Soluble molecules: pentraxins (CRP), collectins (mannose binding lectin), ficolins, complement proteins
 - Recognise LPS, or terminal sugars on microbial cell wall or surfaces
- Cell associated molecules
 - Cell surface: C-type lectins, some toll-like receptors (TLR 2,4,5), scavenger receptors on phagocytes
 - Endosome/phagosome: some TLR (3,7,8,9)
 - Cytosolic: NOD like receptors (NLR), RIG-like receptors, c-GAS
 - Intracellular recognition such as viral or bacterial DNA



- TLR family
 - Recognise PAMPs and trigger a signalling cascade which leads to the translocation of **NF κ B** to the nucleus and ultimately the synthesis of **pro-inflammatory cytokines**.
- Membrane
 - TLR1&2: Bacterial lipopeptides
 - TLR2: Bacterial peptidoglycan
 - **TLR4:** Gram-negative bacterial **LPS (endotoxin)**
 - **TLR5** which detects bacterial **flagellin**
 - TLR2&6: Bacterial lipopeptides
- Endosome
 - **TLR3:** viral **dsRNA**
 - TLR7: viral **ssRNA**
 - TLR8: **ssRNA**
 - TLR9: **CpG DNA**

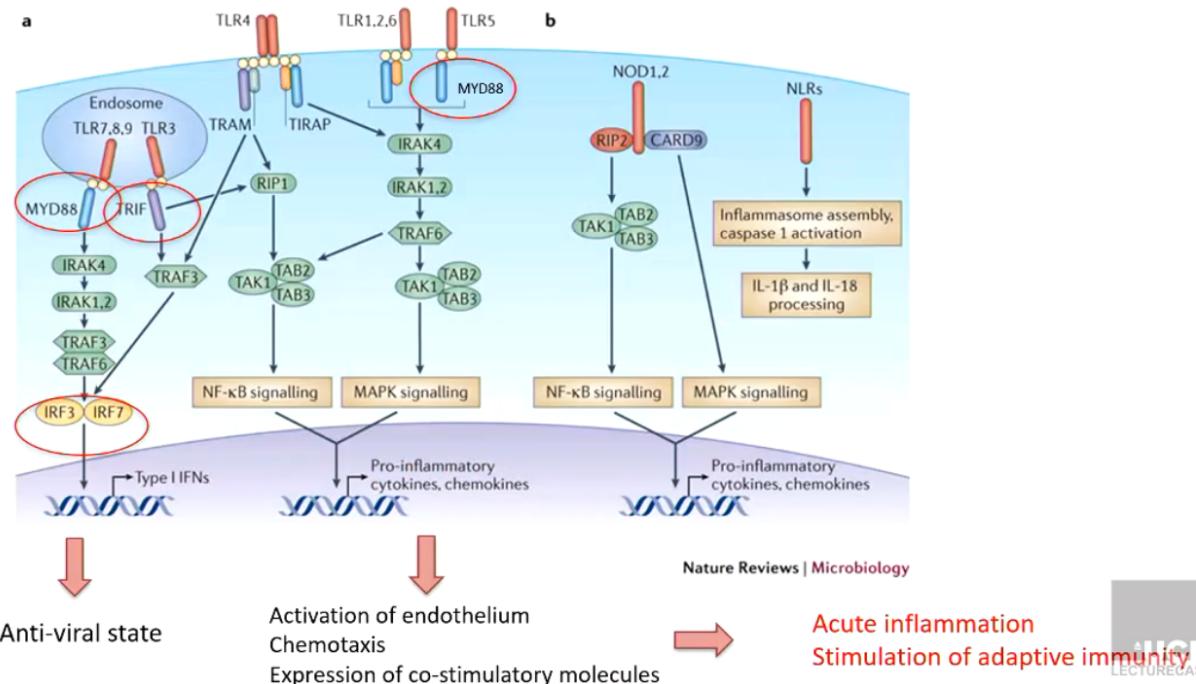
PRR	location	examples	Ligands (PAMP/DAMP)
Soluble			
pentraxins	plasma	C-reactive protein	
collectins	plasma	Mannose binding lectin	Carbohydrates with terminal mannose
ficolins		ficolin	Cell wall components, gram+ bacteria
Cell associated (membrane)			
TLRs	Plasma membrane or endosomal membrane (immune and non-immune cells)	TLR 1-9	Bacterial LPS, peptidoglycans, viral nucleic acids
C-lectin receptors	Plasma membrane of phagocytes	Mannose receptor, dectin 1, dectin 2	Microbial surface carbohydrates with terminal mannose. Glucans from bacterial or fungal cell walls
Scavenger receptors	Plasma membrane of phagocytes	CD36	Microbial diacylglycerides
Cell associated (cytosol)			
Nod-like receptors (NLR)	Cytosol of phagocytes, epithelial cells	NOD1/NOD2 NLRP family (inflammasomes)	Bacterial cell wall peptidoglycans Intracellular damage: (urate crystals, ATP, lysosomal damage)
RLG like receptors (RLR)	Cytosol of phagocytes and other cells	RIG-1, MDA	Viral RNA
Cytosolic DNA sensors	Cytosol of many cell types	AIM2, c-GAS, other sensors activating STING	Bacterial and viral DNA

Cytosolic receptors for PAMPs and DAMPs

- Important for pathogens that found in the cytosol or damage to host cells
- 3 major classes
 - NOD like receptors
 - NOD1/2 bacterial cell wall (peptidoglycan)
 - NLRP3 forms part of inflammasome (bacterial products, DAMPs: ATP, crystals, ROS)
 - Inflammasomes activate inactive proteins rather than having transcription and translation of new proteins
 - RIG like receptors
 - Viral RNA (RIG I, MDA5)
 - Cytosolic DNA sensors
 - Microbial DNA in cytoplasm (C-GAS/STING pathway)

PRR: signalling

- Key inflammatory cytokines are driven by the PRR



Upon recognition, **extracellular microorganisms** can be engulfed by **phagocytic cells** whereas **intracellular organisms** are dealt with by **cytotoxic T-cells** (adaptive) or by **NK cells** (classically considered 'innate').

Intrinsic immunity

- Always present in the uninfected cells (part of the normal cellular processes, can act very early during viral infection)
- Apoptosis, autophagy (prevent virus from spreading), RNA silencing, antiviral proteins
- Cellular proteins that inhibit viral replication and represent a **first line of defense** against viral pathogens (TRIM5, APOBEC3, Tetherin)
 - Interfering with processes of viral spreading
- Sometimes called "restriction factors"

Cells of innate immunity are involved in Phagocytosis, inflammation, cytotoxicity

- Macrophages
- Neutrophils
- Eosinophils

- Basophils
- Mast cells
- Dendritic cells
- NK cells

Phagocytosis

Phagocytosis is a process by which certain cells of the innate response (phagocytic cells: namely **monocytes/macrophages/dendritic cells** and **neutrophils**) engulf foreign organisms.

It brings the engulfed pathogens into contact with microbicidal molecules, many of which are packaged as granules inside structures called **lysosomes**.

Before phagocytosis can occur, the microbe must first **adhere** to the surface of the phagocytic cell.

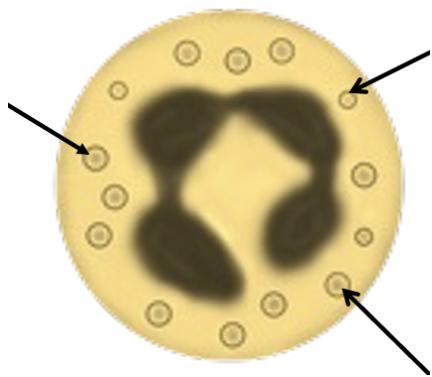
The resulting signal initiates the ingestion phase by activating an actin–myosin contractile system, which results in pseudopodia being extended around the particle.

The pseudopodia eventually fuse to form a vacuole called a phagosome.

The lysosomes of the phagocytic cell then fuse with the phagosome and discharge their microbicidal granules (phagolysosome) .

Neutrophils: granules - killing mechanisms

Different types of granules with different molecules that are antimicrobial peptides crucial in killing mechanisms



- Primary granules (azurophilic)
 - Myeloperoxide
 - Elastin
 - Lysozyme
 - Defensins
- Secondary granules (specific)
 - Lysozyme
 - Lactoferrin
 - Collagenase
- Polymorphic nuclear: multiple bulbs

- Really quick and efficient in phagocytosis
- Tertiary granules (secretory)
 - Gelatinase
 - MMP9
 - Cathepsins
- Phagolysosome
 - NADPH
 - H₂O₂ (ROS)
 - HOCl⁻

Monocytes / Macrophages



- Phagocytosis
 - Important in homeostasis removing dead cells and keeping tissue healthy
- Pro-inflammatory cytokines (TNF-α, IL-1, IL-6)
 - Local effects - allow cells to come into the local tissue
 - Systemic effects (liver, bone marrow, brain) - spread through circulation and cause acute phase response

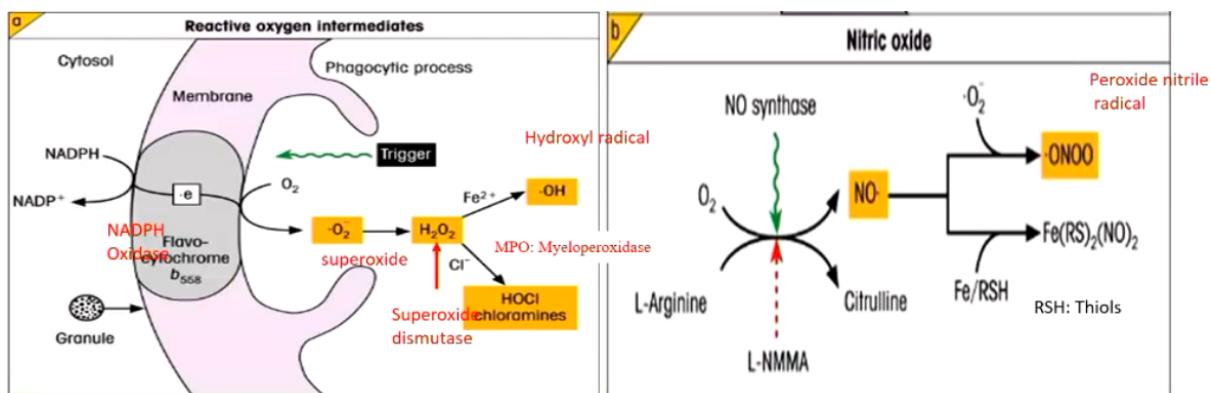
Tissue resident - sentinel cells - raising the alarm when detect pathogens and cytokines will recruit other cells

Phagocytosis and killing of a bacterium (neutrophils and macrophages)

- Recognition and attachment
 - Pathogen (microbes) bind to phagocyte receptors
 - The pattern (PAMP) get recognised
- Engulfment

- Phagocyte membrane zips up around microbe and form a phagosome with ingested microbe
- If in macrophages, it will fuse with a lysosome to form phagolysosome
- Degradation of microbes by lysosomal enzymes in phagolysosome
 - Killing of microbes by ROS and NO
- If in neutrophils different types of granules fuse with phagosome and digest the pathogen

Microbial mechanisms of phagocytic cells



Reactive oxygen pathway - more dominate in **neutrophils**

- There is a dramatic increase in activity of the hexose monophosphate shunt generating NADP⁺, the reduced form of NADPH.
- Electrons pass from the NADPH to the plasma membrane cytochrome ($cytb558$), which reduces molecular oxygen directly to **superoxide anion**.
- The superoxide anion undergoes conversion to **hydrogen peroxide** under the influence of **superoxide dismutase**, and subsequently to **hydroxyl radicals $\cdot OH$** .
 - Each of these products are formidable microbial agents; $\cdot OH$ in particular is one of the most reactive free radicals known.
- Furthermore, the combination of **hydrogen peroxide, myeloperoxidase (MPO)** and **halide ions** constitutes a potent halogenating system capable of killing both bacteria and viruses.

Other killing mechanisms include **nitric oxide** formed by inducible NO synthase (iNOS) - more dominant in **macrophages**

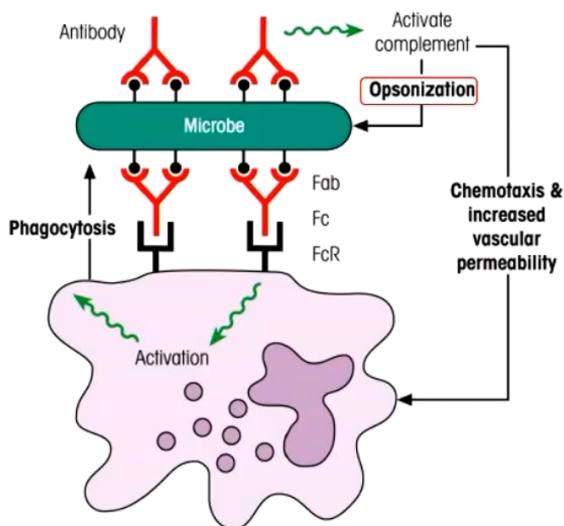
- Nitric oxide by NO synthase and converted to peroxide nitrile radicals

Oxygen-independent mechanisms

C Oxygen-independent mechanisms	
Cathepsin G Low mol. wt defensins High mol. wt cationic proteins Bactericidal permeability Increasing protein (BPI)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall
Lactoferrin	Complex with iron
Proteolytic enzymes Variety of other hydrolytic enzymes	Digestion of killed organisms

- The pathogens are also subjected to a family of cationic peptides called **defensins** that reach very high levels within the phagosome and act as disinfectants against a wide variety of bacteria, fungi and enveloped viruses.
- Further damage is inflicted on the bacterial membranes by **neutral proteinase (cathepsin G)** and by the bactericidal **lysozyme** and bacteriostatic **lactoferrin**.
- Finally, the killed organisms are digested by hydrolytic enzymes and the degradation products released to the exterior.

Antibody and complement help phagocytosis



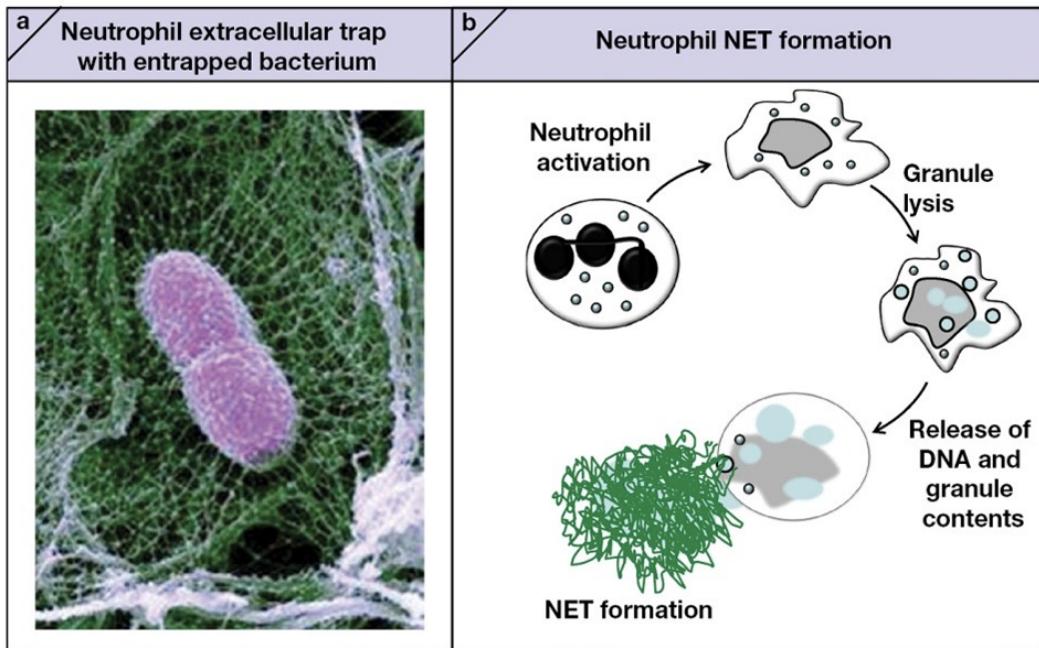
- Opsonisation of microbes by antibody increases the efficiency of phagocytosis
 - If the microbe is re-encountered
 - Antibodies have FC regions which can bind to FC receptors on phagocytes such as neutrophils
- An example of cooperation between innate and adaptive immunity
- A neutrophil takes up a microbe in 30 seconds

Phagocytosis

- Ingestion and killing of extracellular microbes
- Phagocytes: Neutrophils, monocytes, macrophages
- Binding via PRR
- Respiratory bursts: -O₂-dependent killing
- Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated
- Neutrophils are first cells respond to infection - rapid migration from blood into infected tissues
- Monocytes enter tissue - become macrophages
 - If more phagocytes are required, more monocytes migrate into tissue following neutrophils (macrophages are in the tissue already)

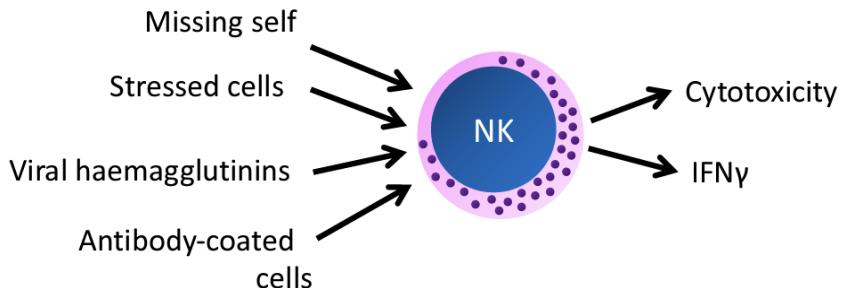
Neutrophils can form extracellular traps (NETs)

- A network of extracellular strings of DNA that trap pathogenic microbes



Natural Killer (NK) cells

Activated
by:



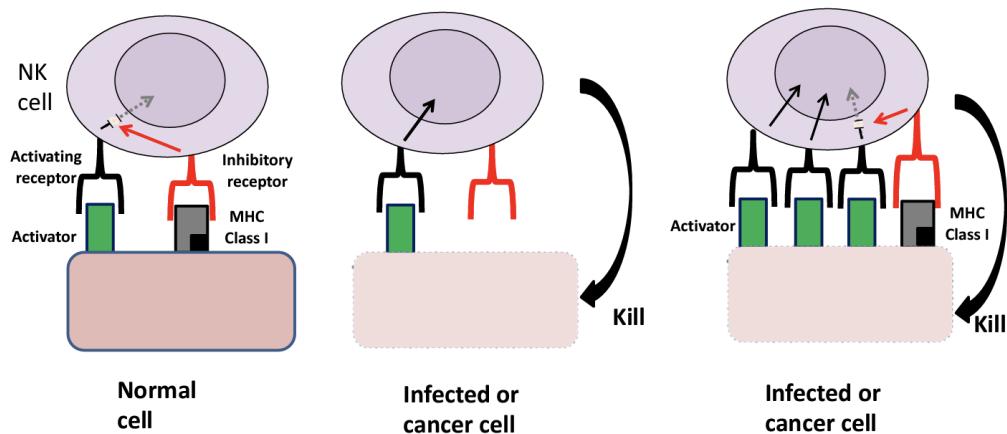
- Stressed cells - damage
- Viral haemagglutinin - on infected cell surface
- Antibody-coated cells - NK cells have FC R
- NK cells are large granular lymphocytes
 - Lymphoid derivatives (vs. myeloids)
- 5-20% of circulating leukocytes
- NK cells destroy abnormal host cells (without sensitisation)
 - Virally-infected, tumour cells, stressed cells
 - Activated by missing self signals
- Granules contain killing molecules

- Important source of interferon gamma - antiviral molecules

Activation of NK cells

- Patrol the body looking for cells that have lost expression of MHC class I (missing self)
- Such abnormal cells are usually either **malignant** or **infected** with a microorganism that interferes with MHC class I expression.
 - Tumours: e.g. lymphoma, melanoma
 - Infection: e.g. Adenovirus 腺病毒, Cytomegalovirus 巨细胞病毒

Mechanisms of NK cell killing



Two sets of receptors on NK cells are involved

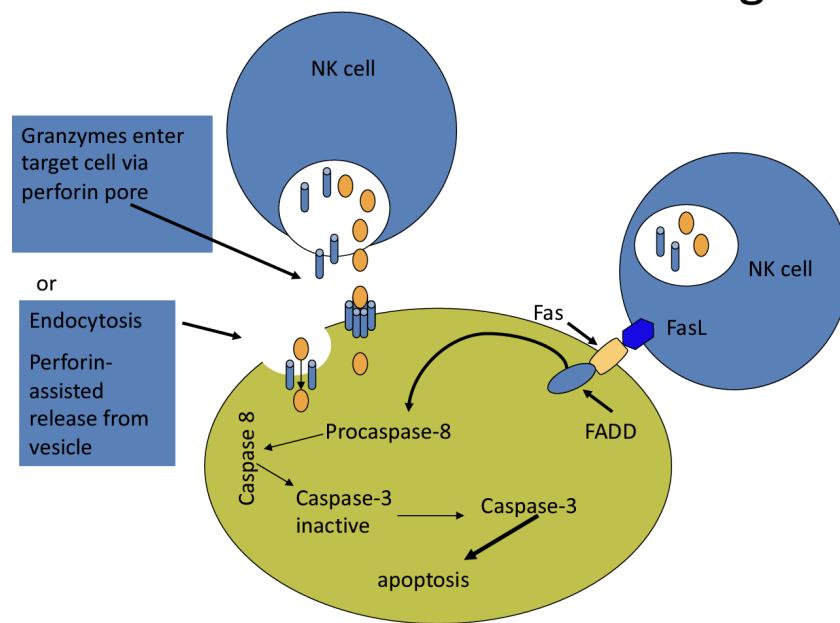
- **Activating receptors** that recognise self molecules that are expressed at increased levels following infection ('induced self')
- **Inhibitory receptors** that recognise MHC class I molecules and interrupt cell signalling from the activating receptors.

Upon ligation, the activating receptors signal the NK cell to kill the target cell.

- Any nucleated cell lacking MHC class I will not engage the inhibitory receptors and only trigger the activating receptors, resulting in its being killed by the NK cell.
- Even MHC Class I is present, more activator presents result in an imbalance between the positive and negative signals will result in killing

NK contain granules that contain proteins (perforin, granzymes) which are released by the process of exocytosis and mediate killing of target cells.

Mechanism of NK cell killing



Surface interaction: Fas - FasL

- NK cells have Fas ligand (FasL) which binds to death receptor (Fas) on the target cell, inducing caspases and apoptosis

Granzyme-mediated or perforin mediated killing

- Perforin puncture the the cell membrane, which allow granzymes enter target cell and kill
- Or Endocytosis
- Release in the immunological synapse and the two cells come close together

Dendritic cells - link with adaptive immunity

- First described by Paul Langerhans, several types
- Immature DCs found in various tissues
 - Sentinel cells
- Mature in inflamed tissues following antigen capture and cytokine
 - Mature only once, change functions, appearance, gene expression
- Mature DCs migrate into LNs and present antigenic peptides to T cells
- Professional antigen presenting cells (APCs)

Activation and maturation of DC

Response to microbial products, inflammatory cytokines, damaged host cells (danger signals)

Immature DC - patrolling barrier tissue

- High antigen uptake (highly phagocytic)
- Low surface MHC class II
 - Important for antigen presenting
- Low co-stimulatory molecules
 - Activation of T cells
- CCR7+ (CCR1, 2, 5, 6+)
 - Low ability to migrate
- More motile than mature DCs to search for pathogens and antigens
- DC sampling the outside
- Getting antigen from the periphery (e.g. skin)
- Langerhan's cell (tissue resident DC) - in transit, Afferent lymphatics (veiled cells) - LN, T cell area (interdigitating DC)

Mature DC - antigen presenting

- Low antigen uptake (poorly phagocytic)
- High surface MHC expression
- High co-stimulatory molecules
- CCR7+++
 - High ability to migrate to LN

DCs are also **phagocytic** to take up antigen when their PRRs recognize PAMPs.

- Following activation, the DCs stop being phagocytic and migrate to the local lymph node where they **present antigen to T-cells**.
 - The antigen is intracellularly processed by proteolytic cleavage into short **peptides** prior to presentation by **major histocompatibility complex (MHC) class II** molecules on the dendritic cell surface.
 - These MHC molecules present the peptides to the T-cell antigen receptor (**TCR**) on the surface of **CD4+ helper T-cells**.

Follicular dendritic cells in lymph nodes and spleen constitute an **entirely different cell type**.

- They do not possess MHC class II molecules, do not engulf antigens and are **not involved in antigen presentation** to helper T-cells.
- They do, however, have **Fc receptors** for IgG (Fc_{gamma}R) and **complement receptors** on their cell surface.
 - This allows them to pick up **immune complexes** (antibody-antigen complexes, which can additionally have complement bound to them) and to **present the native antigen** in these complexes directly to **B-cells**.

Dendritic cells in the skin are mostly MHC II positive (no matter if it is immature or mature)

- CD80 and 86 are upregulated upon activation