A Brief Introduction to Immunology
Learning Objectives

By the end of this Lecture you should know:

• The cells and soluble factors that make up the immune system
• The structure of antibodies
• What the innate immune system is
• How to describe inflammation
• How immune cells sense microbes
• How immune cells leave the circulation to a site of infection
• How immune cells eat microbes
Immunology

- Study of the immune system
- Immunity – ‘immunitas’ exemption for civic duties offered to Roman senators.
- Has evolved to protect us from pathogens
Immune System

- Must discriminate self from non-self

- **Innate Immunity** – Non-specific, instinctive, does not depend on lymphocytes

- **Adaptive Immunity** – Specific ‘Acquired’ immunity, requires lymphocytes, antibodies

- Is made up of cells and soluble proteins (humoral)
Basic Immunology - outline

- **Components of the Immune System**

- **Innate Immunity**

- **Adaptive Immunity (next lecture)**
ORIGIN OF CELLS

Haematopoietic pluripotent stem cell

Bone Marrow

- BFU-E
- CFU-erythrocyte
- Megakaryocyte
- CFU-Meg
- CFU-monoc
- CFU-gran
- CFU-eosin
- CFU-basophil
- Pro-B Cell
- Pre-B Cell
- Thymocyte

Blood

- Erythrocyte
- Platelets
- Monocyte
- Neutrophil
- Eosinophil
- Basophil
- B Cell

Tissue

- Macrophage
- Neutrophil
- Eosinophil
- Basophil
- Plasma Cell
- Lymph node

- LPS
- INFγ
- IL-4
- IL-13
- TGFβ
- M1
- M2

Thymus

- T-Reg
- T-helper
- Cytotoxic T cell

Lymph node

- Plasma Cell

- Thymus

- Myeloid Progenitor
- CFU-GM
- Myelomonocytic stem cell

- Lymphoid Progenitor

- Pro-B Cell
- Pre-B Cell

- B Cell

- T-Reg
- T-helper
- Cytotoxic T cell
Neutrophils

Polymorphonuclear leukocyte

Size 10-14µM, 3-11,000 per mm³ blood (65%)
Lifespan – 6h-12d, express CD66b

* Play important role in innate immunity
  - Phagocytosis

Have 2 main intracellular granules:
Primary lysosomes - contain myeloperoxidase, muramidase, acid hydrolases, proteins (defensins)

-Secondary granules containing lactoferrin and lysozyme

Primary lysosomes combine with phagosomes containing microbes to digest them.
Have Fc and complement receptors
Can kill microbes by secreting toxic substances (superoxides)
Monocytes

Mononuclear leukocyte

Size 14-24μM, 100-700 per mm$^3$ blood (5%)
Lifespan – months, express CD14

* Play important role in innate and adaptive Immunity, Phagocytosis & Ag presentation

Differentiate into Macrophages in the tissues

Main role – remove anything foreign (microbes) or dead

Have lysosomes containing peroxidase that can kill microbes

Have Fc, complement receptors also Pattern Recognition Receptors (PRR) Toll-like and mannose receptors – can bind to all kinds of microbes
Macrophages

‘Large eaters’

Reside in tissues, Lifespan – months/years
Eg. Kupffer cells – liver, microglia - brain

Play important role in Innate and Adaptive Immunity – Phagocytosis & Ag presentation

Most often first line of non-self recognition

Main role – remove foreign (microbes) and self (dead/tumour cells)

Have lysosomes containing peroxidase (free radicals)

Have Fc, complement receptors also Scavenger, Toll-like and mannose receptors – can bind all kinds of microbes

Present Ag to T-cells
Eosinophil

Polymorphonuclear leukocyte

Size 10-14µM, 100-400 per mm$^3$ blood (5%)
Lifespan – 8-12d, express CD125

Granules stain for acidic dyes (eosin)

Mainly associated with parasitic infections and allergic reactions.

Granules contain Major Basic Protein – potent toxin for helminth worms

MBP – activates neutrophils, induces histamine release from mast cells & provokes bronchospasm (allergy – Done in the 3$^{rd}$ year)
Basophil

Polymorphonuclear leukocyte

Size 10-12 µM, 20-50 per mm$^3$ blood (0.2%)
Lifespan – 2d

Granules stain for basic dyes

Very similar to mast cells

Express high-affinity IgE receptors (FcεR1)

Binding of IgE to receptor causes de-granulation releasing histamine – main cause of allergic reactions

mainly involved in immunity to parasitic infections and allergic reactions.
Mast Cell

Size 10-14µM,

Only in tissues (precursor in blood)

Very similar to basophils

Express high-affinity IgE receptors (FcεR1)

Binding of IgE to receptor causes de-granulation releasing histamine – main cause of allergic reactions (Done in 3rd year)

Mainly involved in immunity to parasitic infections and allergic reactions
T Lymphocytes (T cells)

Mononuclear leukocyte
Size 5-12\(\mu\)M, 300-1,500 per mm\(^3\) blood (10\%)
Lifespan – hrs – yrs, Mature in thymus (T)
Express CD3 (T cell receptor complex)

*Play major role in Adaptive Immunity*

- Recognise peptide Ag displayed by Antigen Presenting Cells (APC)

4 main types
- T helper 1 (CD4 – ‘help’ immune response intracellular pathogens)
- T helper 2 (CD4 – ‘help’ produce antibodies – extracellular pathogens)
- Cytotoxic T cell (CD8 – can kill cells directly)
- T reg (FoxP3) – regulate immune responses ‘dampen’

Found in blood, lymph nodes and spleen
B Lymphocytes (B cells)

Mononuclear leukocyte
Size 5-12 µM, 300-1,500 per mm$^3$ blood (15%)
Lifespan – hrs to yrs, Mature in bone marrow (B)
Express CD19 + 20 (depends on maturity)

* Play major role in Adaptive Immunity

Recognise Ag displayed by Antigen Presenting Cells (APC)

Express membrane bound antibody on cell surface

Differentiate into plasma cells that make Antibodies

Found in blood, lymph nodes and spleen
Natural Killer (NK) Cells

Account for 15% of lymphocytes
Express CD56, Found in spleen/tissues

Look like ‘large granular lymphocytes’

NK cells recognise and kill:
- Virus infected cells
- Tumour cells

By apoptosis – programmed cell death
Soluble factors

- Complement
- Antibodies
- Cytokines, Chemokines
Complement (C’)

Group of ~20 serum proteins that need to be ‘activated’ to be functional.

**Classical** - Ab bound to microbe

**Alternative** – C’ binds to microbe

**Lectin** – activated by mannose binding lectin bound to microbe

More on this later!
Antibodies

Hallmark of Adaptive immunity – they bind specifically to Antigen (Ag)

Immunoglobulin (Ig’s)
- soluble
- bound to B cells as part of B-cell antigen receptor

Ig’s are glycoproteins – 5 distinct classes.

IgG (IgG1-4)
IgA (IgA1 & 2)
IgM
IgD
IgE
Antibody (Ab) – protein produced in response to an antigen. It can only bind with the antigen that induced its formation – i.e. specificity.

Antigen (Ag) – A molecule that reacts with preformed antibody and specific receptors on T and B cells.

Epitope – the part of the antigen that binds to the antibody/receptor binding site.

Affinity – measure of binding strength between an epitope and an antibody binding site. The higher the affinity the better.
Antibody acts as an adapter that links a microbe to a phagocyte.
Immunoglobulin (Ig) G

secreted antibody

The basic structure of IgG1

N terminal end
variable region

antigen-binding sites

hinge region

C terminal end

heavy chain (450 residues)

light chain (212 residues)

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Predominant in human serum – 70-75% of total Ig in serum
IgM

Accounts for 10% of Ig in serum

Pentamer, formation requires J chain

Mainly found in blood – big so not cross endothelium

Mainly primary response, initial contact with Ag

The monomeric form (mIgM) is present as an antigen-specific receptor on B cells.
IgA

Accounts for 15% of Ig in serum

In humans 80% of serum IgA is as a monomer (most animals serum IgA is a dimer)

The predominant Ig in mucous secretions such as saliva, colostrum, milk, bronchiolar & genitourinary secretions - Called Secretory IgA (sIgA)

sIgA is held together with a J chain and a secretory component
IgD

Accounts for 1% of Ig in serum

A transmembrane monomeric form (mlgD) is present on mature B cells
IgE

Accounts for ~0.05% of Ig in serum

Basophils and Mast Cells express and IgE-specific receptor that has high affinity for IgE

Basophils and Mast Cells are continually saturated with IgE

Binding Ag triggers release of histamine by these cells

Associated with allergic response and defence against parasitic infections
Cytokines - proteins secreted by immune and non-immune cells

Interferons (IFN) - induce a state of antiviral resistance in uninfected cells & limit the spread of viral infection
- IFNα & β - produced by virus infected cells
- IFNγ - released by activated Th1 cells

Interleukins (IL) – produced by many cells, over 30 types
- Can be pro-inflammatory (IL1) or anti-inflammatory (IL-10)
- Can cause cells to divide, to differentiate and to secrete factors

Colony Stimulating Factors (CSF)
- Involved in directing the division and differentiation on bone marrow stem cells – precursors of leukocytes

Tumour Necrosis Factors (TNFα & β)
- Mediate inflammation and cytotoxic reactions
Chemokines - ‘Chemo’tactic cyto’kines’

Group of approx 40 proteins that direct movement of leukocytes (and other cells) from the blood stream into the tissues or lymph organs by binding to specific receptors on cells

CXCL – mainly neutrophils (but also T & B lymphocytes)

CCL – monocytes, lymphocytes, eosinophils, basophils

CX3CL – mainly T lymphocytes & NK Cells

XCL – mainly T lymphocytes

They attract leukocytes to sites of infection/inflammation – like magnets
Defence Mechanisms

**INNATE**
(Non-Specific)

- 1st line of defence
- Provides barrier to antigen
- Is present from birth

**ADAPTIVE**
(Specific)

- Response specific to antigen
- Memory to specific antigen
- Quicker response
Innate Immunity

Hallmarks:

- Primitive (spread across species)
- ‘un-learned/instinctive’ response
- Does not depend on immune recognition by lymphocytes
- Does not have long lasting memory
- Integrates with Adaptive response
Innate Immunity

Includes:

- Physical and chemical barriers
- Phagocytic cells (neutrophils and macrophages)
- Serum proteins (complement, acute phase)
Anatomical Barriers

- Skin
- Sebum (skin secretions) pH 3-5
- Intact skin

Prevents penetration
Prevents growth (low pH)
Physical Barriers

### Exterior defenses

- Lysozyme in tears and other secretions
- Commensals
- Skin: physical barrier
- Fatty acids
- Commensals
- Low pH and commensals of vagina
- Bronchi: mucus, cilia
- Gut: acid, rapid pH change, commensals, flushing of urinary tract
- Removal of particles by rapid passage of air over turbinate bones

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Mucous Membranes

- Saliva
- Tears
- Mucous secretions
- Mucous - entrapment
- Cilia – beating removes microbes
- Commensal colonies – attachment, nutrients
Physiological Barrier

- Temperature (pyrexia)
  - Chickens have high body temperature and are Anthrax resistant

- Fever response inhibits micro-organism growth
Physiological

- pH

- Gastric acidity (*Helicobacter pylori*)
  - Neonate stomach less acidic than adult
  - and so susceptible to infection

- Oxygen tension - aerobes/anaerobes
Inflammation

- Sometimes the barriers are breached
  - Tissue damage (trauma) or infection

Response

- Stop bleeding (coagulation)
- Acute inflammation (leukocyte recruitment)
- Kill pathogens, neutralise toxins, limit pathogen spread
- Clear pathogens/dead cells (phagocytosis)
- Proliferation of cells to repair damage
- Remove blood clot – remodel extracellular matrix
- Re-establish normal structure/function of tissue
Inflammation

‘A series of reactions that brings cells and molecules of the immune system to sites of infection or damage’.

Hallmarks:

- Increased blood supply
- Increased vascular permeability
- Increased leukocyte transendothelial migration ‘extravasation’
Inflammation

- **Acute Inflammation**
  - Complete elimination of a pathogen followed by resolution of damage, disappearance of leukocytes and full regeneration of tissue

- **Chronic Inflammation**
  - Persistent, un-resolved inflammation
Sensing Microbes

In blood – Monocytes, Neutrophils
In tissues – Macrophages, Dendritic cells

PRR – Pattern Recognition Receptors (on cells)

PAMP – Pathogen-Associated Molecular Patterns (on microbe)
Sensing Microbes – Toll-Like Receptors

TLRs recognise Pathogen-Associated Molecular Patterns expressed by microbes

TNFα, chemokines, ICAM-1 etc
Complement (C’)

Overview of the complement activation pathways:
- Classical pathway activators: C1q, C1r, C1s
- MBL (MBL-1, MASP-1, MASP-2)
- Lectin pathway activators: C1q, C1r, C1s

The membrane attack pathway:
1. Membrane attack pathway (C5b-8)
2. C5 convertase (C6, C7, C8)
3. C9

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Complement

C' can:

- Lyse microbes directly (Membrane Attack Complex)
- Increase chemotaxis (C3a and C5a)
- Opsonisation (C3b)
Extravasation

Leukocyte migration across endothelium

- Shear force and high charge prevent adhesion
- High shear
- Endothelium
- Low charge
- Adhesion molecules
- Migration
- Venules
- Capillaries
Extravasation

Lumen of post-capillary venule

Endothelium

Infection

Tissue

Neutrophil

Chemokine

Integrin (CD18/CD11b)

E-Selectin

Adhesion Molecule (ICAM-1)

CD15

Chemokine Receptor

GAG

CD31/PCAM-1

TNFα

BUG
Phagocytosis

Phagocytosis – 'cell eating'
Mainly by Macrophages and neutrophils (also by dendritic cells - adaptive)

Phagolysosome

Phagocytosis mediated by opsonic receptors

1. binding
   - bacterium
   - C3b
   - carbohydrate

2. engulfment
   - FcR
   - CR
   - MR

3. phagosome formation
   - acidification
   - cytotoxic molecules
   - proteolysis
   - FA/11 (macrophalin, CD68)

4. lysosome fusion
   - digestion
   - MHC class II
   - antigen presentation
   - secretion
     - H₂O₂, NO, TNFα, etc.

5. membrane disruption/fusion

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N = nucleus
B = Phagocytosed Bacteria
Phagocytosis

http://www.youtube.com/watch?v=UeuL3HPfeQw


http://www.youtube.com/watch?feature=player_detailpage&v=fpOxgAU5fFQ
Phagocytosis

Human macrophage eating a polystyrene bead and *Mycobacterium tuberculosis* (see arrow). These can live in macrophages.
Mechanisms of Killing

Two killing pathways present in polymorphs and macrophages.

- **O₂-dependent**

  Reactive Oxygen Intermediates (ROI)
  
  Superoxides (O₂⁻) converted to H₂O₂ then ·OH (free radical)
  
  Nitric Oxide (NO) - vasodilation (Viagra) increases extravasation but also directly anti-microbial.

- **O₂-independent**

  Enzymes - defensins (insert into membranes), lysozyme
  
  pH, TNF
Inflammation – accessory molecules

- Acute phase proteins (present in blood and increase during infection)

C Reactive Protein
Serum protein produced by liver, binds to some bacterial cell walls (pneumococci). Promotes opsonisation, binds to C1q and activates C’

Mannose binding lectin (MBL)
Binds to lectin on microbes, promotes opsonisation (via MBLR) and activates C’

Surfactant protein-A (SP-A)
Binds haemagglutinin in influenza – reduces ability of virus to infect cells
INNATE IMMUNITY

- Effective but limited
- Can be evaded
- Supplements and augments Adaptive immunity.

Adaptive immunity has:-

- antigen specificity and diversity
- immunological memory
- specific self/non-self recognition
Defence Mechanisms

**INNATE**  
*(Non-Specific)*
- 1st line of defence
- Provides barrier to antigen
- Is present from birth
- No memory
- Not require lymphocytes

**ADAPTIVE**  
*(Specific)*
- Response specific to antigen
- Memory to specific antigen
- Quicker response
- Requires lymphocytes

Innate and adaptive responses are integrated
Why do we need Adaptive Immunity?

It’s a WAR

- Microbes evade innate immunity (Dr Stafford) (proteases, decoy proteins, etc)
- Intracellular viruses and bacteria ‘hide’ from innate immunity
- Need memory to specific antigen – ‘seen it before so faster response’
  - Cell Mediated - T cells - intracellular microbes
  - Humoral (Ab) - B cells - extracellular microbes
Recap

Antibody acts as an adapter that links a microbe to a phagocyte.
Cell-Mediated Immunity

Interlay between:
- Antigen Presenting Cells (APC)
  - Macrophages
  - Dendritic Cell
  - B cells
- T cells

Requires intimate cell to cell contact
- control Ab responses via contact with B cells
- directly recognise and kill viral infected cells
Cell-Mediated Immunity

Also Requires
Major Histocompatibility Complex (MHC)
Intrinsic/Endogenous (intracellular) antigens
Extrinsic/Exogenous (extracellular) antigens
Recognise ‘Self’ or ‘Non-Self’
T Cells

**DO NOT** respond to soluble antigens only intracellular antigens ‘presented antigens’

T cells that recognise ‘self’ are killed in the foetal thymus as they mature (called **T cell selection**)

TCR recognises foreign antigens in association with **Major Histocompatibility Complex (MHC)**
T Cell Receptor

T Cell Receptor (TCR) - structure similar to Fab Ig’s

Heterodimers – 90% are αβ, 10% γδ
T Cells recognise Ag with MHC
Major Histocompatibility Complex (MHC)

Display peptides from **self OR non-self proteins** (eg. degraded microbial proteins) on the cell surface – invasion alert

In humans coded by **Human Leukocyte Antigen (HLA)** genes

- **MHC I** – coded by HLA (A, B & C genes) - glycoproteins on **ALL** nucleated cells (graft rejection).
- **MHC II** - coded by HLA (DP, DQ& DR) - glycoproteins **ONLY** on APC.
- **MHC III** – code for secreted proteins (complement)
MHC

Class I
- α1
- α2
- α3
- β2m

8-10 aa peptide

On all Cells

Class II
- α1
- β1
- α2
- β2

13-24 aa peptide

On APC
### MHC & T Cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>MHC</th>
<th>T cells</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td><strong>Intrinsic</strong></td>
<td>Class I</td>
<td>Tc (CD8)</td>
<td>Kill infected cell with intracellular pathogen</td>
</tr>
<tr>
<td>Intracellular</td>
<td>All cells</td>
<td></td>
<td></td>
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<tr>
<td>(eg. virus)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Extrinsic</strong></td>
<td>Class II</td>
<td>Th (CD4)</td>
<td>Help B cells make Ab to extracellular pathogen, can help directly kill</td>
</tr>
<tr>
<td>Extracellular</td>
<td>APC only</td>
<td></td>
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<tr>
<td>(phagocytosis)</td>
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Phagocytosis
T Cell Ag Recognition & Activation

Much more complex than this – as usual!!

Involves co-stimulatory molecules
CD28 on T cell bind to CD80/CD86 on APC

This is required for full activation

Activation
IL-2 is secreted & binds to IL-2R on T cells (autocrine)

Leads to:
division, differentiation, effector functions, memory
Functional T cells

**IFNγ Helps Kill Intracellular pathogens**

**IFNγ**

**Naive**
- **αβ T**
- **CD4**
- **CD8**

**IL-12 hi**
- **Th1**

**IL-12 lo**
- **Th2**

**IL-2**
- **IL-4,5,10**

**Ab production**

Kill Intracellular pathogens directly
Tc (CD8) Activation

CD8 + MHCI/peptide = Tc / CTL (effector cell)
CTL forms proteolytic granules & releases perforins and granulysin
Also induces apoptosis (cell suicide)
Th1 (CD4) Activation

APC presents Ag with MHC II to naïve CD4 T cell
Stimulation with high levels of **IL-12** activate naïve cells to Th1 cells
Th1 cells go to secondary lymphoid tissue (spleen, lymph nodes)
Activated Th1 (CD4) cells proliferate (clonal expansion)
Th1 cell recognises Ag on infected cells (with MHC II) via TCR (CD4)
Th1 secretes INFγ – stop virus spread (and apoptosis)
Humoral adaptive immunity

B cell activation

- B cells express membrane bound Ig (IgM or IgD monomer)

- Each B cell can only make 1 Ab that will only bind one epitope on one Ag

- We are born with more than $10^9$ immature B cells – enough to detect every single possible epitope on all antigens - ever!

- B cells that recognise self are killed in BM
B Cells Present Ag to T Cells via MHC II

Monomeric IgM (or mlgD) binds Ag

Phagocytosis

Peptide displayed on surface with MHC II

TCR of naive Th (CD4) binds to MHC II

Lots of other co-stimulatory molecules required.
T cells love helping B cells

APC eats Ag (extrinsic) and presents it to naïve CD4+ T cells (via MHC II)

These turn into Th2 cells

Th2 cells bind to B cells that are presenting Ag (via MHC II). This Ag has been captured using the mIgR on cell surface.

Th2 cell now secretes cytokines (IL-4, IL-5, IL-10 and IL-13)

These cause B cells to divide – CLONAL EXPANSION and differentiate into

Plasma cells (AFC = antibody forming cell) and Memory B cells (Bm)
**B cell activation - Summary**

- Upon binding an Ag that ‘fits’ B cells become activated.

- Activated B cells go to the lymph nodes where they **proliferate** *(clonal expansion)* and **differentiate** into plasma cells.

- These plasma cells secrete Ab of same specificity but are generally IgM – these later turn into IgG but still have same specificity to the same Ag *(class switching)*.

- Some still express cell surface mIgM and recalculate for months *(Memory B cells)*.

- Re-stimulation of Memory B cells leads to secondary response - *This is very quick*.
Ab effector Functions

Specific secreted Ab may

- Neutralise toxin by binding to it
- Increase opsonisation – phagocytosis
- Activate complement

**Link between Innate and adaptive immunity**
Vaccination

- **Eg.** Tetanus vaccine
  - Tetanus toxoid from *Clostridium tetani* causes muscle contractions/spasms

- Treat purified toxoid in the lab with formalin – loss of toxicity but **NOT** epitopes (shape)
Vaccination

Principle of vaccination

- Vaccination
  - Toxoid
- Primary antibody response
  - Toxin
- Natural infection
- Secondary antibody response
  - Acquired immunity

Antibody response over time:
- Memory cells formed
Summary of Adaptive Immunity

Functions of different types of lymphocyte

- **Th1**
  - Activation
  - Antigen presentation
  - Macrophage

- **Th2**
  - Activation
  - Antigen presentation
  - B cell
  - Antibody production

- **CTL**
  - Cytotoxicity
  - Virally infected cell and some tumor cells

- **LGL**

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Reading


Any problems e-mail – c.murdoch@sheffield.ac.uk