



Summary - complete - immunology

Immunology 1 (University of Technology Sydney)



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Immunology

Introduction to the Immune System: Organs and Cells

The structural and functional complexity of the immune system (innate vs adaptive)

Describe structure and distribution of lymphatic network linking all lymphoid organs

Describe basic structure, cellular population of primary, secondary, tertiary lymphoid organs

The process of haematopoiesis (immune cell production)

Immunology - study of the immune system, protection of the body from invading pathogens or foreign macromolecules (viruses, bacteria, protozoa, parasites, fungi). Can protect against our own aberrant cells in tumour immunity. Can develop immune responses against our own proteins in autoimmunity.

The Immune System - Recognises invading pathogens or foreign substances and defends the body by producing an immune response. In many species, can be classified into sub-systems: innate (non-specific) and adaptive (specific).

Pathogens - invading microorganisms that can cause disease. Eg. virus, fungi, bacteria, protozoa, parasites.

Foreign substances include toxins and pollution.

How does the immune system recognise a pathogen?

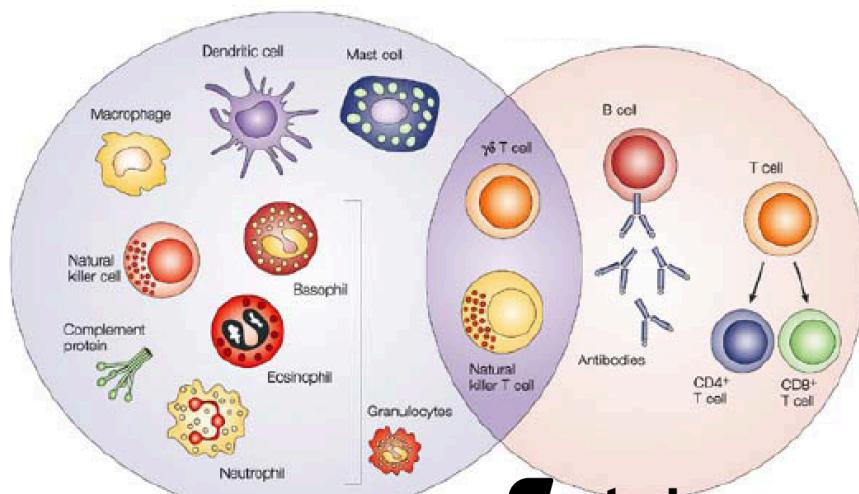
- Invading pathogens have antigens (Ag) on their surface, which the immune system recognises as non-self.
- Ag can be a cell surface protein, glycoprotein, liposaccharide, lipids.
- Non-specific innate immune cell receptors bind Ag directly.
- The presence of Ag causes immune response to be activated to destroy pathogen.

Innate immune cells - rapidly recognise pathogen Ags via invariant (non-specific) receptors that trigger killing, phagocytosis and/or Ag presentation.

Adaptive immune cells - recognise pathogen Ags following presentation by APCs to activate immune cell expansion followed by pathogen killing.

Immune System: Innate vs Adaptive

Innate	Adaptive
Non-specific receptors Responds quickly No memory	Specific receptors Responds slowly the 1st time Memory



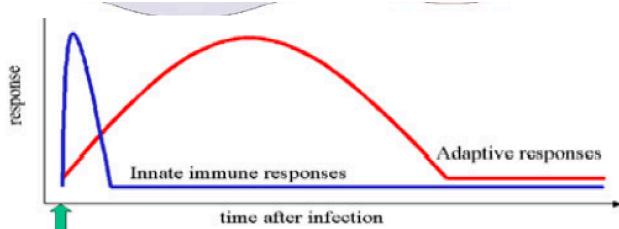
1st Line of Defence - Mechanical/Chemical

Surface barriers protect against invading pathogens:

- Hair
- Keratin is the key structural material making up outer layer of skin
- Mucous membrane
- Antimicrobial secretions destroy pathogens before they enter the body

2nd Line of Defence - Innate Immunity

3rd Line of Defence - Adaptive Immunity



Organs of the Immune System

Specific organs required for development and function of the immune system

- Primary lymphoid organs - bone marrow and thymus, sites of immune cell origin and lineage commitment
- Secondary lymphoid organs - lymph nodes, spleen and liver, trap antigens and provide an environment for interaction with mature immune cells
- Tertiary lymphoid tissues - import immune cells and APC during an inflammatory response, aggregates of cells in the lamina propria of:
 - MALT (mucosal associated lymph tissue)
 - GALT (gut associated lymph tissue)
 - BALT (bronchial associated lymph tissue)
- All lymphoid organs connected by lymphatic system

Lymphatic System

- Lymph system similar to cardiac system, has one way flow (valves), begins in the tissues and ends at the heart
- 3 main parts: vessels carry fluid, lymph (fluid in vessels), lymph nodes (filters lymph of pathogens)
- Plasma from blood vessels seeps into tissues
- Lymphatic vessels depend of muscle contraction for lymph flow back to heart
- Lymph and lymphocytes empty into the left subclavian vein of the heart

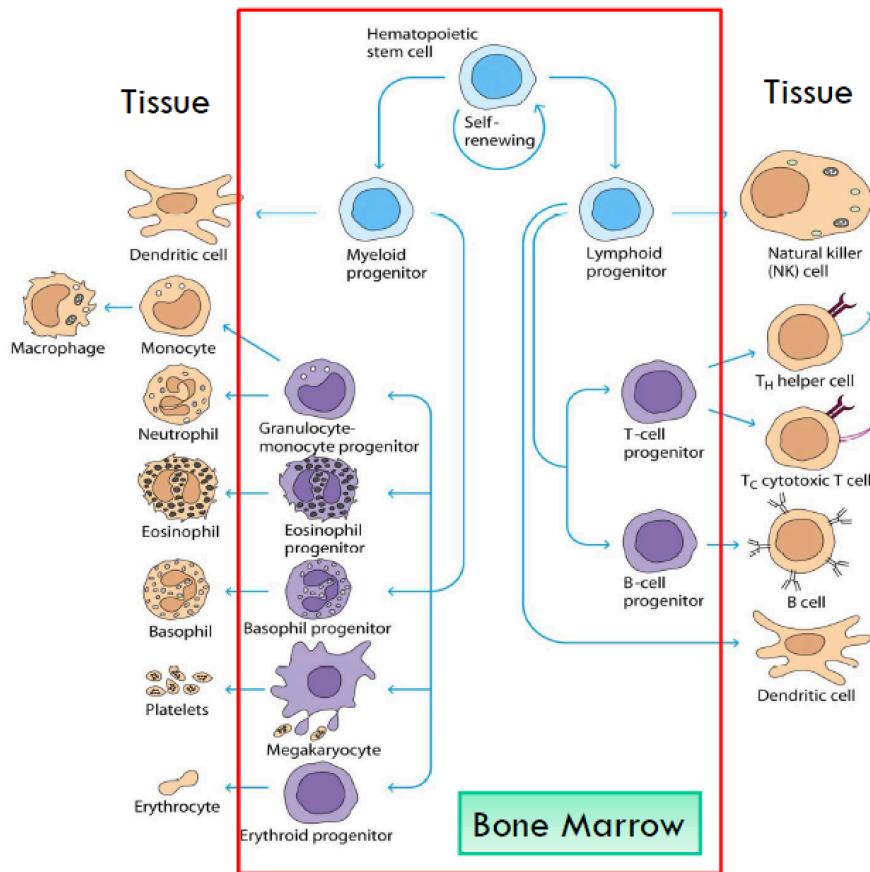
LYMPHOID PRIMARY ORGANS (Development)

Bone Marrow

- Site of haematopoiesis, formation of all blood cells from CD34+ HSC, B cell receptor assembly
- BM MSC assist B cell maturation by:
 1. Providing a source of self Ag → B cell apoptosis
 2. Cytokine (IL-7) for B cell development

Haematopoiesis

- HSC (CD34+ Haematopoietic Stem Cell) - reside in bone marrow, pluripotent, 1/50000 BM cells, extremely proliferative if need arises
- HSC differentiate into common lymphoid or myeloid progenitor cells (lineage commitment)
- Growth factors, cytokines determine cell lineage
- Mesenchymal stromal cells (MSC) support HSC development in BM and in vitro
- Haematopoietic Growth Factors - and cytokines determine cell lineage commitment:
 - Myeloid Growth Factors
 - Multi-CSF (IL-3)
 - M-CSF (Macrophage CSF)
 - G-CSF (Granulocyte CSF)
 - GM-CSF (Granulocyte Monocyte CSF)
 - Erythropoietin (Epo) - induces production of RBCs

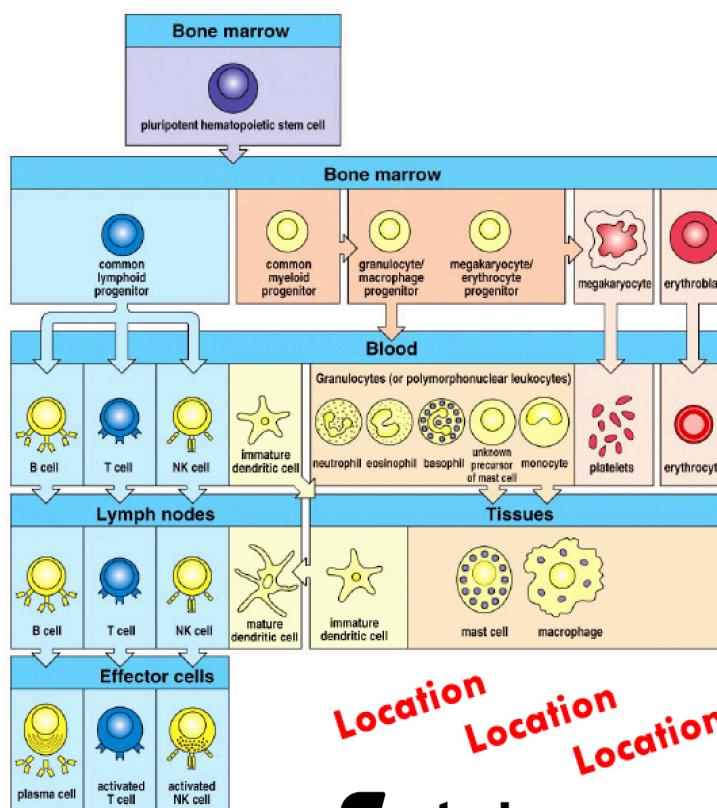
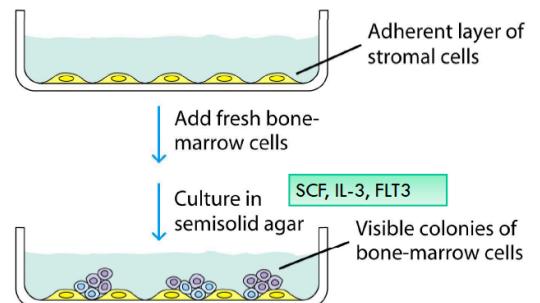


BM isolation for stem cell culture *in vitro*:

Preparation of human BM:

1. Collect BM from upper iliac crest using aspiration needle
2. Dilute human BM 7:1 with buffer and pass cells through 100microlitre filter to remove bone fragments and cell clumps
3. Layer 35mL diluted cell suspension of 15mL Ficoll-Paque in 50mL conical tube
4. Centrifuge at 445xg for 35min at RTP

In vitro culture of CD34⁺ HSC



Thymus

- Bilobed organ on top of heart
- T cells move from BM to thymus for T cell receptor assembly and selection for release into periphery
- Involves 2 steps:
 1. Thymic stromal cells express high levels of MCH I & II, present various Ag and T cells
 2. T cells recognise self-Ag → apoptosis, T cells that fail to recognise foreign Ag → apoptosis
- 95-99% of T cells die in thymus

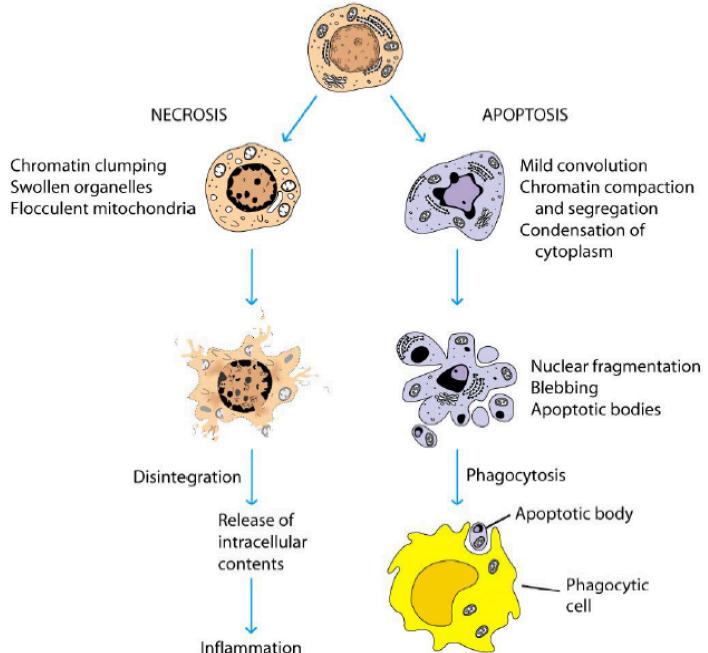
Cell Death

Apoptosis - orderly

- Reduction in Cell Volume
- Chromatin condensation
- DNA degradation
- Mφ Ingest membrane bound bodies
- No inflammation

Necrosis - chaos

- Bursting of cell due to injury
- Contents released to environment
- Inflammation



LYMPHOID SECONDARY ORGANS (Maturation)

Lymph Node

- Ags trapped by dendritic cells
- DC process and present Ag with MCH class II
- Activates Th cells, secretes cytokines, allows activation of B cells
- B cells interact with soluble Ag
- Activated lymphocytes proliferate

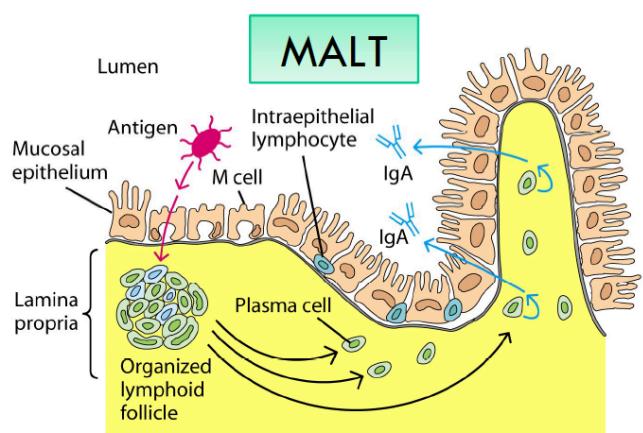
Spleen

- Functions differently from lymph nodes.
 - Lymph nodes trap Ag from tissues - Spleen traps Ag in circulatory system, allows spleen to activate immune system in response to systemic infection
- Ag in blood captured by dendritic cells, presented to Th cells with MCH II
- Activation of Th allows subsequent activation of B cells
- Blood leaving spleen enriched with activated lymphocytes and antibodies

LYMPHOID TERTIARY TISSUES (MATURATION)

Mucosal Associated Lymphoid Tissue (MALT)

- Mucous membranes SA = 400 m²
- MM most common pathogen entry site, MM protected by MALT
- Organisation varies
- M cell allows AG entry, unique architecture allow B cell activation of IgA secretion that prevents pathogen attachment



Introduction to the Immune System: Functional Cells

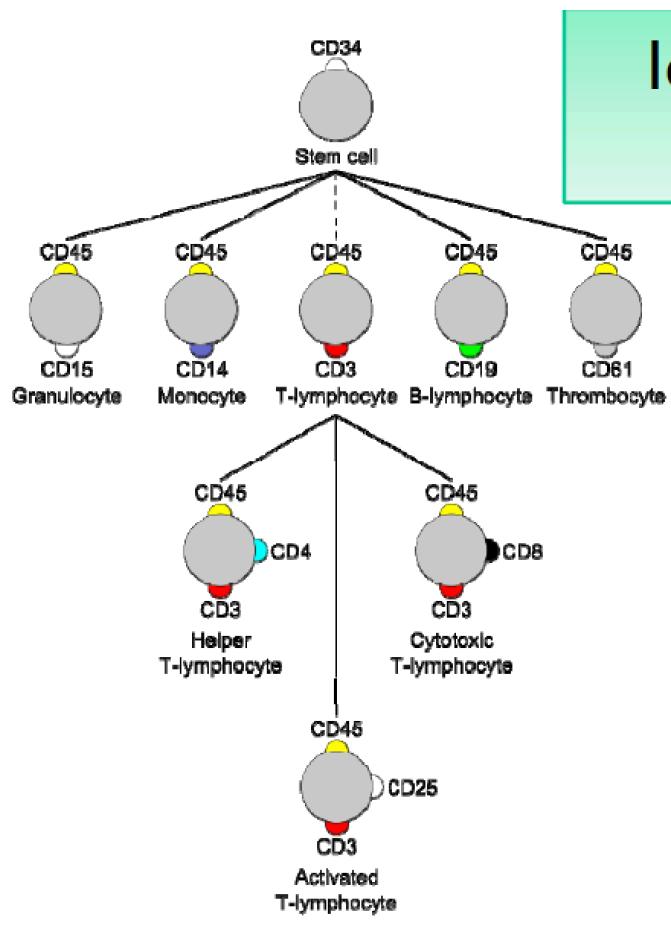
Structural and functional complexity of immune system
Identify immune cell type involved in adaptive and innate immune responses

Cells of the Immune System – leukocytes (WBC – innate or specific), Neutrophils, Eosinophils, Basophils/Mast cells, Monocytes/Macrophages/Dendritic cells

Cell Type	Cells / mm ³	% total leukocytes
Red blood cells	5 x 10 ⁶	
Platelets	2.5 x 10 ⁵	
Leukocytes	7.3 x 10 ³	
Neutrophils	3.7-5.1 x 10 ³	50-70
Lymphocytes	1.5 - 3.0 x 10 ³	20-40
Monocyte	1 - 4.4 x 10 ²	1-6
Eosinophil	1 - 2.2 x 10 ²	1-3
Basophil	<1.3 x 10 ²	<1

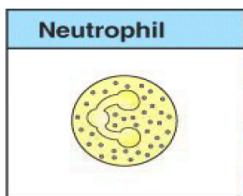
Identifying Cells – CD (Cluster of Differentiation)

- Over 350 human CD markers
- T cells, CD4, CD8, CD3
- B cells, CD19
- NK cells, CD56
- Monocytes/Macrophages, CD14
- Dendritic cells, CD11c (human), CD11c (mouse)



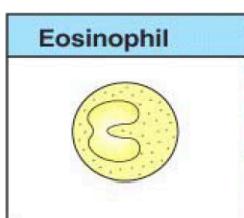
NEUTROPHILS

- Only enter tissues when instructed
 - Phagocytosis
 - Bactericidal killing
- Eg. Respiratory burst



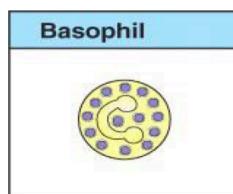
EOSINOPHILS

- Attack and killing of large antibody coated parasite such as worms



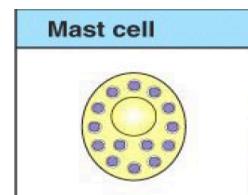
BASOPHILS

- Smallest WBC in circulation
- Function unknown
- Maybe similar to mast cell



MAST CELLS

- Mainly in tissues, release of granules containing histamine and other compounds
- Important in inflammation and allergy



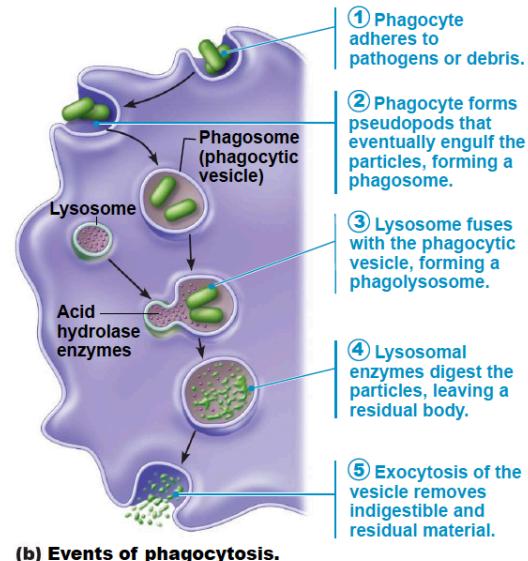
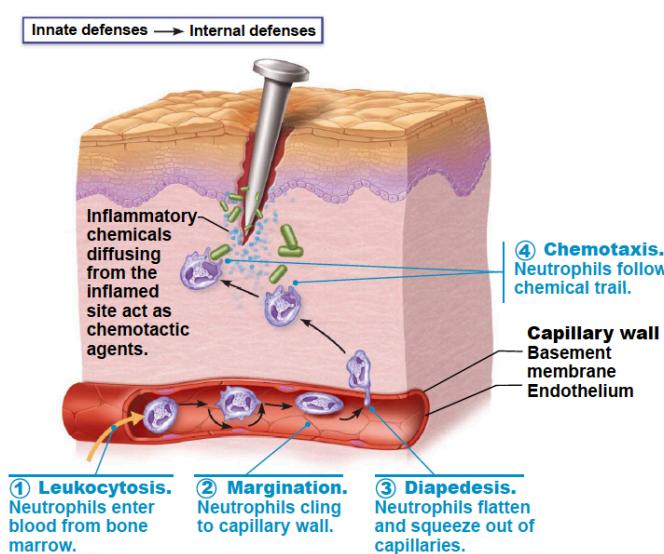
MONONUCLEAR CELLS

- Innate immune cells
 - Monocytes in blood, MΦ in tissues (monocytes 5-10 times smaller)
 - MΦ increases phagocytic ability
 - Secretes cytokines and produces hydrolytic enzymes
 - Named based on tissue they reside – alveolar (lungs), Kupffer (liver), Microglial (brain), Osteoclasts (bone)
 - Activated by phagocytosis of cytokines
 - Antigen presenting capacity via MHC II
- LYMPHOID CELLS**
- Adaptive immune cells, B-cells, T-cells, Null cells (NK cells)
 - 20-40% of body's leukocytes, 99% of lymph node
 - Nucleus occupies almost entire cell, 6 µm diameter

Phagocyte Mobilisation

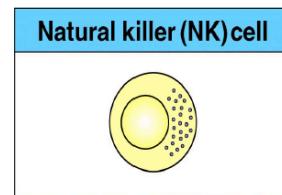
Steps for phagocyte mobilisation

1. **Leukocytosis** – release of neutrophils from bone marrow in response to leukocytosis-inducing factors from injured cells
2. **Margination** – neutrophils cling to walls of capillaries in inflamed area in response to CAMs
3. **Diapedesis** – of neutrophils
4. **Chemotaxis** – inflammatory chemicals promote positive chemotaxis of neutrophils



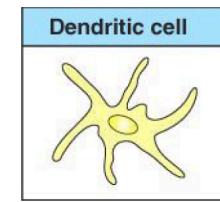
NK CELLS

- Innate immune cells, do not express lymphocyte markers
- Eliminate tumour cells, virally infected cells
- Use CD16 they carry out ADCC
- Reduction of MHC I can activate them (loss of self)



DENDRITIC CELL

- Antigen recognition/uptake
- Antigen presentation-produce cytokines to direct T cell response
- Several types – Langerhans (skin), Circulating DCs
- Interstitial DCs, populate organs eg. Heart, lungs, liver, intestines
- Interdigitating DCs, T-cell areas of lymph nodes and thymic medulla
- Scarce cell type, grown in vitro early 90s, intense research area (tumour treatment)

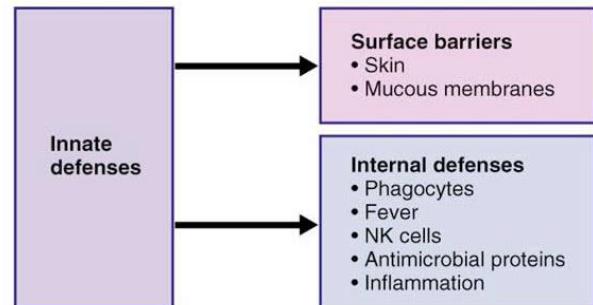


Innate Immunity I

All eukaryotes have defence mechanisms against microbial; and viral pathogens. Vertebrates evolved two 'types' of immune systems: innate and adaptive/acquired immunity

Characteristics of Innate Immunity

- Ancient – in all eukaryotes
- Immediate/rapid response to pathogen – first line of defence
- Recognition of molecular patterns common across pathogens
- Low specificity, lack of memory
- Directs adaptive immune system



1ST LINE OF DEFENCE – SURFACE BARRIERS

SKIN

- Heavily keratinised – physical barrier
- Weakly acidic (pH3-5), inhibits bacteria/fungi growth
- Sebum secreted by the sebaceous gland contains antimicrobial substances

MUCOSAL MEMBRANES

- Line all body cavities that open to exterior (digestive, reproductive, respiratory, urinary)
- Secretion of HCl and enzymes into stomach that destroy pathogens
- Enzymes in saliva (eg. Lysozymes) break down microbes
- Production of mucus traps mucus (contains numerous anti-microbial molecules)
- Modifications eg. Cilia trap and sweep away microbes from lower respiratory tract

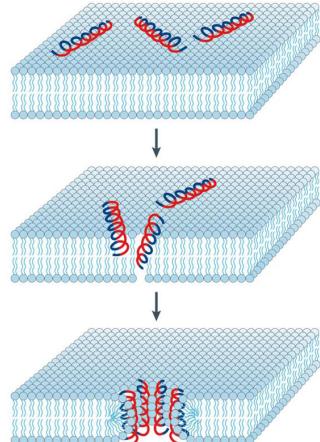
Antimicrobial peptides (AMPs)

- Two families of AMPs: cathelicidins and defensins
- Cationic short polypeptides found on epithelial surfaces
- Preferential interaction with bacterial membranes: (+) charged AMP bind to (-) phospholipid
- Weak interaction with eukaryotic/host cell membrane: (-) phospholipids not found on extracellular face

AMPs bind to bacterial membrane

Oligomerisation of AMPs creates a pore in bacterial membrane

Lysis of bacteria



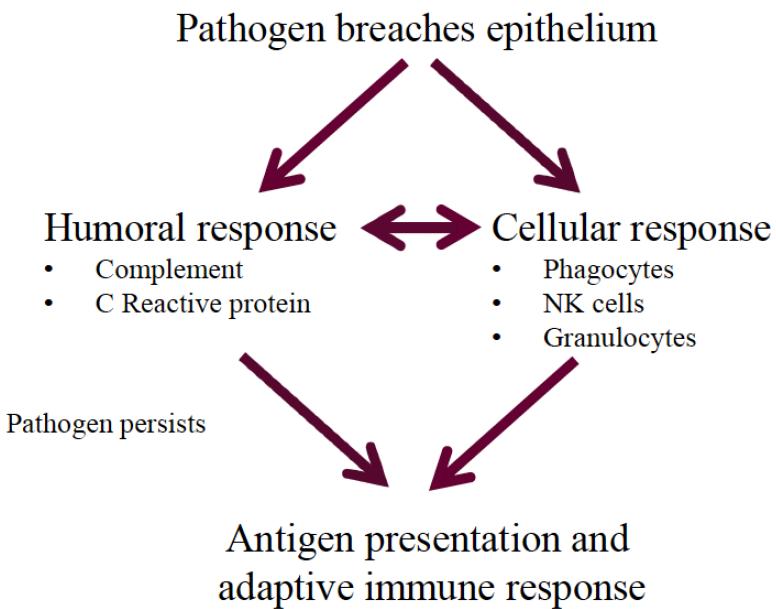
Nature Reviews | Microbiology

Other types Antimicrobial Found in Epithelium

- Immunoglobulins – especially IgA, part of adaptive immune system, bind to bacteria and enhance phagocytosis
- Surfactants – bind to carbohydrates expressed by bacteria and enhance phagocytosis
- 'Normal' flora found in epithelium, competition with pathogenic microbes

Antimicrobial Barriers

	Skin	Gut	Lungs	Eyes/Nose
Mechanical	Epithelial cells joined by tight junctions			
	Longitudinal flow of air/fluid		Mucus movement by cilia	
Chemical	Fatty acids	Low pH Enzymes (pepsin)		
	Antibacterial peptides (defensins)		Salivary enzymes (lysozyme)	
Microbiological	Normal flora			



Humoral Immunity

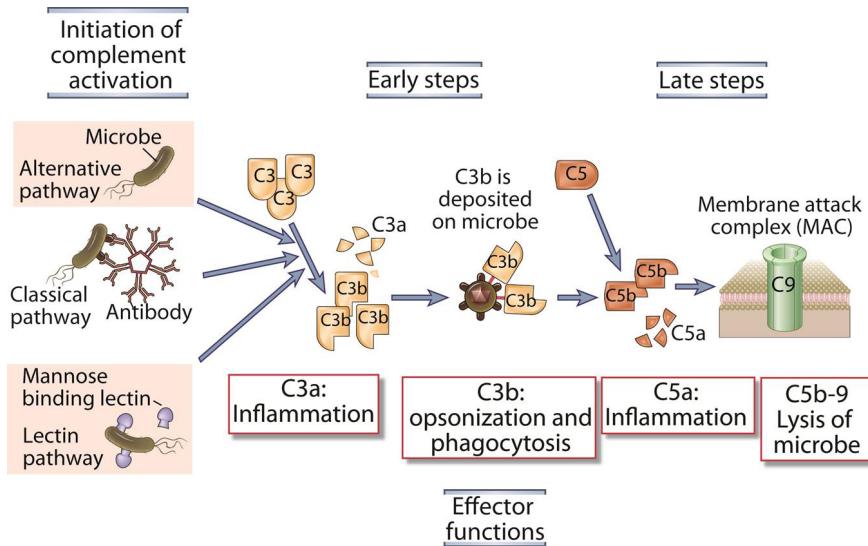
- Soluble factors (non-soluble) mediate immunity, generally proteins
- 3 types – antibodies, complement system, C-Reactive protein
- Antibodies part of adaptive immune system (can have innate properties)
- Complement and CRP innate immune system

Complement System

- Group of >30 soluble proteins (enzymes) found in serum in inactive form
- Frequently referred to as a cascade – sequential activation by cleavage
Deficiency in one complement protein can affect whole system
- Three pathways – classical, lectin, alternative
- Results in microbial killing, opsonisation, inflammation

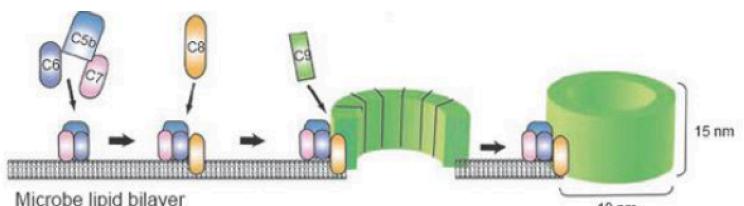
Complement Activating Proteins

- Classical pathway – induced by C1q binding directly to microbial surface components, indirectly or binding antibodies already attached to microbial surfaces
- Alternative pathway – spontaneous hydrolysis and activation of C3, binds directly to microbial surfaces
- Lectin pathway – carbohydrate binding proteins (mannose binding lectin) bind to carbohydrate structures on microbial surfaces

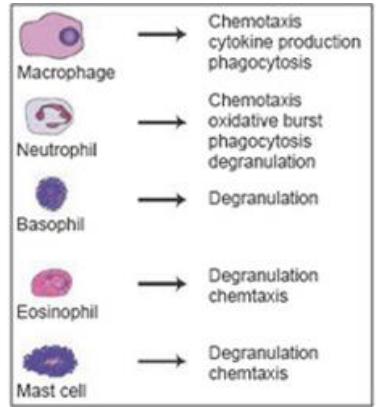


Effector Functions of Complement System

1. Direct lysis of pathogens via Membrane Attack Complex (MAC)
 - Complement components form complex, exposure of hydrophobic phase allows insertion to microbial membrane
 - C9 binds to complex and polymerizes forming a pore



- Enhancement of phagocytosis (opsonisation)
 - Receptors on surface of cell recognize complement components attached to microbial surface or to antibodies
- Recruitment and activation of immune cells
 - Interaction of complement fragments with receptors on endothelial cells and mast cells produce local inflammatory responses
 - Phagocytic cells recruited



Cells of Innate Immunity

- Phagocytes – neutrophils, macrophages, dendritic cells
- NK cells
- Granulocytes – neutrophils, eosinophils, basophils

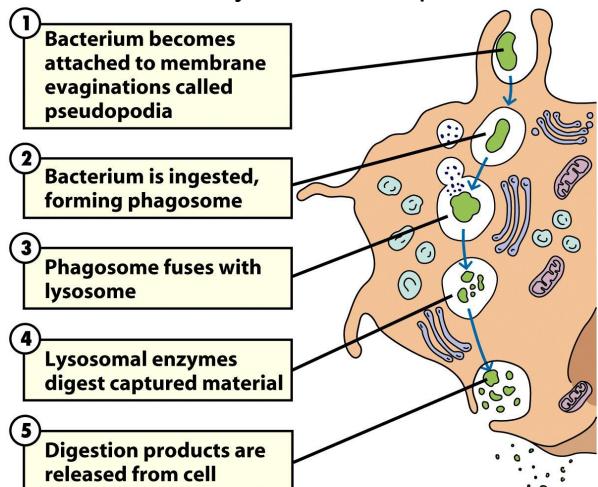
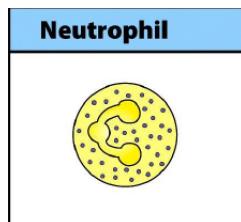


Figure 3-4b
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Neutrophils

Function – Phagocytosis and activation of bacterial mechanisms

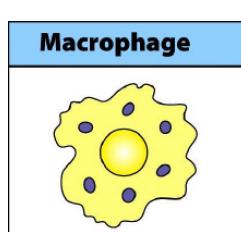
- Differentiate from multi-potential stem cells in BM, released mature into blood, 30-70% of WBCs in peripheral blood.
- Contain lysosomal granules containing lysozyme, acid hydrolases, proteases, peroxidase
- Does not recirculate.



Macrophages

Function – phagocytosis and activation of bacterial mechanisms, antigen presentation

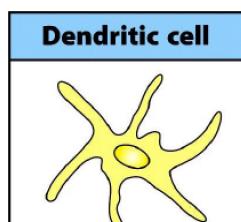
- In blood - matured from monocytes, in tissues specialized – Kupffer cells in liver
- Differentiate from BM stem cells, released as monocytes (phagocytic, bactericidal)
- 2-7% WBCs in peripheral blood, emigrate to tissues, differentiate to macrophages
- Contain hydrolytic enzymes



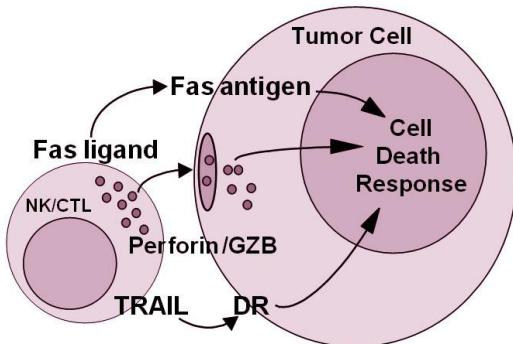
Dendritic Cells

Function – antigen uptake in peripheral sites, antigen presentation

- Most important cell types in immune system, different types in different body parts
- Main function – take up and process antigenic material, present it to immune system (bridge between adaptive and innate)
- Heterogenous, derived from monocytes, endogenous within skin, etc



Innate Immunity II



NK Cells

- Innate lymphoid cell, not considered professional phagocyte
- Respond to virally infected cells, tumour cells
- Contain cytotoxic granules, perforin (pore forming protein), granzymes (proteases enter through perforin pore)
- Expression of ligands that activated “death” receptors on target cells

How do NK cells recognise tumour/virus-infected cells?

- One mechanism to evade adaptive immune system – down-regulate MHC class I expression on virus-infected or malignant cells
- Normal cells express MHC I on surface
- NK cells ‘sense’ lack of MHC I expression on abnormal cells and kill them
Inhibitory and activation receptors combined

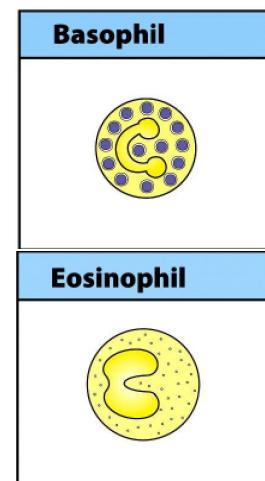
What are granulocytes?

- Type of leukocyte characterised by granules in cytoplasm
- Granules – intracellular vesicles contain a variety of antimicrobial substances, released upon activation (defensins, proteolytic enzymes, cytokines)
- Neutrophils, basophils, eosinophils

Basophils

Function – unknown

- Contain large basophilic granules – contain histamine, heparin, proteolytic enzymes
- Least common granulocyte, constitute 0.1-0.3% WBCs in peripheral blood
- Important role in certain inflammatory reactions



Eosinophils

Function – killing of antibody-coated parasites

- Contain eosinophilic granules containing histamine, peroxidase, lipases
- 1-5% of WBCs in peripheral blood
- Important role in parasite infections, allergy, asthma

How do innate immunity cells know what's good and bad?

Basic Rules of Immunology

1. Immune system is there to stop invading pathogens
2. Immune system has a sense of self
3. Immune system has a sense of non-self (eg. Pathogens)
4. Immune system can effectively remove pathogens with minimal harm to self

How does the innate immune system recognise self from non-self?

- Pathogen-Associated Molecular Patterns (PAMPs)
- Molecules expressed by a group of pathogens that are not present in host

Bacterial Cell Membranes

- Lipoteichoic acid, lipopolysaccharide (LPS) and peptidoglycan are unique to bacterial
- Membranes serve as potent PAMPs to innate immune system

PAMPs

- Double stranded RNA synthesised by viruses (eg. Rotavirus)
- Unmethylated CpG motifs produced by viruses
- Flagellin on bacteria (eg. Helicobacter)
- Zymosan – carbohydrate found on some fungal species

Pattern Recognition Receptors (PRRs)

- PRRs recognise PAMPs
- Expressed on surface or intracellularly by innate immune cells (toll-like, C-lectin, NOD-like, RIG-I-like receptors)
- PRR ligation by PAMP “activates” innate cells – phagocytosis, release of cytotoxic mediators, secretion of proinflammatory cytokines

Toll-Like Receptors (TLRs)

- Major PRR gene family
- “leucine-rich repeat” zipper structure
- Recognise conserved pattern motifs found on variety of pathogens

Signalling:

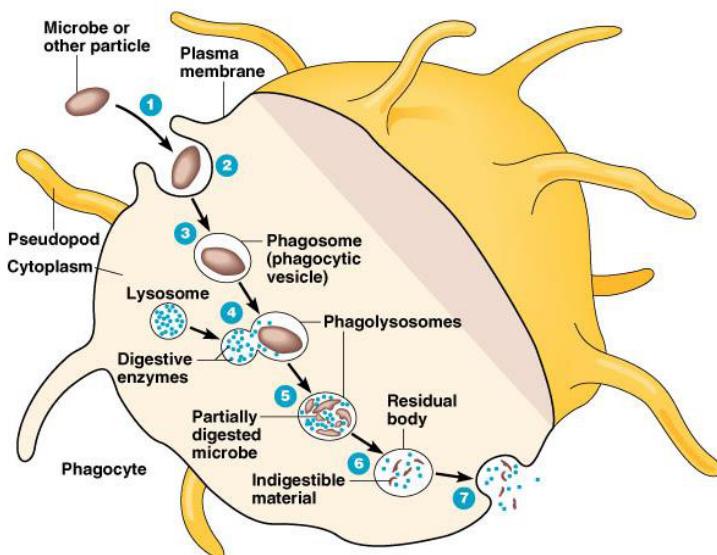
- Ligation of TLRs by pathogen activates cell
- Activation results in expression of proinflammatory cytokines
- Cytokines – activate nearby immune cells, help induce antiviral state

What happens when a macrophage encounters extracellular microbe?

1. Microbe coated in humoral mediator eg. Complement proteins
2. Macrophage captures microbe by PRR and complement receptor, begins to express lysosomes
3. Microbe is phagocytosed
4. Lysosomes fuse with phagosome – contain enzymes, oxidases pump free radicals into phagolysosome
5. Microbe digested, microbe derived peptides transported via MHC proteins to cell surface, presented to T cells
6. Indigestible material expelled

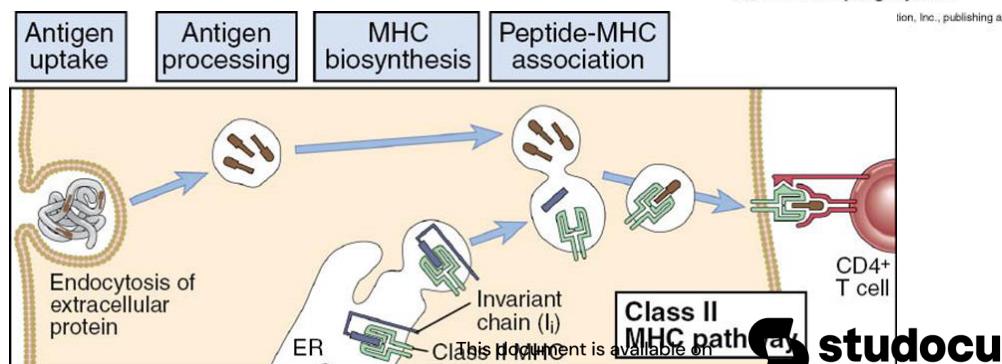
Dendritic cells act as bridge between innate and adaptive immunity

- Endocytose/phagocytose pathogen (antigen)
- Digest pathogen into small peptides
- Peptides bound to MHC, transported to cell surface for T-cells



(a) Phases of phagocytosis

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Adaptive Immunity

Differences between innate and adaptive immunity
Diversity and specificity in adaptive immune system

Role of B cells in adaptive immunity

Ag and antibodies

B cell activation – Clonal Selection

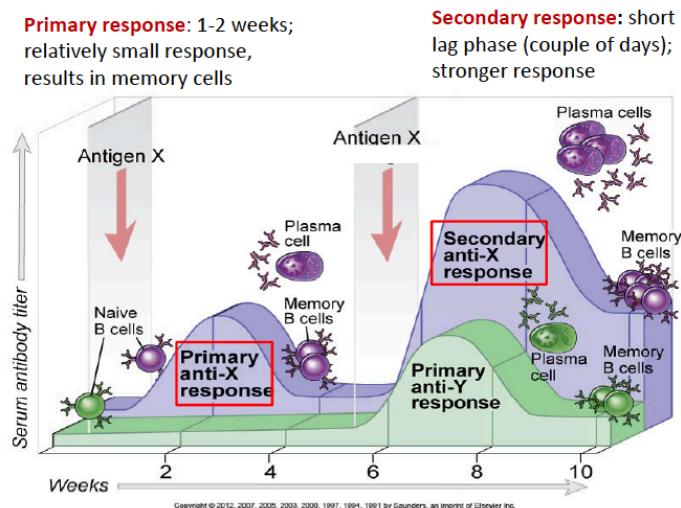
Germinal centre reaction – somatic hypermutation (Affinity Maturation)

Adaptive Defences – Humoral immunity (B cells)

- Cellular Immunity (T cells)

	Adaption
Self/non-self discrimination	Present, reaction is against foreign
Lag Phase	Present, response takes at least a few days
Specificity	High, response directed only to the agents that initiated it
Diversity	Extensive, resulting in wide range of antigen receptors
Memory	Present, subsequent exposures to same agent induced amplified responses

Dynamics of the adaptive immune response



Innate and Adaptive Immunity Work Together

	Innate	Adaptive
Number of pathogen receptors	$10^2 - 10^3$	10^9
Time to develop response	Immediate	Days/weeks
Memory	No	Yes
Strength of response after 2 nd challenge	Similar	Greater

Adaptive (Specific) Immune System

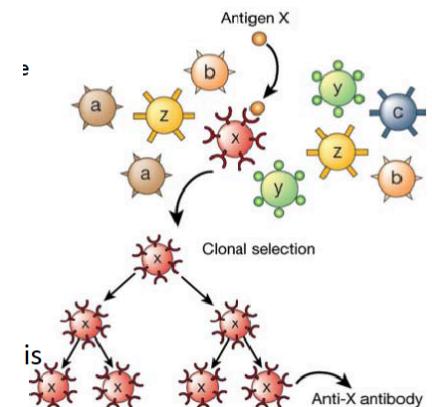
- Two cell types comprise system – T lymphocytes, B lymphocytes
- From lymphoid precursor: 'B' bursa of fabricius, 'T' thymus
- T Cells – primary development organ is thymus
- B Cells – Primary development organ is bone marrow and spleen, diverse in antigen recognition

What are Antigens?

- Antigens – Antibody Generators, proteins that elicit immune response
- Proteins, carbohydrates, nucleic acids, lipids, haptens + carrier eg. Nickel

How does the Adaptive Immune System recognise so many antigens?

- Each T or B cell clone has one specificity or antigen receptor (TCR or BCR (antibody)), so one cell can recognise one antigen
- Unique antigen receptors generated by random rearrangement of gene segments which encode specificity of antigen receptor
- B cells with receptors for self are deleted in bone marrow
- RESULT: huge pool of B cells and T cells with different Ag specificities



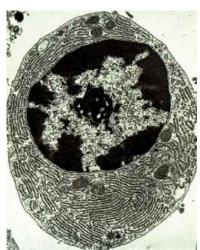
The Clonal Selection Theory

Clonal Selection – adaptive immune cells which encounter antigen in periphery induced to survive, proliferate and differentiate, generating antigen-specific clones

- Clonally selected lymphocytes differentiate into effector cells eg. Memory cells
- Theory explains specificity and memory

B Cells

- Type of lymphocyte, round cell with small cytoplasm, round nucleus (mononuclear)
- ~5% all WBCs
- Primary role – secrete antibodies as plasma cells which have large cytoplasm, rough ER, protein factory (~2000 Abs/s)

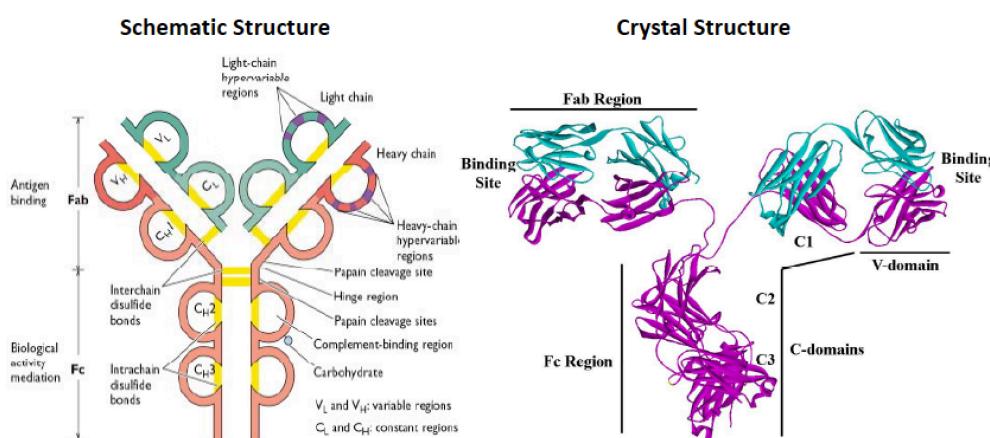


Plasma Cells

- Large cytoplasm with numerous sheets rough ER
- Protein factory – can synthesise
- Low expression BCR
- Small number found in blood – reside primarily in bone marrow and spleen
- Short lived 1-2 weeks

Antibodies

- Membrane bound (BCR) or secreted (by plasma cells) soluble molecules
- Fab region – responsible for antigen binding (via domain)
- Fc region – interacts with Fc receptors on immune cells, recruits complement
- One antibody – one antigen



B cell Receptor (BCR) is an antibody

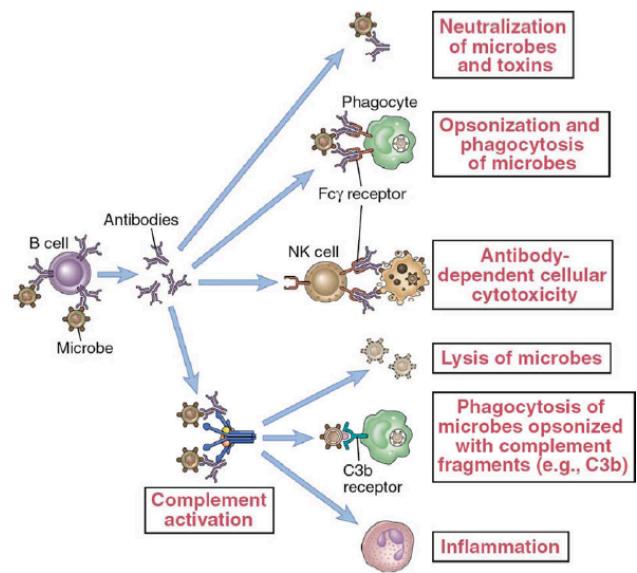
Each B cell clone expresses unique antibody receptor (BCR)

How is the unique binding site on an antibody generated?

- Antigen binding occurs in 'variable domain' called VH or VL
- During B cell development, gene encoding heavy or light chain variable regions generated at random from selection of gene segments

VDJ recombination of heavy and light chain Variable Regions

- Gene encoding heavy chain – variable domain 'stitched' together from three different gene segments
 - V: variable
 - D: diversity
 - J: joining
- Process relies on Recombination Activating Gene (RAG) 1 and 2
- Heavy chain VH comprised of 1V, 1D, 1J
- Light chain VH comprised of 1V, 1J

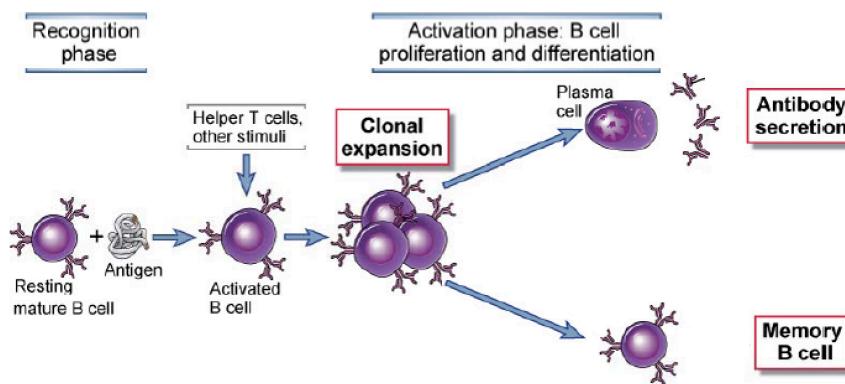


Negative Selection – Controlling Autoreactive B Cells

- Stromal cells present self antigen to B cells in bone marrow
- ~10% B cells reach periphery
- Receptor editing may save some B cells

When B Cells leave the BM – Clonal Selection

- Each naïve B cell clone expresses unique BCR (antibody) on their surface
- When B cell binds to antigen it is specific for, it becomes activated and clonally expands

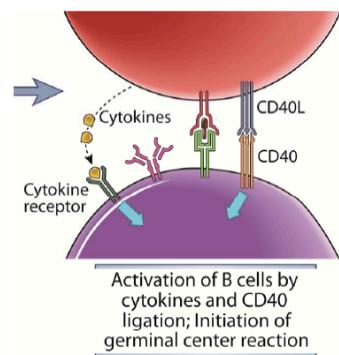


The B Cell Receptor (BCR)

Two components – Antibody, Igα and Igβ, signalling occurs through Igα and Igβ complex only

2nd and 3rd Signals – T cells Activate B cells

T cells provide – CD40 ligation, Cytokines



The Germinal Centre (GC) – B Cell Activation and Differentiation

- Mass of leukocytes that form within lymph nodes during infection
- Primarily composed of proliferating (and differentiating) B cells

Fine Tuning the Immune Response

Somatic Hypermutation

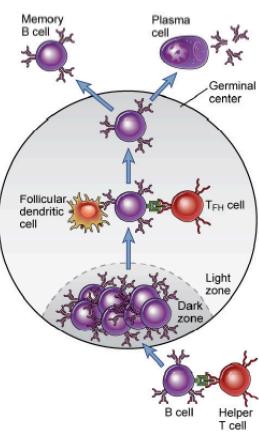
- Introduction of point mutations into rearranged V_L, V_H genes
- Caused by enzyme: Activation-Induced (Cytidine) Deaminase or AID
- C region unaffected

Affinity Maturation

- B cells with BCRs that have increased antigen affinity selected for survival

The GC Reaction

- Activated B cells travel to dark zone of GC and proliferate (clonal expansion)
- Fine tuning the BCR: Dark zone B cells (Centroblasts) undergo somatic hypermutation and begin to express BCRs with higher or lower affinity for antigen
- B cells migrate to light zone (centrocytes), encounter follicular DCs that present antigen to B cells (natural selection)



Activation of B cells and migration into germinal centre

B cell proliferation

Somatic mutation and affinity maturation, isotype switching

Exit of high-affinity antibody-secreting memory and B cells

B cells and Autoimmune Disease

- Secrete auto-antibodies
- Secrete inflammatory cytokines
- Autoimmune diabetes: relies on auto-reactive B cells in NOD mice

Regulatory B Cells in Autoimmune Disease

- IL-10 secreting B cells protective in models of autoimmune disease: NOD mice (T1 diabetes)
- EAE (multiple sclerosis): adoptive transfer of Bregs ameliorates disease

Antibody Isotypes

Understand functions of different antibody isotypes
The process of isotype (class) switching

Antibodies (Immunoglobulins)

- Made up of heavy and light chains, each with constant and variable regions (linked by disulphide bonds)
- Can be found in serum (secreted by plasma cells) or attached to surface of B cells as part of B cell receptor (BCR) complex
- Variable regions – encode antigen specificity of antibody
- Constant region of heavy chain – biological effector function of antibody
- 5 different isotypes with varied function defined by constant region
- IgA, IgD, IgE, IgG, IgM

When a B cell becomes activated for the first time during primary immune response, it secretes IgM. When B cell activated – receives signals to switch isotype
Different isotypes of antibodies have different biological effects

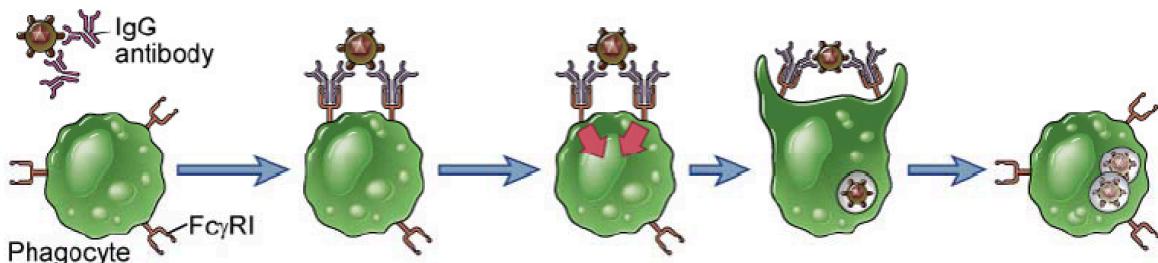
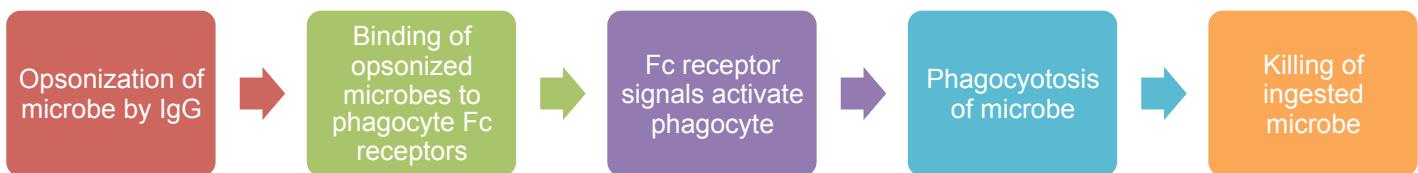
Antibody Effector Function

- Bind to surface of microbes
- Induce phagocytosis
- ADCC – Antibody Dependent Cellular Cytotoxicity
- Lysis of microbes
- Induce inflammation

Immunoglobulin G (IgG)

- G heavy chain, 80% of Ab serum
- Long half life, 7-25 days
- Monomer – 2 identical antigen binding site
- Three constant regions – IgG1, IgG2, IgG3
- Fc portion:
 - Recruit complement (Complement Dependent Cytotoxicity),
 - Engage Fc receptors on macrophages
 - Engage DCs and neutrophils to enhance phagocytosis
 - Engage Fc_γ receptors on NK cells to induce target cell cytotoxicity (Antibody Dependent Cellular Cytotoxicity)

Enhancement of Phagocytosis by IgG



- Phagocyte expresses multiple Fc receptors
- Crosslinking – induces phagocyte to phagocytose microbe

Antibody Dependent Cellular Cytotoxicity

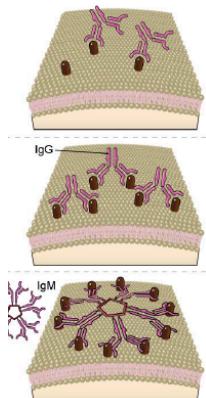
- Antibodies bind to surface of infected cell
- NK cell (expresses Fc gamma receptors) crosslinked by antibody
- Antibody coated cell is killed

Complement Dependent Cytotoxicity (CDC)

- Antibody binds to surface, Fc portion recruits c1q
- Recruitment of the complement cascade results in lysis of the target cell

Immunoglobulin M (IgM)

- M heavy chain, 5-10% serum Ab
- Short half life ~5 days
- 1st antibody secreted by B cells when activated, other isotypes take longer to develop (isotype switching in GCs)
- Exists as pentamer in serum, J chains connect individual IgMs
- Can recruit complement (CDC) via c1q
- Binds FcR a/m on macrophages – enhances phagocytosis of microbes
- High avidity



Avidity vs Affinity

Affinity – strength of interaction for one binding site

Avidity – combined strength of multiple binding sites, IgG = 2 binding sites =
Affinity x 2

FAB fragments same affinity but lower avidity

Valency of Interaction	Avidity of Interaction
Monovalent	Low
Bivalent	High
Polyvalent	Very High

C1q Binding by IgM (and IgG)

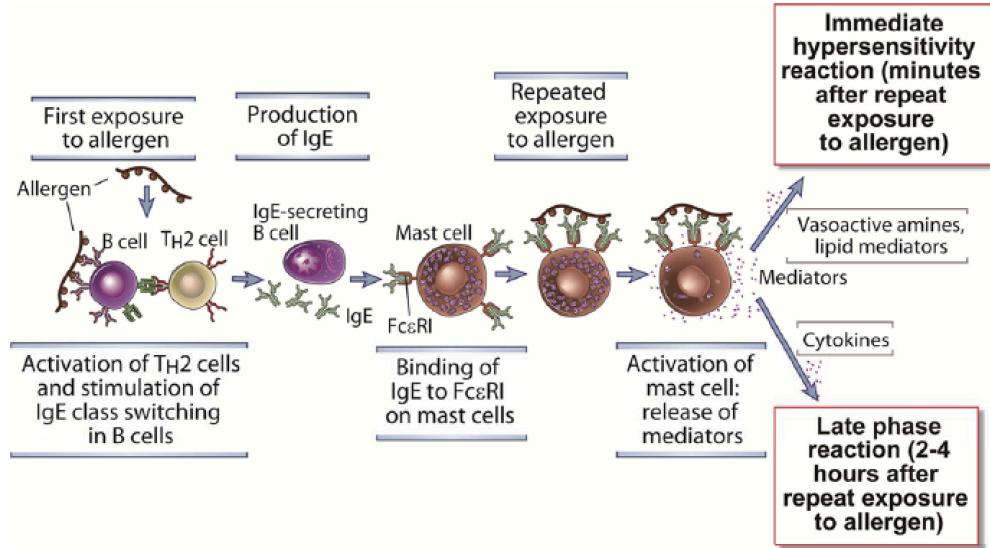
- In serum – unable to bind complement, conformational changes occur when complexed with antigen - allow c1q to bind
- Makes c1q binding site accessible in IgM and IgG Fc region
- Complement only activated when antibody bound to antigen on surface

Immunoglobulin A (IgA)

- A heavy chain, highest daily production, 10-15% serum Ab
- Predominant in external secretion – breast milk, saliva, tears, mucus
- Prevents pathogens binding to mucosa, neutralisation of bacteria, viruses, toxins
- Exists as monomer in serum, but polymer in secretion (high avidity)

Immunoglobulin E (IgE)

- E heavy chain, very low serum concentration (0.002% of Ig)
- Mediates allergy
- 4 heavy chain C domains (constant)
- Potent biological actions (low conc.)
- Provides immunity to helminths BUT responsible for allergy
 - Hay fever
 - Asthma
 - Hives
 - Anaphylaxis
 - Peanut allergy
 - Eczema
- IgE sensitizes mast cells to antigen by binding FcRe



B cell expressing antibody specific for allergen, binds to it, switches, begins secreting IgE.

Allergen reenters body, crosslinks on surface of mast cell
Degranulation of mast cell, release of inflammatory mediators.

Immunoglobulin D (IgD)

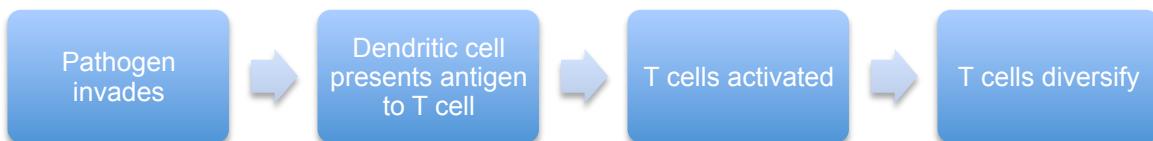
- D heavy chain, low serum concentration (0.2% serum Ig)
- As a secreted component, no biological effect
- Primarily found on B cells as part of B cell receptor complex
- Naïve B cells express both IgM and IgD

Germinal Centre Reaction Review

- Activated B cells received 1st signal, B cell receptor recognition of antigen, travel to dark zone and proliferate.
- Dark zone B cell undergo somatic hypermutation, express BCRs with higher or lower affinity for antigen
- B cells migrate to light zone, cells with strongest specificity selected (others die by apoptosis)
- Centrocytes receive signals from T cells to undergo isotype switching, leave GC and become memory B cells or antibody secreting plasma cells

Class Switching in GC

- B cells that enter GC receive signals from cytokines to undergo antibody isotype switching
- Isotype switching allows transmembrane expression and secretion of other isotypes
- Cytokines secreted by CD4+ helper T cells present in light zone



Isotype Switching allows a given V_H exon to associate with different C_H genes

- Antigen dependent
- Irreversible (deletional) DNA recombination stimulated by external signals (DNA looped out)
- Heavy chain VH remains the same, light chain unaffected, antibody retains same specificity for antigen

Cytokines secreted by T follicular helper cells determine the antibody isotype

- Cytokine from Tfh determines isotype
 - IFNg induces IgG switch
 - IL-4 or IL-3 induces IgE
 - TGF- β induces IgA switch
- Without appropriate cytokines, B cells do not switch, keep producing IgM
- CD40 ligation +
 - IFNg induces IgG switch
 - IL-4 or IL-13 induces IgE
 - TGF- β induces IgA switch

X-linked Hyper IgM

- T cells do not express CD40L
- Cannot isotype switch
- Susceptible to upper respiratory tract infection

