Imperial College London

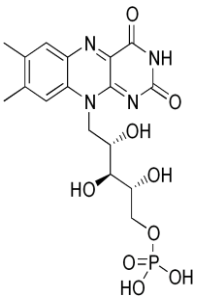
School of Medicine

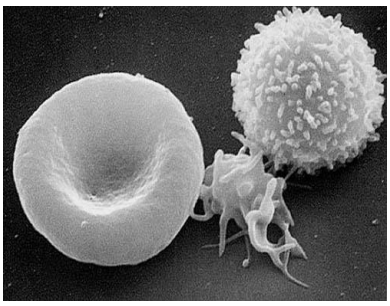
Year 1 – 2012/13

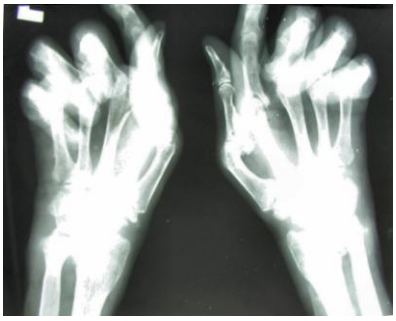
Molecules Cells and Disease

Lecture notes volume 2

Nov 6 - Dec 11, 2012







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MCD Year 1 Lecture notes – volume 2

This volume contains notes for lectures given from 6th November to 11th December 2012. They are provided in the order of presentation and are so printed that you can disassemble this book and reassemble notes in Course order to assist with revision, if you wish.

SOLE FEEDBACK – Molecules, Cells and Disease

The following pages provide you with templates on which you can record your thoughts as the course proceeds. At the end of the course you can enter your views onto SOLE.

Please answer all questions by selecting the response which best reflects your view.

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
The content of this module is useful.	☐	☐	☐	☐	☐
The support materials available for this module (e.g. handouts, web pages, problem sheets) are helpful.	☐	☐	☐	☐	☐
I receive sufficient feedback and guidance.	☐	☐	☐	☐	☐
Overall, I am satisfied with this module.	☐	☐	☐	☐	☐

Please use this box for constructive feedback and suggestions for improvement.

SOLE FEEDBACK - INDIVIDUAL LECTURERS

Please note that for SOLE, a Lecturer's name will only appear once. This template gives you the opportunity to record your comments about each lecture in the order of delivery.

On the following section, you have an opportunity to record any comments and constructive feedback you have for each lecturer.

	The lecture(s) are well structured					**The lecturer explains concepts clearly**					**The lecturer engages well with the students**				
Lecturer and Lecture Title	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Birgit Leitinger Tissues 3: Extracellular matrix biology I	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peter Clark Tissues 4: Fluid compartments of the body	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Birgit Leitinger Tissues 5: Extracellular matrix biology II	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Alexandra Blakemore Genetics 7: Complex genetic diseases – can genes make us fat?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Charles Bangham Immunology 1: Introduction to immunology	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keith Gould Immunology 2: Immune cells and organs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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Sohag Saleh Tissues 6: Nerve	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keith Gould Immunology 3: Innate immunity	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sohag Saleh Tissues 7: Muscle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sohag Saleh Tissues 8: Signalling between cells I	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keith Gould Immunology 4: Antibodies	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ingrid Muller Immunology 5: B-Lymphocytes	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
David Holden Microbiology 1: Bacterial properties	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keith Gould Immunology 6: T-Lymphocytes and antigen recognition	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sohag Saleh Tissues 9: Signalling between cells II	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ingrid Muller Immunology 7: Effector T-Lymphocytes	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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Ingrid Muller Immunology 8: Regulation of lymphocyte responses	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shiranee Sriskandan Microbiology 2: Bacterial diseases	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Andrew Edwards Microbiology 3: Hospital acquired infection and antibiotic resistance	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rob Goldin Cell pathology 1: Cell injury	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peter Openshaw Immunology 9: Host defence overview	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wendy Barclay Microbiology 4: Viral properties	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Elaine Bignell Microbiology 5: Fungal infection	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wendy Barclay Microbiology 6: Patterns of viral infection	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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Lecturer and Lecture Title	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
James Carton Cell pathology 2: Haemodynamic disorders	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mary Thompson Cell pathology 3: Inflammation	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mike Osborn Cell pathology 4: The autopsy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wendy Barclay Microbiology 7: Prevention and treatment of viral disease	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rathi Ramakrishnan Cell Pathology 5: Cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wendy Barclay Microbiology 8: Evolution and emergence of new viruses	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ian Feavers Microbiology 9: Defence and vaccination against bacteria	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rob Goldin Cell Pathology 6: Cell pathology case studies	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

TISSUES 3:
Extracellular matrix biology I

Dr. Birgit Leitinger

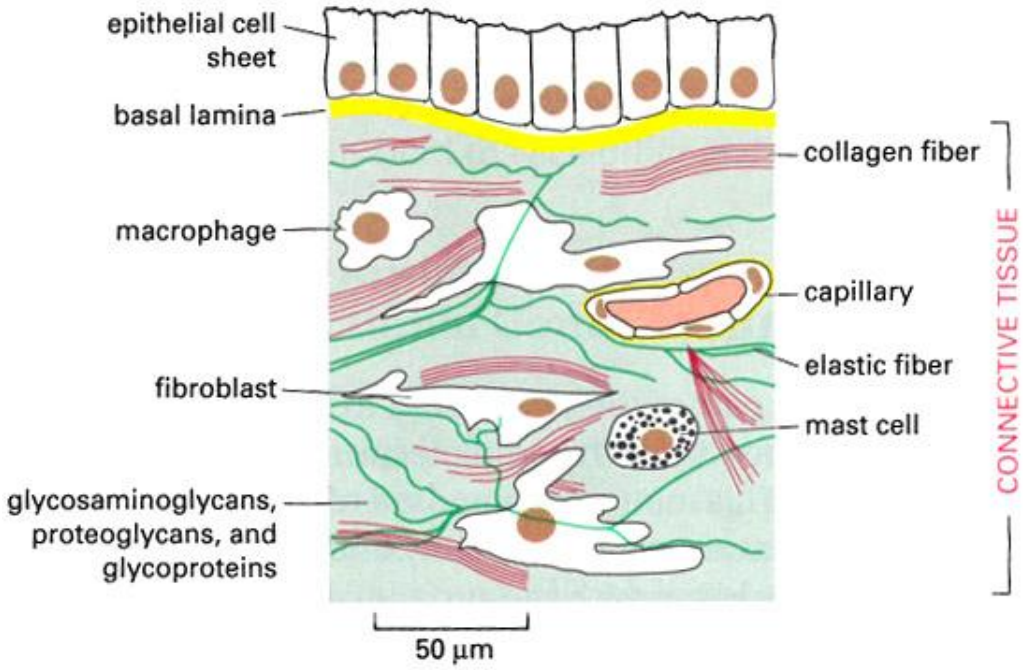
Extracellular matrix (ECM)

The ECM is a complex network of macromolecules (proteins and carbohydrates) deposited by cells. It becomes immobilised outside the cells and fills the spaces between cells.

The ECM plays both architectural (mechanical stability) and instructional roles (influences cell behaviour).

Connective tissues are tissues rich in ECM.

The main components of ECM are collagens, glycoproteins and proteoglycans.



Collagens

A family of fibrous proteins found in all multicellular organisms

Are the most abundant proteins in mammals

Major proteins in bone, tendon and skin

At least 28 different collagen types are known

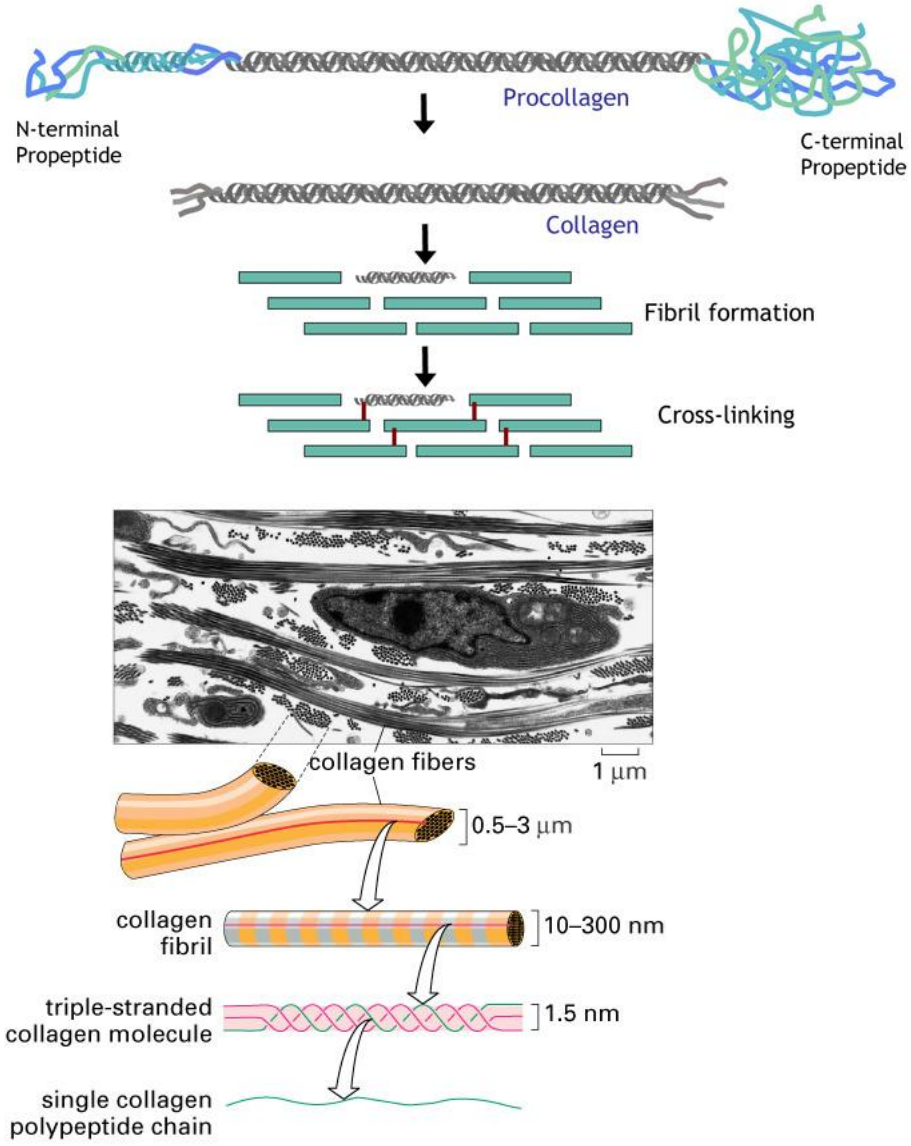
Each collagen molecule comprises three a chains, forming a triple helical structure
In the triple helix every third position must be occupied by glycine, as there is no room near the helix axis for the side chain of any other residue

Collagen biosynthesis

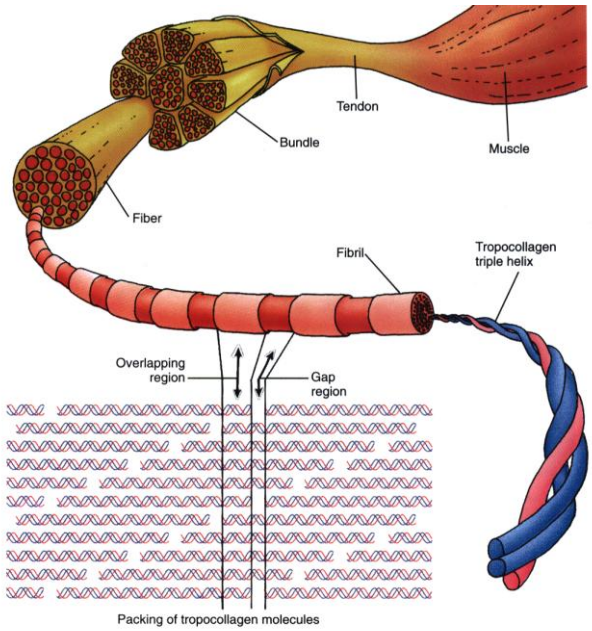
Collagen biosynthesis and secretion follows the normal pathway for a secreted protein, but the collagen a chains are synthesised as longer precursors, called pro-α chains, by ribosomes attached to the endoplasmic reticulum. The pro-α chains undergo a series of covalent modifications and fold into triple-helical procollagen molecules before their release from cells.

After secretion from cells, extracellular peptidases remove the propeptides from procollagen. In the case of fibrillar collagens, the resulting collagen molecules associate laterally to generate fibrils. There are covalent cross-links between collagen molecules in the fibril, providing tensile strength and stability.

Assembly of collagen into fibrils and fibers

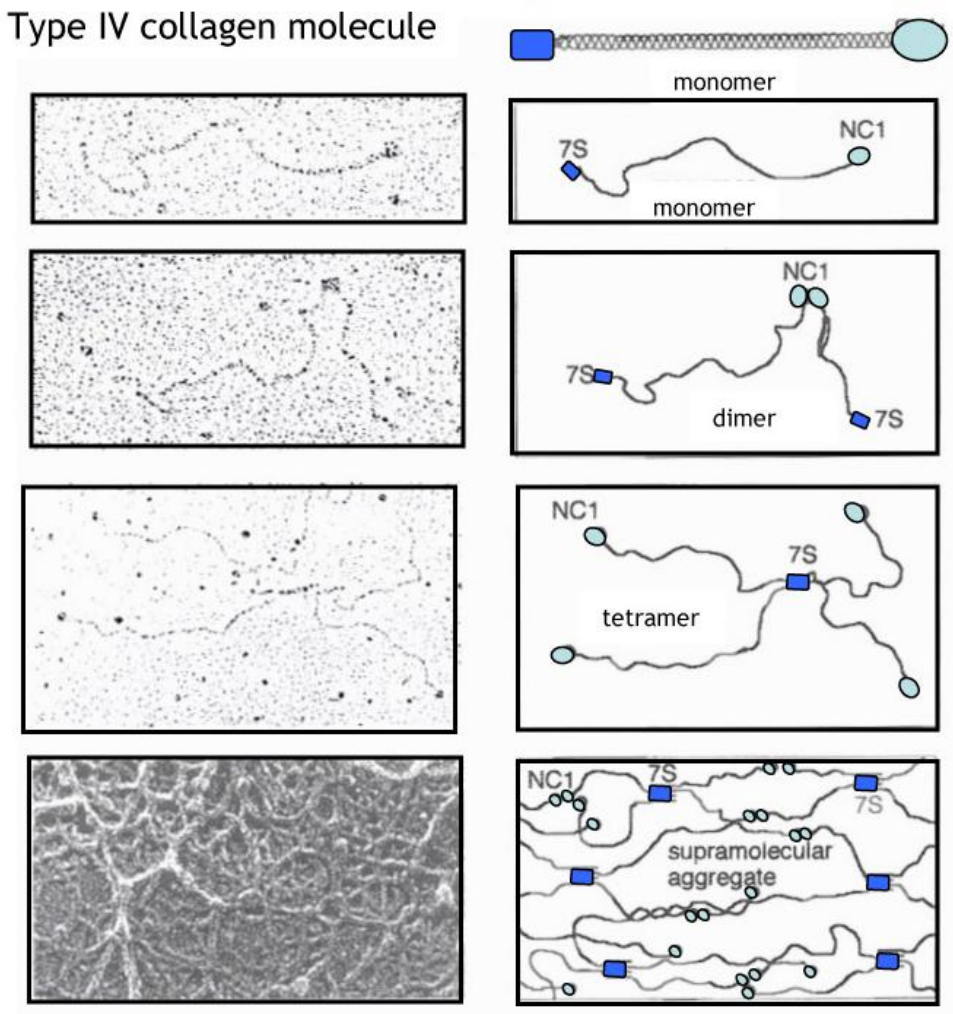


The parallel bundles of collagen fibres in the tendon resist tensile force in one direction



Non-fibrillar collagens

Some collagens are fibril-associated and regulate the organisation of collagen fibrils in tissues



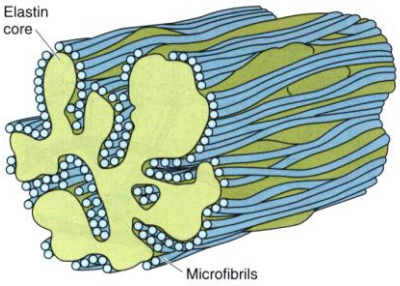
An important non-fibrillar collagen is the net-work forming collagen type IV, which is present in all basement membranes.

In the collagen IV net-work, the collagen type IV molecules can associate laterally between triple-helical segments as well as head-to head and tail-to tail between the globular domains to give dimers, tetramers and higher order complexes.

Elastic fibers

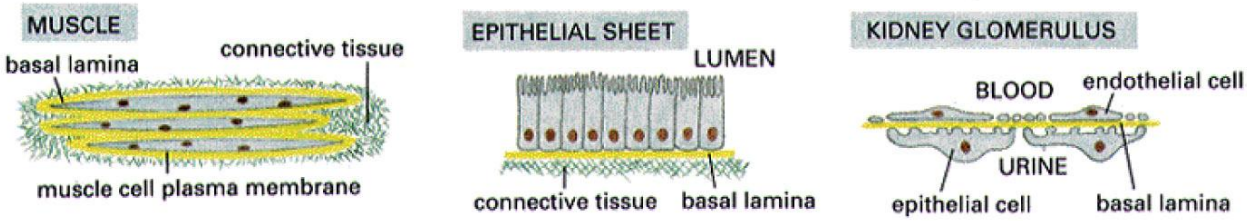
Another type of protein fiber. While collagens are important for the tensile strength of tissues, elastic fibers are important for the elasticity.

Consist of a core made up of the protein elastin, and microfibrils rich in the protein fibrillin.

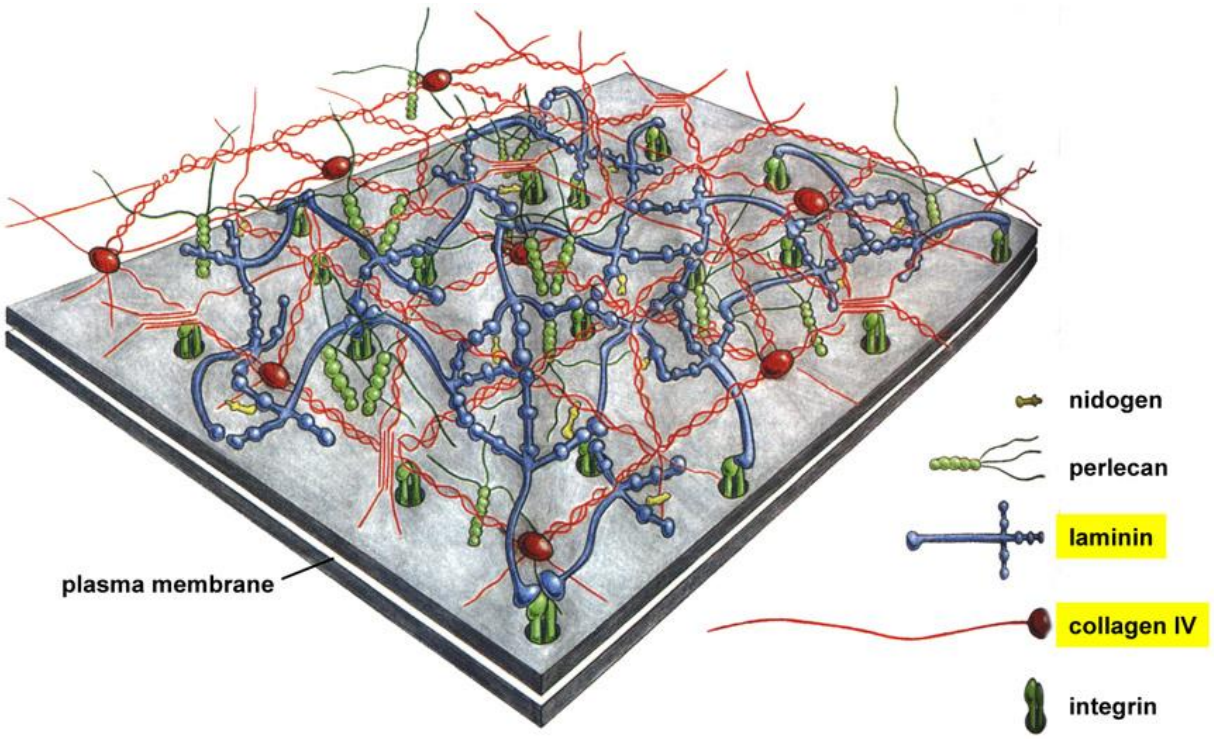


Basement membranes (BMs)

Are also called basal lamina
Are flexible, thin mats of ECM underlying epithelial sheets and tubules
The main components of BMs are collagen type IV and laminins
BMs separate cells from the underlying connective tissue
In the kidney glomerulus, the BM acts as a highly selective filter



Model of basement membrane structure



TISSUES 4
Fluid Compartments of the body
Dr Peter Clark

Learning objectives

- List the main fluid compartments in the body, and give the approximate size of each.
- List the main features of the composition of each compartment.
- Define osmosis, diffusion and permeability.
- Define haemolysis. Describe the composition of a solution that would cause haemolysis of red blood cells.
- Explain how tonicity is different from osmolarity.
- Briefly describe the main mechanisms by which solutes exchange across cell membranes. For each mechanism give an example solute and state whether this is an active or passive process.
- Define oedema, and outline its causes.
- Describe the main types of exchange across the capillary wall, including exchange through endothelial cells and through the pores between endothelial cells.

Lecture Outline

A. Size and composition of fluid compartments
B. Tonicity vs Osmosis. Membrane permeability
C. Transport across membranes
D. Exchange across capillary wall

Use the following notes along with the images in the powerpoint file on the Intranet.

A. Main Fluid Compartments & Sizes in a 70 kg man
Intracellular (IC, inside cells) = 23 L = 55% of body water.
Extracellular (EC, outside cells) = 19 L= 45% of body water.
Interstitial fluid (IF, between cells) = 15 L =36% of body water. It is the fluid between cells.
Blood plasma (fluid part of blood, no cells) = 3 L = 7% of body water.
Transcellular fluid (fluid within epithelial lined spaces) = 1 L = 2% of body water. It includes, for example, cerebrospinal (around brain and spinal chord), ocular (in eye), synovial (in joints) fluid.

Composition of Main Fluid Compartments. The table gives concentrations in units of mmol/l (= 10^{-3} mol/l). Plasma values are given as an example of extracellular fluid, and skeletal muscle values are given as an example of intracellular fluid.
Na^+ is main extracellular cation.
K^+ is main intracellular cation.
Free Ca^{2+} has very low intracellular concentration. It is an important intracellular signalling ion. Low concentration means that adding or removing a small number of ions makes a big change in concentration.
Cl^- is main extracellular anion.
Organic phosphates are main intracellular anions.
Proteins (highly charged anions) is in both extra- and intracellular fluid, but is higher in intracellular fluid.
pH = -log of H^+ concentration in mol/litre. For example, fluid with a H^+ concentration 10^{-7} mol/l has a pH of 7.0. Note that H^+ concentration is *very* low in both fluids, and is higher in intra- than extracellular fluid.

Osmolarlity is the *same* in extra- and intracellular fluids because most cells and tissues are permeable to water (except a few very exceptional locations in the body, for example parts of the kidney).

B. Tonicity vs Osmosis. Membrane permeability

Osmolarity is the concentration of all the particles in a solution. For example, 1mmol/l NaCl solution contains 2mmol/l particles because each NaCl dissociates in Na^+ and Cl^- in water.

Osmosis moves water to the area of higher osmolarity (=area of lower water concentration).
Diffusion moves solutes to an area of lower solute concentration

Permeability**:** how easily a solute crosses a membrane.

3 examples follow showing the effects of diffusion and osmosis one cell volume:
(1) **Initial osmolarity inside cell > osmolarity outside cell**. Membrane permeable to (lets through) both H_2O and solute.
End point reached when:
Solute concentrations equal, so no further diffusion of solute
Water concentrations equal, so no further osmosis
Final volumes same as initial volumes.

(2) **Initial osmolarity inside cell > osmolarity outside cell**. Membrane permeable to H_2O, but **impermeable** to all solutes (does not let solutes through).
End point reached when:
Solute concentrations equal, so no further diffusion of solute
Water concentrations equal, so no further osmosis
Final volumes different than initial volumes; cell has swelled up.

(3) **Initial osmolarity inside cell > osmolarity outside cell.** Membrane permeable to H_2O, and permeable to one type of solute, but **impermeable** to the other.
This case is different than (2) because the difference (inside vs outside) in osmolarity of the impermeant solute is greater now.
End point reached when:
DISASTER! cell bursts when enough water enters cell to stretch membrane to its breaking point. Red blood cell bursts (haemolysis) when membrane area approximately doubles.

Take-home-point is that osmolarity of a solution, which was the same in (2) and (3), is not a reliable guide to its effects on cell volume. More useful is...

Tonicity defines the "strength" of a solution as it affects final cell volume.
Tonicity depends on both cell permeability and osmolarity. Osmolarity does not depend on cell permeability.
Tonicity depends on both the extracellular solution and also on the cell types. The membranes of different cell have different degrees of permeability to many different solutes.

- if cell shrinks in the solution, that solution is **hypertonic**.
- if cell swells in the solution, that solution is **hypotonic**.
- If cell volume is unchanged, that solution is **isotonic**.

C. Transport Across Cell Membranes

Passive - DOWN an electrochemical gradient (gradient of charge & conc).

a. Through lipid - examples: lipids, oxygen, carbon dioxide, steroid hormones.

b. Through pores (channels) - examples - water, ions, urea.
characteristics – specific.
some are gated (open & closed states) by chemical ligands or by voltage.

c. On carriers.
characteristics – specific binding of carrier to solute, then conformational change.
example: facilitated diffusion, such as, transport of lactic acid out of skeletal muscle cells intointerstitial fluid.

Active - can transport UP an electrochemical (charge & conc) gradient.
On carriers. characteristics – specific binding of carrier to solute, then conformational change.

a. primary active transport - uses energy from ATP hydrolysis coupled to "up-hill" movement of a different solute; example - Na/K pump.
b. secondary active transport - uses energy from "down-hill" movement of one solute coupled to "up-hill" movement of a different solute. There are many examples of Na^+ moving "down-hill" into cell and another solute (H^+, Ca^{2+}, glucose, etc) moving "up-hill".

Endocytosis and exocytosis – encapsulation in membrane as solute enters or leaves the cell.
characteristics - generally large molecules.
examples: Endocytosis of nerve growth factors (proteins) entering. Exocytosis of peptide hormones from endocrine glands.

D. Exchange across the Capillary Wall

Oedema (edema) is swelling of a tissue due to excess interstitial fluid.
Causes:

1) imbalance of forces causing fluid to move between the blood plasma, interstitium, and lymphatic vessels. (Lymph vessels return excess interstitial fluid to the blood)

2) increased permeability of capillary walls to plasma proteins.
Relevant structures: endothelial cells and clefts between endothelial cells.

Small, water-soluble substances pass through the clefts between cells.
Lipid-soluble substances pass through the endothelial cells.
Exchangeable proteins are moved across endothelial cells by vesicular transport.

***Note**:* Plasma proteins generally *cannot* cross the capillary wall unless it is damaged (for example due to trauma).

TISSUES 5
Extracellular matrix Biology II
Dr. Birgit Leitinger

ECM molecules are large, modular glycoproteins
Most ECM proteins are very large and have a modular architecture. They are composed of characteristic protein domains of 50-200 amino acids. By being built in this way, ECM proteins can be multifunctional and interact with a number of different interaction partners. Most major ECM proteins are glycosylated and are either glycoproteins or proteoglycans.

Laminins
Consist of three very large chains (α, β, and γ), forming a cross-shaped molecule.

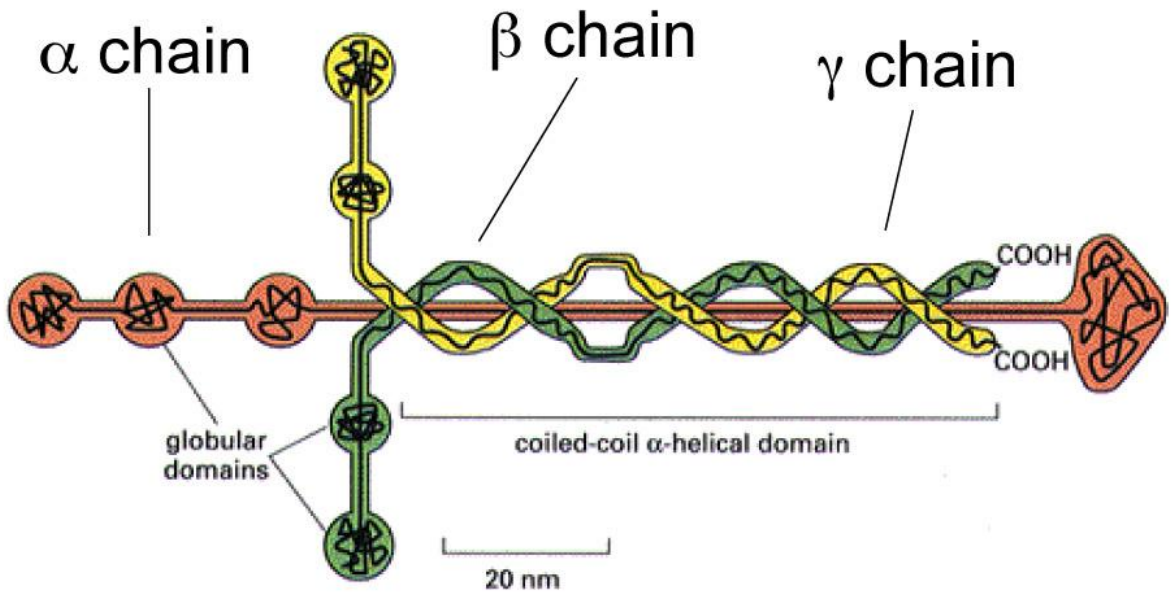
Interact with cell surface receptors such as integrins and dystroglycan.

Can self-associate as part of the basement membrane or associate with other BM components such as collagen IV, nidogen and proteoglycans.

Specific chain mutations are associated with inherited diseases, such as muscular dystrophy or epidermolysis bulosa.

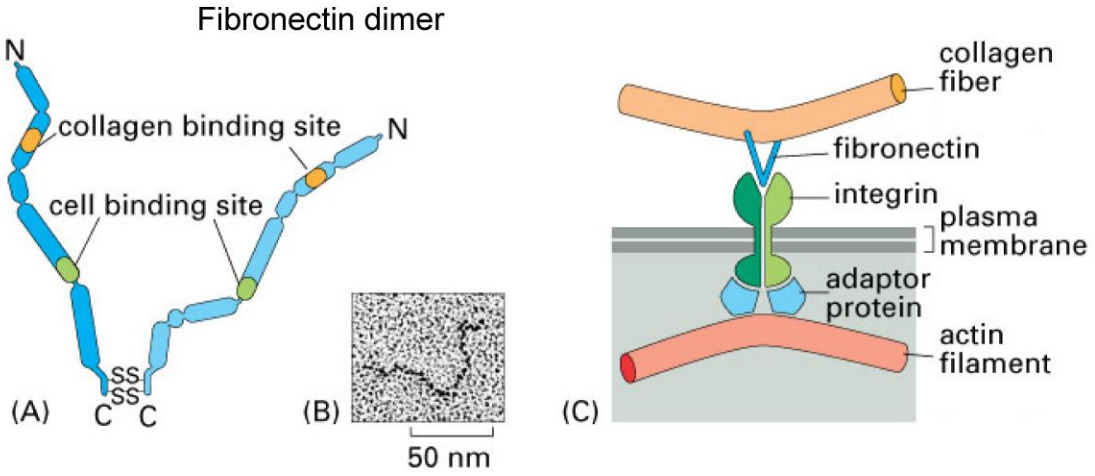
Have many functions such as tissue differentiation, cell-matrix junction formation, cell migration.

In congenital muscular dystrophy the α2 chain of laminin 2 is lacking.



Fibronectins
Fibronectins are a family of closely related glycoproteins of ECM and body fluids.
They can exist as an insoluble fibrillar matrix in the ECM or as soluble plasma proteins.
One gene encodes fibronectin; alternative splicing generates different isoforms.
Fibronectins have important roles in regulating cell adhesion, migration, embryogenesis and tissue repair. There are no known human mutations, consistent with the idea that fibronectin is essential for life.
Fibronectin is a multi-domain, modular protein that interacts with many ECM components and cell surface receptors. It forms disulfide-linked dimers.

Fibronectin forms a mechanical continuum with the actin cytoskeleton of many cell types. Integrin receptors at the cell surface bind to fibronectin on the outside of the cell, and are linked to the actin cytoskeleton inside the cell.



Proteoglycans
Proteoglycans are composed of a core protein to which one or more glycosaminoglycan (GAG) chains are attached. GAG chains are long unbranched sugars consisting of repeating disaccharide units. GAGs occupy a huge volume relative to their mass. They can form hydrated gels that are resistant to compression.

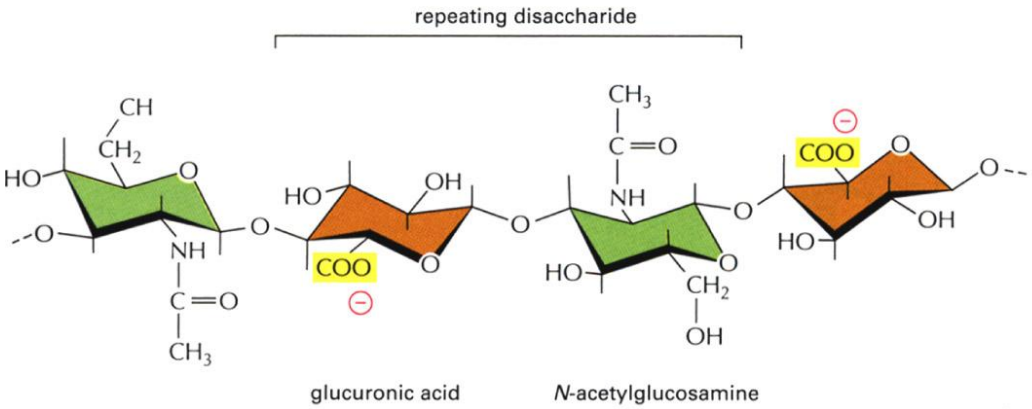
Many GAG chains are sulfated and highly negatively charged. Small proteoglycans (e.g. decorin) have a single GAG chain attached, whereas some large proteoglycans carry up to 100 GAG chains (e.g. aggrecan).

Depending on the nature of the repeating disaccharide unit, the GAG chains are called chondroitin sulfate, dermatan sulfate, heparan sulfate, or keratan sulfate.

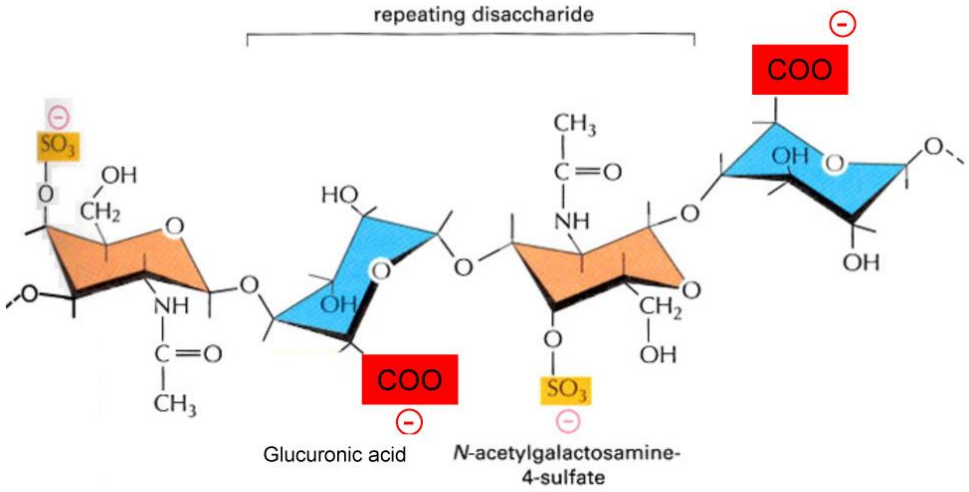
Hyaluronan (also called hyaluronic acid) is spun out directly from an enzyme embedded in the plasma membrane. All other GAGs are synthesised and attached to their core proteins in the endoplasmic reticulum and Golgi apparatus inside the cells.

Hyaluronan is unique in being simply a carbohydrate chain. All other GAGs are attached to core proteins, forming proteoglycans.

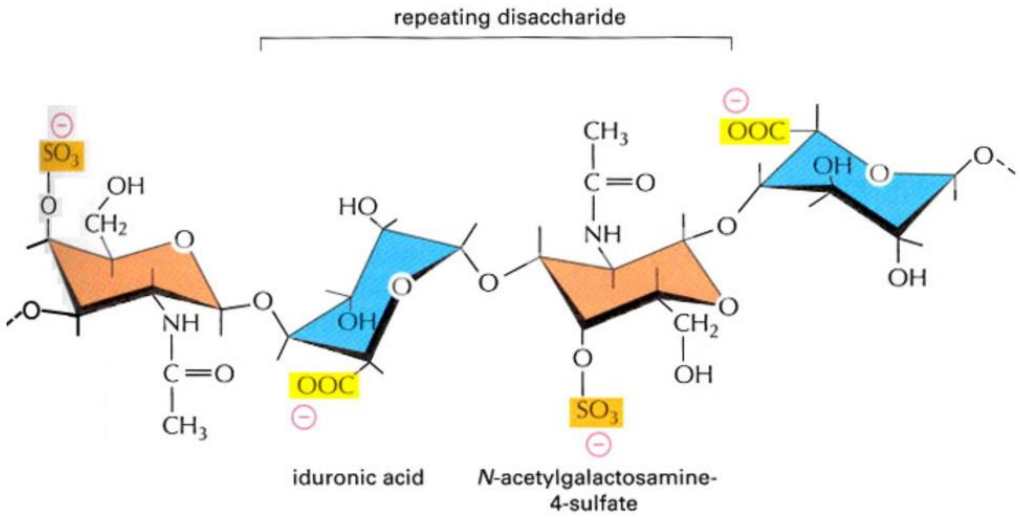
Hyaluronan



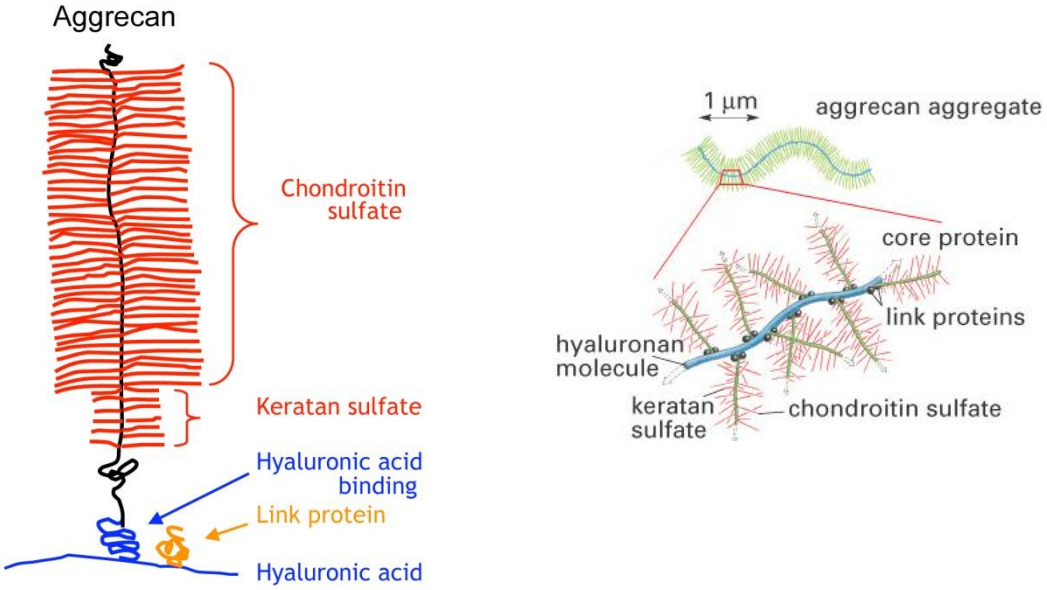
Chondroitin sulfate



Dermatan sulfate



Aggrecan
A major cartilage matrix constituent is the proteoglycan aggrecan. It associates with hyaluronan and forms supramolecular complexes (aggrecan aggregates). There are three types of molecules in the aggregates: aggrecan (a proteoglycan), hyaluronan (a GAG) and link protein. Aggrecan is highly sulfated and negatively charged. It can retain large quantities of water. Under compressive load, water is given up, but regained upon reduction of the pressure. In this way, aggrecan can resist compressive forces in the cartilage.



Osteoarthritis
Osteoarthritis is an erosive disease resulting in excessive ECM degradation.
The cushioning properties of cartilage over the end of bones are lost. With age aggrecan is cleaved by aggrecanases and metalloproteinases. This results in loss of aggrecan fragments to synovial fluid.

Decorin
A small proteoglycan with a single dermatan sulfate chain. Decorin binds to collagen fibers and regulates fibrillogenesis. Mice that cannot make decorin have a fragile skin with reduced tensile skin.

GENETICS 7
Complex genetic diseases – can genes make us fat?

Dr Alexandra Blakemore

What is a complex genetic disease? Heterogeneity.

How do we know whether a condition is “genetic”?
Twin studies can be used to assess heritability (the proportion of variance due to genetic effects). Monozygotic twins are (nearly!) genetically identical, whereas dizygotic twins share 50% of genes. Differences between MZ twins are largely environmental, whereas DZ twins have both genetic and environmental differences. Comparison of **concordance rates** between MZ and DZ twin pairs allows us to estimate heritability. Adoption or other types of study can also help.
http://tinyurl.com/28olgda

Heritability of obesity is similar to that of height.
http://www.ajcn.org/cgi/reprint/87/2/275.pdf

Monogenic obesity is caused by defects in the leptin-melanocortin pathway, which operates in the brain (hypothalamus) to regulate eating behavior and energy expenditure. Single gene defects in this pathway are found in up to 1 in 20 morbidly obese children. The most common is *MC4R* deficiency, which is autosomal dominant.

There are over 40 genetic diseases or syndromes which include obesity:
Prader-Willi syndrome, Bardet-Beidl syndrome, WAGR-O, etc.

Common obesity:
Genome wide SNP association studies have revealed that common SNPs in over 30 genes are associated with obesity, but the effects are subtle and these markers (including FTO) have poor predictive power for individual subjects.

The findings do not explain the high heritability of obesity. Potential reasons include:

- Inflated estimates of heritability
- Epigenetic factors
- Rare rather than common variants are involved
- Other types of genetic variant, eg. genomic copy number variants

http://jcem.endojournals.org/cgi/content/full/93/11_Supplement_1/s51
Two separate CNVs at 16p11.2 have recently been shown to cause obesity. Both have very high penetrance. One accounts for almost 1% of morbid obesity and the other raises BMI by around 5 points
http://www.nature.com/nature/journal/v463/n7281/full/nature08727.html
Mendelian forms of obesity are not rare. Implications for personalised medicine…….

IMMUNOLOGY 1
Introduction to Immunology

Prof. Charles Bangham (c.bangham@imperial.ac.uk).

After this lecture you should be able to

- Explain the importance of immunology for human health.
- Outline the basic principles of immune responses, and the timescales in which they occur.
- Define the terms antigen, antibody, B lymphocyte, T lymphocyte, active immunity, passive immunity, primary and secondary immune responses.
- Outline the concept of clonal selection, and its role in immune responses.
- Understand the roles of *natural selection* and the *physical organization* of the immune system in its function.

Basic principles of the immune response and the timescale

Immune system recognizes non-self material that enters the body and responds to it.
Innate immune system works rapidly (minutes) and has broad specificity.
Adaptive immune system takes longer (days) and has exquisite specificity.

Definition of terms

Antigens are molecules which react with antibodies or T cells. However not all antigens can *induce* an immune response in the host: those that can are termed ***immunogens****.*

Antibody molecules can be found in the blood stream and the body fluids and bind specifically to particular molecules termed antigens. They are the *acquired* component of the *humoral* immune response.

Lymphocytes are mononuclear cells which are part of the leukocyte (white blood) cell lineage. They are subdivided into B (Bone marrow-derived) and T (Thymus-derived) lymphocytes. Lymphocytes express *antigen receptors* on their surface to enable recognition of a specific antigen

Naïve lymphocytes have never encountered the antigen to which their cell surface receptor is specific and thus have never responded to it.

Memory lymphocytes are the products of an immune response, enabling the specificity of their specific receptor to remain in the pool of lymphocytes in the body.

Innate immunity An early phase of the response of the body to possible pathogens, characterized by a variety of non-specific mechanisms (e.g. barriers, acids or enzymes in secretions) and also molecules and receptors on cells which are ***Pattern Recognition Molecules*** which recognize repeating patterns of molecular structure found on the surface of microorganisms. The innate immune response does not generate *memory*.

Adaptive immunity is the response of antigen-specific lymphocytes to antigen, and includes the development of immunological memory. Adaptive responses can increase in magnitude on repeated exposure to the potential pathogen and the products of these responses are specific for the potential pathogen. Also known as ***Specific Immunity*** or ***Acquired Immunity.***

Active Immunity is the induction of an immune response by the introduction of antigen.

Passive Immunity is immunity gained without antigen induction i.e. by transfer of antibody or immune serum into a naïve recipient.

Primary Response is the response made by naïve lymphocytes when they first encounter their specific antigen.

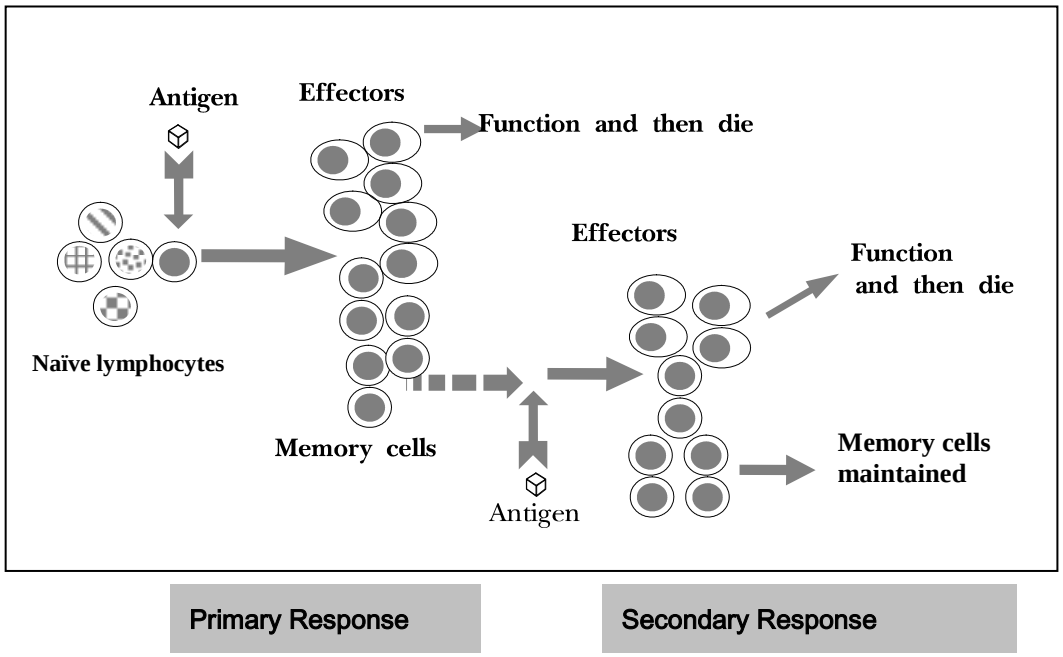
Secondary Response is the response made by memory lymphocytes when they re-encounter the specific antigen.

T cells Originate in the thymus. They recognize antigen presented at the cell surface by MHC molecules. Surface markers found on T cells are CD3, CD4, & CD8.

B cells Originate in the bone marrow. They recognize free antigen in the body fluids Surface markers associated with B cells are CD19, Surface Immunoglobulin Class II MHC

Clonal selection and its role in immune responses.

T and B cells produced in the primary lymphoid organs are released into the peripheral lymphoid pool. Those that meet their specific antigen proliferate and produce effector and memory cells. Those that do not meet there antigen die. The size of the peripheral lymphoid pool is regulated by homeostatic mechanisms



Primary and secondary responses

In the primary response, naïve lymphocytes are activated by antigen to ensure proliferation of a lymphocyte with the appropriate receptor for the antigen. This is at the centre of the immune response. After the antigen has been removed a few lymphocytes with the appropriate receptor specific for that antigen remain (the rest die). These are memory lymphocytes and memory of a specific infection can last for years.

So some years later there can be a *secondary* response which is more effective than the primary response. This greater effectiveness is due to its greater magnitude and more rapid onset than the primary response.

Physical organization of the immune system is important for its function

The tissues are patrolled by lymphocytes, antibodies and antigen-presenting cells.
For example, the skin contains lymphatic vessels that drain into local lymph nodes.
Gut lymphoid tissue controls responses in the intestinal tract.
Antigens present in the blood are taken to the spleen.

IMMUNOLOGY 2
Immune Cells and Organs

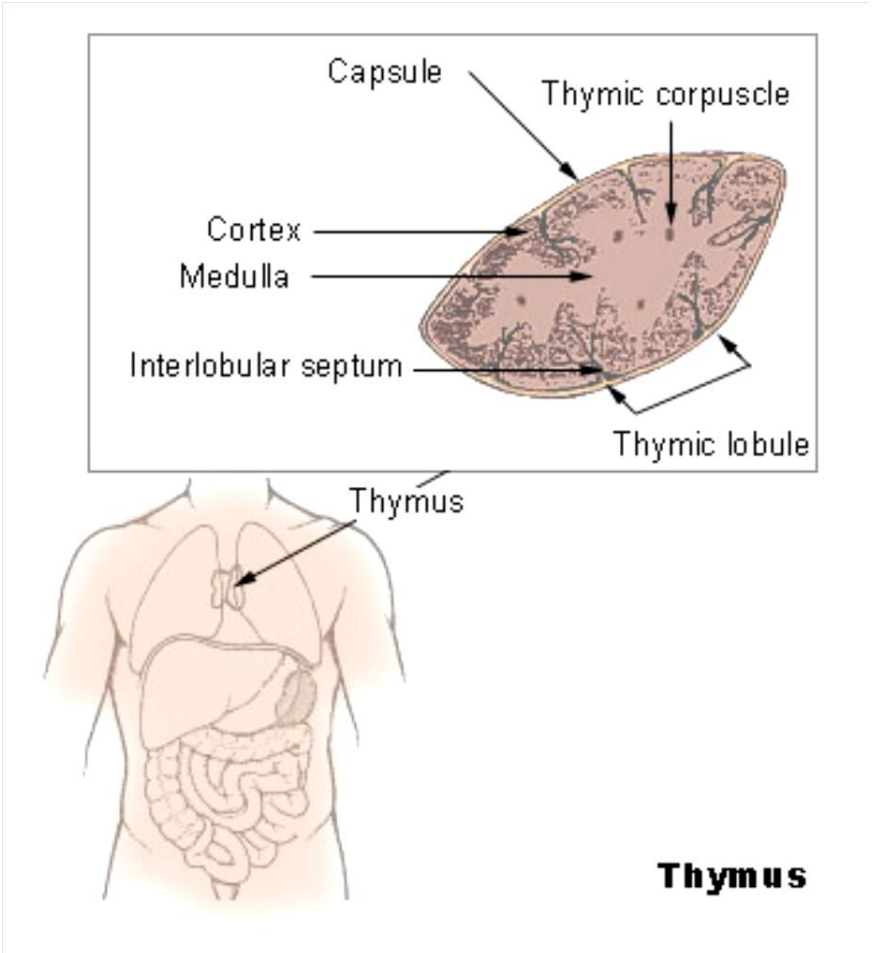
Dr Keith Gould

Learning Objectives

- Name the primary & secondary lymphoid organs and briefly differentiate between their functions.
- Draw simple diagrams to illustrate the structure of the thymus, lymph node, spleen, Peyer's patch, and indicate the changes that occur after stimulation by antigen.
- Outline the re-circulation of lymphocytes.
- Explain the use of CD markers for discrimination between lymphocytes.
- Compare and contrast phenotypic characteristics of B and T cells.
- Give examples of antigen presenting cells and their locations.

Primary Lymphoid Organs are the major sites of lymphopoiesis, the generation of lymphocytes. Here lymphoid stem cells differentiate into mature functional lymphocytes. The primary lymphoid organs are the thymus and bone marrow.

The Thymus is bi-lobed in mammals, located in the thorax.
Each lobe organised into lobules & in each lobule are histologically defined regions of cortex and medulla. The cortex contains the immature thymocytes, some of which are selected to become mature thymocytes in the medulla. There is a great deal of cell death in the thymus and only a small percentage of the cells (about 5%) exit the thymus into the peripheral T cell pool. The mammalian thymus atrophies with age & areas of active T cell production are replaced with adipose tissue.



***The Bone Marrow*;** In the foetus, the liver is an active prior to most bones becoming active sites of production. In later life active sites may include spongy regions at the end of long bones; also vertebral bones, sternum, ribs, flat bones of the cranium and pelvis. Bone marrow produces is stem cells and B lymphocytes. Those stem cells destined to be T lymphocytes migrate to the thymus throughout life. For B cells, differentiation is centripetal with the stem cells under the bone and the most mature phases of the B cell pathway found nearer the centre of the marrow.

Secondary lymphoid organs provide an environment in which lymphocytes can interact with antigen and with other lymphocytes: they are the sites at which antigen, antigen presenting cells and mature lymphocytes come together to initiate an immune response. They have special vascular adaptations to recruit lymphocytes from the blood. Secondary lymphoid tissue includes the spleen, lymph nodes, and mucosa associated lymphoid tissues (MALT).

The spleen contains 2 main types of tissues, the red pulp and white pulp. The red pulp acts as a general filter for blood & the white pulp is the lymphoid tissue and constitutes the major initiator of responses to blood-borne antigens. Around the central arteriole are concentric areas of lymphoid tissue = the periarterial lymphatic sheath (PALS). The region nearest the arteriole is a T cell zone. Periodically, there are B cell follicles, either primary or secondary and around this is the marginal zone which seems to be the primary site of entry of B and T cells into the white pulp.

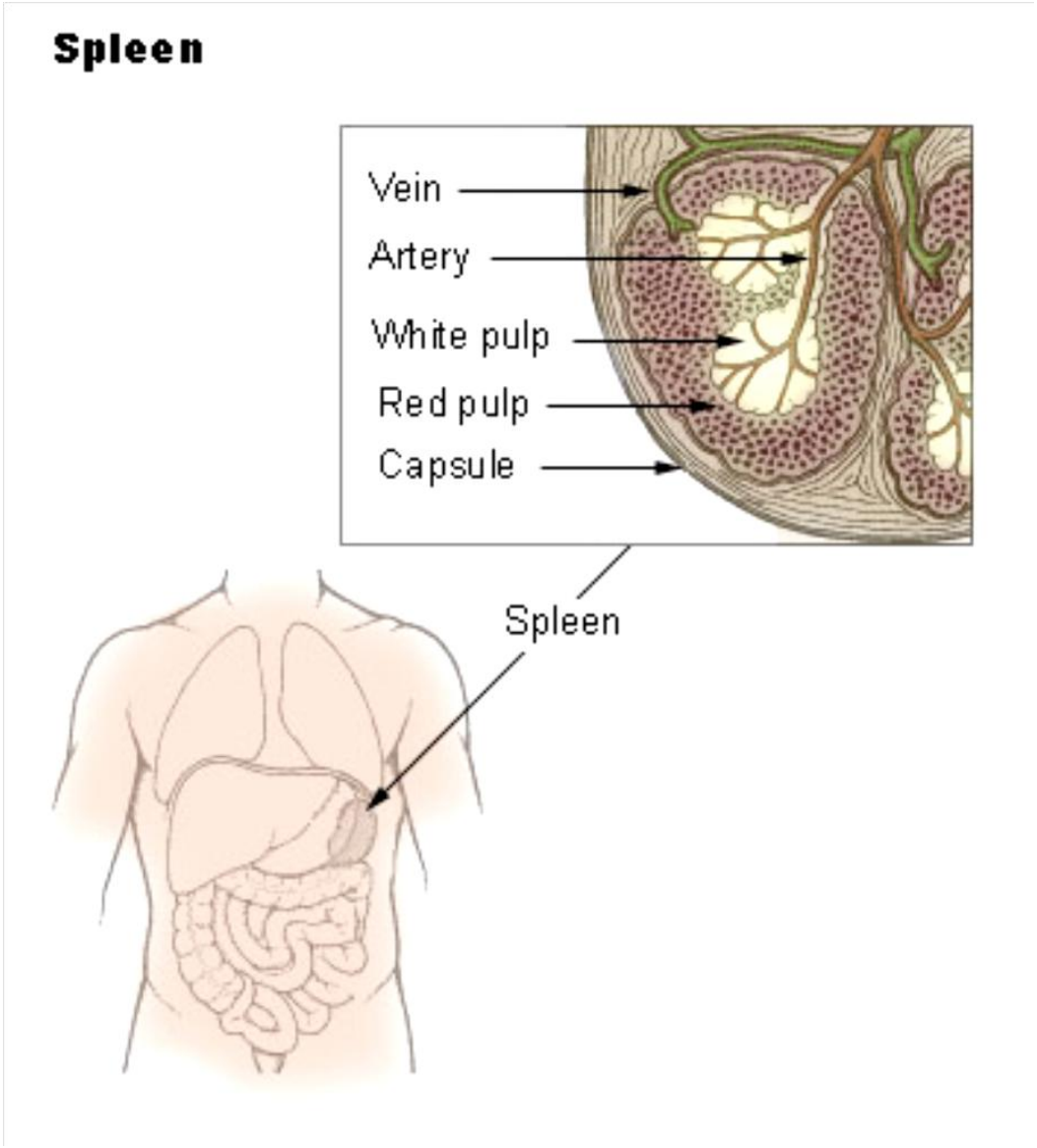
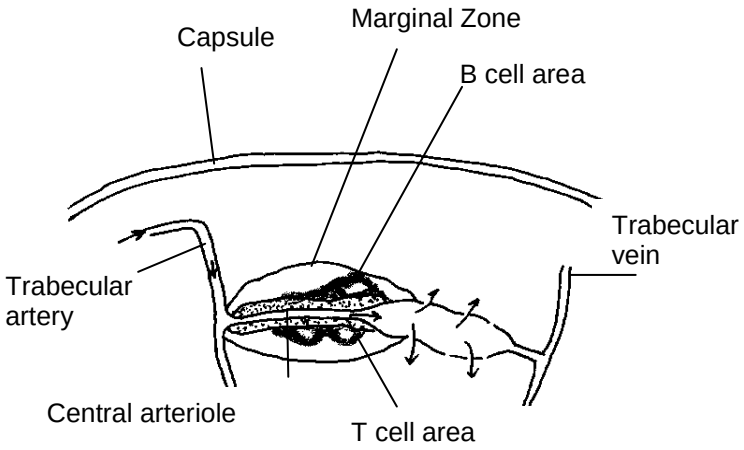
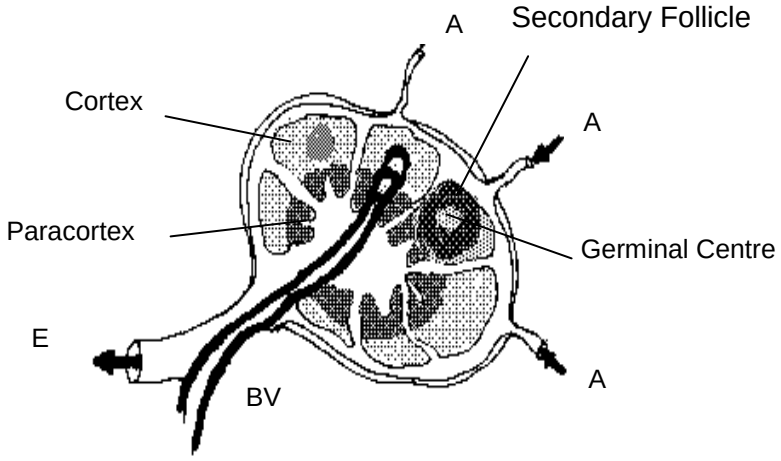


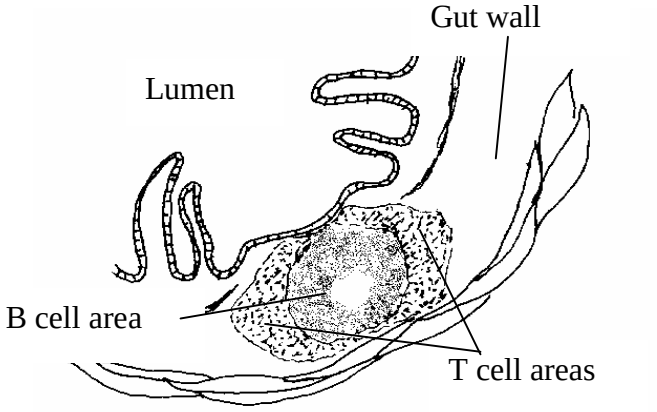
Diagram of part of the Spleen



Human lymph nodes are 1-15 mm across, are round or kidney shaped and have an indentation at the hilus where the blood vessels (BV) enter and leave the node. Lymph arrives at the lymph node through several AFFERENT vessels (A) and leaves through one EFFERENT vessel (E) at the hilus. During passage of lymph through the node there is removal of particulate antigens by the phagocytic cells and then this is transported to the lymphoid regions of the node. The cortex is a B cell area and the paracortex is a T cell area.



Mucosal Associated Lymphoid Tissue (MALT) are aggregates of lymphoid tissue which do not have a tough outer capsule. They are found especially in the lamina propria, and sub-mucosal areas of the gastrointestinal, respiratory and genito-urinary tracts. Typical examples of MALT are tonsils and appendix. Other examples include Peyer's patches (see below). These are organised regions of lymphoid tissue found in the wall of the gut.



Lymphocyte recirculation
B and T lymphocytes which have matured in the bone marrow or thymus and which have not yet encountered antigen are called *naïve* lymphocytes. These cells circulate constantly from the blood into the secondary lymphoid organs, and leave the vasculature through a specialised section of the post capilliary venule known as the high endothelial venule (HEV). They move from the lymph node to the lymphoid vessels and eventually return to the blood via the thoracic duct. In the presence of an infection those cells which recognise the infectious agent are held in the lymphoid tissue where they proliferate and differentiate.

The CD system of nomenclature

The *cluster of differentiation* (CD) system is a way of identifying cell surface molecules present on leukocytes. The name comes from the fact that such molecules were first identified using monoclonal antibodies made in different laboratories around the world, and each set of antibodies that recognise a particular molecule forms a "cluster". Since 1982, International workshops have been held regularly to assign CD numbers to particular surface molecules. The CD system is commonly used as cell markers, and there are now more than 300 CD numbers assigned. Of course, they are not just markers but have important functions.

Comparisons:

T cells	B cells
Recognise processed antigen presented at the cell surface by MHC molecules	Recognise intact, free antigen (not presented by MHC molecules)
Antigen receptor is either $\alpha\beta$ or $\gamma\delta$ TCR	Antigen receptor includes cell surface antibody
All T cell express CD3 $\alpha\beta$ T cells express CD4 or CD8	Express CD19 & CD20 at surface
Produced by Thymus	Produced by Bone Marrow

Antigen Presenting Cells
Cells that can present antigen to lymphocytes in an immunogenic form are collectively termed antigen presenting cells, or APC's.

Cell	Location	Presents to
Dendritic cells	Widespread e.g. skin & mucosal tissue; migrate to draining lymph nodes	T cells
Follicular dendritic cells	lymphoid follicles	B cells
B cells	lymphoid tissue	T cells
Activated Macrophages	lymphoid tissue, peripheral tissues	T cells

TISSUES 6
Nerve

Dr Sohag Saleh NB: Please see corresponding lecture slides for diagrammatic representations

Learning objectives

1. Be able to name the different cell types within the nervous system and be aware of their functions
2. Have a detailed knowledge of the major components of a neurone
3. Understand how the resting membrane potential is generated and how this relates to function
4. Understand the processes of intercellular communication between cells of the nervous system

Background

The central nervous system (CNS) consists of the two cerebral hemispheres, the brainstem, the cerebellum and the spinal cord. The peripheral nervous system (PNS) consists of the nerve fibres originating from the CNS.

The cerebral hemispheres (also known as the telencephalon) have a distinctive convoluted surface appearance where the ridges are called gyri (singular gyrus) and the valleys are called sulci (singular sulcus). Each hemisphere is conventionally separated into four functionally distinct regions or lobes:

i. Frontal: Responsible for executive functions such as personality
ii. Parietal: Contains the somatic sensory cortex responsible for processing tactile information
iii. Temporal: Contains important structures such as the hippocampus (short term memory), the amygdala (behaviour) and Wernicke's area (auditory perception & speech)
iv. Occipital: Processing of visual information

The brainstem consists of the medulla, pons and the midbrain. These structures have a multitude of important functions and are the target or the source of all the cranial nerves.

The cerebellum is located towards the dorsal region of the CNS and is attached to the brainstem. It has an important role in motor coordination, balance and posture.

Finally the spinal cord extends down from the medulla and acts as a conduit for neural transmission but can coordinate some reflex actions

Cells of the nervous system

1. Neurones

The neurones are the most extensively studied cell type within the CNS and due to their polymorphous nature they cannot really be classified on the basis of shape, location or function. A mature neuron is a non-dividing excitable cell whose main function is to receive and transmit information in the form of electrical signals. Although they come in a variety of shapes and sizes they do share a number of distinguishing features

Soma (Cell body, perikaryon): The soma contains the nucleus of the cell and a lot of the protein generating machinery i.e. ribosomes. In neurons it only accounts for around 10% of the total surface area of a cell.

Axons: These are long thin processes that arise from the '*axon hillock'* region of the cell body and are responsible for transmitting the signals from a neuron. Most neurons only have a single axon, which is intermittently covered in a high resistance, low capacitance substance called myelin, which allows faster transmission of electrical signals.

Dendrites: These are also thin but highly branched outgrowths from the cell body and they receive input from other neurones thereby regulating the excitability of the cell body.

2. Astrocytes

Astrocytes are the most abundant cell type in the mammalian brain. They function as structural cells and are known to play an important role in cell repair, synapse formation, neuronal maturation and plasticity.

3. Oligodendrocytes & Schwann cells

Oligodendrocytes are the myelin producing cells of the CNS, whilst Schwann cells perform the same function in the PNS. Each oligodendrocyte cell body sends out numerous projections that form internodes of myelin covering the axons of neurons. Whilst each oligodendrocyte is capable of myelinating a number of axons a Schwann cell only myelinates a single axonal segment.

4. Microglia & Ependyma

Microglial cells are specialised cells that are similar to macrophages and they perform immune functions in the CNS. Ependymal cells are epithelial cells that line the fluid filled ventricles regulating the production and movement of cerebrospinal fluid.

Resting membrane potential (RMP)

There is an ionic imbalance between the extracellular fluid and the intracellular fluid of a neuron with an unequal distribution of the major physiological ions (see table 1). These concentrations are determined by the activities of a variety of membrane bound channels and transporters.

Table 1: Ionic composition of extracellular and intracellular fluids

Ion species	Intracellular concentration	Extracellular concentration
Sodium (Na^+)	5-15 mM	140-155 mM
Potassium (K^+)	140-160 mM	2-5 mM
Calcium (Ca^{2+})	~100 nM	1-2 mM
Chloride (Cl^-)	70-140 mM	5-10 mM

The relative concentrations of these ions is one of the factors that gives the cell membrane an electromotive force (emf) or a potential difference between the inside and the outside of the cell. Conventionally the outside of the cell is referred to as the zero reference point and has a voltage of 0mV, the inside of the cell (in particular the area immediately adjacent to the cell membrane) has a negative membrane potential of around -50 to -90 mV in neurones. Thus neurones are said to have a resting membrane potential (RMP) of around -70 mV.

Action potential

The differences in ionic composition (see table 1) are exploited by neurones to manipulate the membrane potential. If, for example the membrane potential becomes more negative the cell is said to be hyperpolarised whereas if the membrane potential becomes more positive the cell is said to be depolarised. When a cell is sufficiently depolarised an action potential is generated, where there is a brief depolarisation spike in the membrane potential (to around +10 mV) before returning back to the RMP. This action potential is transmitted along the membrane and axon by means of cable transmission and it is the ability to propagate action potentials, which makes these cells 'excitable'.

Ion movement during an action potential

Na^+ and K^+ ions play the most important roles in the generation of a neuronal action potential.

- At the RMP voltage-gated Na^+ channels (VGSCs) and voltage-gated K^+ channels (VGKCs) are in the closed configuration
- Membrane depolarisation causes opening of the VGSC and Na^+ influx results in depolarisation
- The VGKC opens at a slower rate and causes the efflux of K^+ from the cell causing membrane repolarisation (return to the RMP from the depolarised state)
- The action potential leaves an imbalance in the ionic concentrations, which need to be restored
- The Na^+-K^+-ATPase (pump) performs the function of restoring the ion gradients
- In the 'resting' configuration Na^+ enters the vestibule and upon phosphorylation the ions are transported through the protein
- The Na^+ is removed from the cell in the 'active' configuration and K^+ enters the vestibule
- As the pump returns to the resting configuration the K^+ is transported back into the cell and the ion balance is restored

Saltatory conduction

Although unmyelinated axons do exist, the vast majority of neuronal axons are intermittently wrapped in myelin produced by the oligodendrocytes. The unmyelinated regions between each covered area are known as the nodes of Ranvier and these areas have a high density of VGSCs and VGKCs. Since the myelin wrapping has a high resistance and a low capacitance an action potential will 'jump' from one node of Ranvier to the next, thus propagating the action potential along the axon at a faster rate. This type of electrical transmission utilised exclusively by neuronal axons is known as saltatory conduction.

Neuronal intercellular signalling

Although the action potential can be transmitted along a continuous axon by 'jumping' between the nodes of Ranvier they are unable to jump from one neurone to another. The small gaps that exist between two neurones are known as synapses.

The synapse

The synapse itself is a junction consisting of a pre-synaptic nerve terminal (e.g. the axon terminal), which is separated from the postsynaptic cell (e.g. the dendrite of another neurone) by an extracellular space known as the synaptic cleft. Since the electrical signal cannot jump over the synaptic cleft it is converted into a chemical signal to cross the synapse and then back into an electrical signal on the post-synaptic cell.

Chemical transmission at a synapse

Transmission at chemical synapses is based on an elaborate sequence of events and the process is initiated when an action potential arrives at the terminal of the presynaptic neuron:

1) The action potential leads to the opening of voltage-gated Ca^{2+} channels in the presynaptic membrane.
2) Opening of these channels causes a rapid influx of Ca^{2+} into the presynaptic terminal
3) This elevation allows synaptic vesicles, containing neurotransmitter, to fuse with the plasma membrane of the presynaptic neuron.
4) Fusion of synaptic vesicles with the terminal membrane causes the neurotransmitters to be released into the synaptic cleft. This is known as exocytosis.
5) Following exocytosis, the neurotransmitter diffuses across the synaptic cleft and binds to specific receptors on the membrane of the postsynaptic neuron.
6) The binding of neurotransmitter to postsynaptic receptors cause channels in the postsynaptic membrane to open or close, thus changing the ability of ions to flow into or out of the postsynaptic cells.
7) The released neurotransmitter is subsequently either metabolised within the synaptic cleft or taken up by transport proteins into the presynaptic terminal where it is recycled.

Although this type of chemical transmission is conventionally attributed to neurones there is a growing body of evidence which has shown that astrocytes are also able to release neurotransmitters in response to Ca^{2+} influx and they are also involved in the reuptake of neurotransmitter.

Learning objectives revisited

1. **Be able to name the different cell types within the nervous system and be aware of their functions**

Neurones: excitable cells that contain a cell body (soma), an axon and dendrites. They are involved in communication
Astrocytes: star shaped cells that provide support for neurones and are involved in cell repair and neurotransmitter reuptake
Oligodendrocytes & astrocytes: myelin producing cells
Microglia: neuronal macrophages

2. **Have a detailed knowledge of the major components of a neurone**

Soma: the cell body with nucleus and intracellular organelles; Axon: thin outgrowth responsible for the conduction of an action potential, generally covered in myelin; dendrites: highly branched outgrowths of the soma.

3. **Understand how the resting membrane potential is generated and how this relates to function**

RMP exists due to ionic imbalance, high extracellular Na^+ and high intracellular K^+. Opening on voltage-gated Na^+ and K^+ channels causes ion movement down concentration gradients i.e. influx of Na^+ (leading to depolarisation) and efflux of K^+ (leading to repolarisation). The brief depolarisation is called an action potential and is propagated along an axon by cable transmission and saltatory conduction.

4. **Understand the processes of intercellular communication between cells of the nervous system**

Intercellular communication between neurones occurs at synapses. At the axon terminal (or the presynaptic junction) the arrival of an action potential triggers the influx of Ca^{2+} into the cell. The Ca^{2+} influx results in exocytosis of neurotransmitter containing vesicles. The neurotransmitter is released into the synaptic cleft and binds to receptors located on the postsynaptic cell. The neurotransmitter is then recycled and repackaged into vesicles.

IMMUNOLOGY 3
Innate Immunity

Dr Keith Gould

Learning objectives:

- Briefly describe the functions of the important phagocytic cells: neutrophils, monocytes/macrophages.
- Define cytokines and describe their general properties.
- Define complement, list its major functions, and draw a ***simple*** diagram of the complement pathways.
- Describe a typical inflammatory response to a localised infection involving recruitment of neutrophils, and phagocytosis and killing of bacteria.
- Briefly outline the events involved in a systemic acute phase response.
- Outline the phenotype and functions of natural killer (NK) cells.

INNATE IMMUNITY

Present from birth ("inbuilt"); discriminates between self and non-self using *pattern recognition receptors* which recognise *pathogen-associated molecular patterns* (e.g. lipopolysaccharide), but does not use antigen-specific receptors (as used by lymphocytes); not enhanced upon second exposure (no *immunological memory*); uses cells and soluble components in body fluids; provides a *rapid response* (minutes to hours) that cooperates with and directs subsequent antigen-specific, adaptive immunity.

PHAGOCYTIC CELLS

engulf invading organisms, try to kill them, release signals to alert other cells to the infection.

Neutrophils (polymorphonuclear leukocytes); abundant relatively short-lived leukocytes, circulate in blood and migrate out into tissues to sites of damage/infection. Highly phagocytic cells, antigen uptake is more efficient after *opsonisation*, the process of coating of micro-organisms with proteins that are able to be bound by phagocytes. *Antibody* and *complement* function as *opsonins* in this way. Neutrophils have multiple killing mechanisms, the most important of which involve the oxygen-dependent *respiratory burst*. Neutrophil deficiency is associated with infections due to extracellular bacteria and fungi.

Macrophages are the other main phagocytic cells, found in tissues.

Monocytes are the blood form of macrophages. As well as engulfing and trying to kill invading organisms, macrophages are particularly important for their role in secreting "*alarm" cytokines*, in particular *interleukin-1 (IL-1), IL-6, IL-8* and *tumour necrosis factor alpha (TNF-α)*.

Dendritic cells found in the skin and near mucosal epithelia also recognise pathogens, secrete cytokines, engulf pathogens and migrate to local lymph nodes to present antigens to the adaptive immune system.

CYTOKINES: *many different small secreted proteins involved in cell to cell communication*; *have biological effects at very low concentrations, are short-lived, and generally act locally*. *They often have effects on more than one cell type, and are produced in combinations rather than individually in isolation, leading to a "cytokine milieu"or "cocktail"*.

Different categories are:

Interleukins - communicate between leukocytes

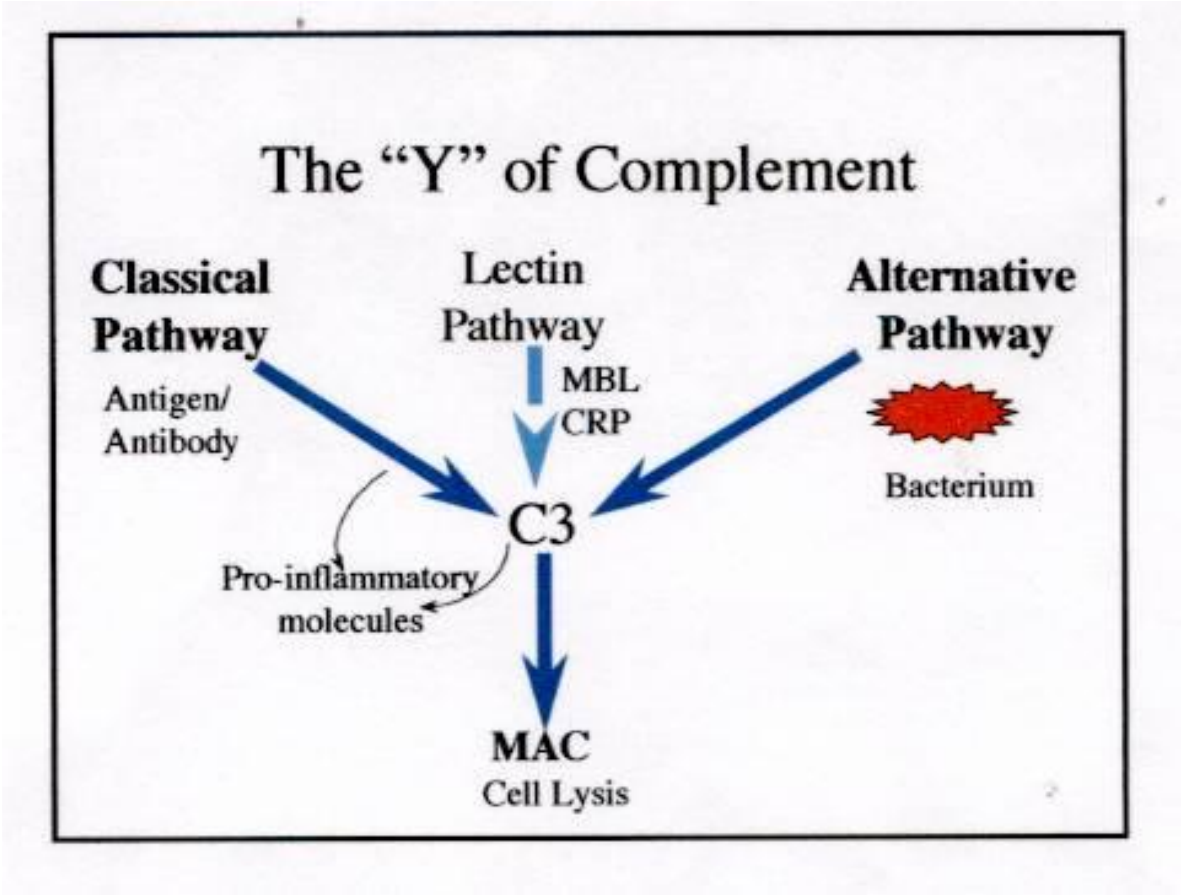
Interferons - have anti-viral effects

Chemokines - required for chemotaxis and recruitment of cells to particular sites

Growth factors - required for development of cells of the immune system

Cytotoxic – can induce cell death e.g. tumour necrosis factor

COMPLEMENT: *a complex series of proteins in serum and in tissues which form a triggered enzyme cascade system, leading to opsonisation of micro-organisms, direct killing of micro-organisms, promotion of inflammation and recruitment of leukocytes*. **Mast cells** may be activated to degranulate by complement products (*anaphylatoxins)* leading to vasodilation and increased vascular permeability. A simple diagram of the complement pathways:

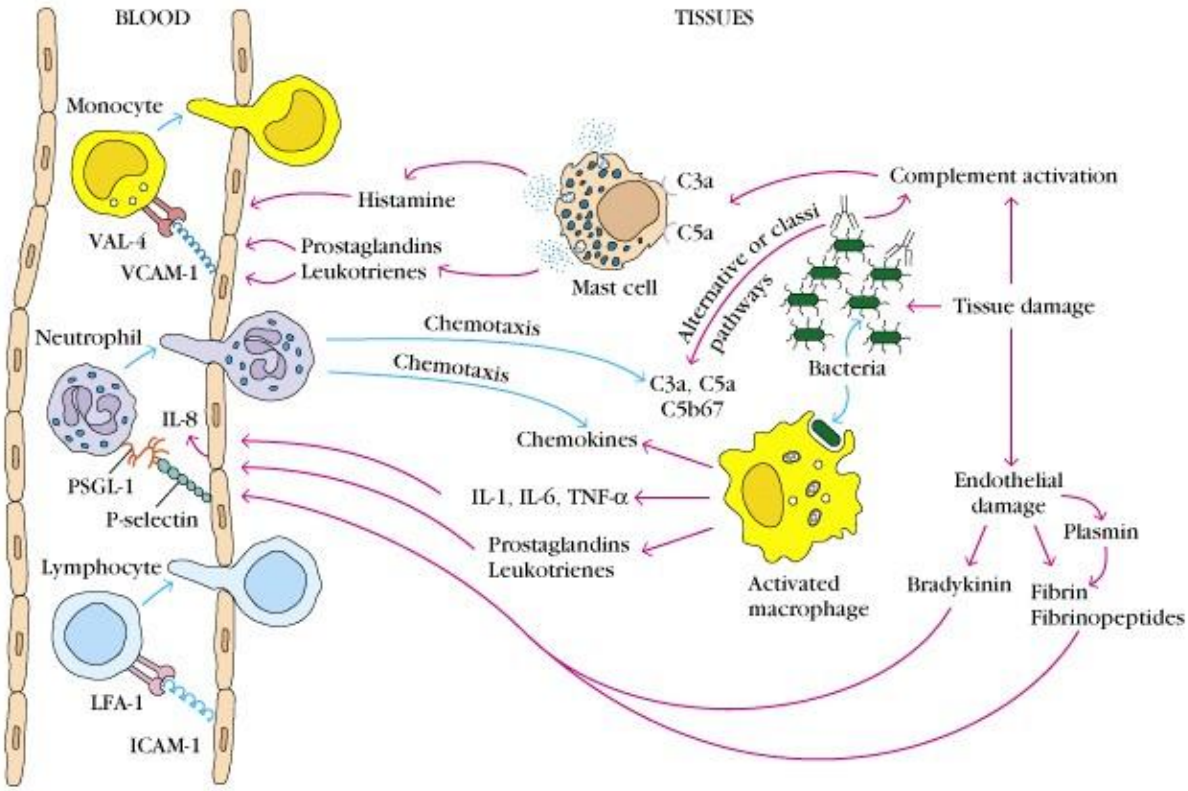


MBL= mannan-binding lectin

CRP= C-reactive protein

A LOCAL ACUTE INFLAMMATORY RESPONSE

Macrophage activation, cytokine secretion, endothelium activation, leukocyte extravasation and chemotaxis, neutrophil phagocytosis and killing.



Complement activation, opsonisation, mast cell degranulation, increase in vascular permeability.

Kinin cascade activation, increase in vascular permeability.

A local inflammatory response may be accompanied by an **acute phase** systemic response 1-2 days later; *fever, increased production of leukocytes, production of **acute phase proteins** by the liver*; this is a cytokine induced reaction (IL-1, IL-6 and TNF-α). Acute phase proteins include: **C-reactive protein, mannan –binding lectin, serum amyloid A, complement components, fibrinogen** (for clotting); all these help fight the infection.

NATURAL KILLER CELLS: *large granular lymphocytes (5-10% of peripheral blood lymphocytes) with cytotoxic activity, important in defence against tumours and viral infections (Herpes viruses in particular). No antigen-specific receptor, but express complex sets of both activating and inhibitory cell surface receptors: whether to kill or not is a balance of different signals. Activated NK cells also secrete the cytokine interferon-γ.*

SUMMARY OF INNATE IMMUNITY:
CELLS: phagocytes (bacteria); NK (viruses)
HUMORAL: complement (bacteria); interferons (viruses)

TISSUES 7
Muscle

Dr Sohag Saleh NB: Please see corresponding lecture slides for diagrammatic representations

Learning objectives

5. Understand the ultrastructure of skeletal muscle and its general functional characteristics
6. Understand the process of excitation-contraction coupling in skeletal muscle
7. Be able to name all the major structural features of a sarcomere
8. Understand the 'sliding filament' theory of muscle contraction
9. Understand the basics of cardiac muscle and how it differs from skeletal muscle
10. Be aware of the characteristics that distinguish smooth muscle from striated muscle

Background

Muscles are specialised cells that are responsible for movement through the generation of force. Within vertebrates there are three types of muscle:

1. Skeletal muscle: is attached to bone and produces movement of the body relative to the external environment
2. Cardiac muscle: pumps blood around the body through the blood vessels
3. Smooth muscle: exists within the lining of all the hollow organs (e.g. blood vessels, gastrointestinal tract) and provides the propulsion to move substances within the body.

Although, there are a number of differences between these types of muscle the mechanism through which force is generated is reasonably similar in all three.

Skeletal muscle

Skeletal muscles connect the bones in the arms, legs, and spine and are used in complex coordinated activities. Antagonist muscle pairs consist of a flexor (e.g. bicep) and an extensor (e.g. quadriceps). There are considered to be two main types of muscle contraction:

1. **Isometric contraction**: This is where tension develops but the muscle length does not change
2. **Isotonic contraction**: This is when the muscle changes length while the tension remains the same. During concentric contraction the muscle shortens and during eccentric contraction the muscle lengthens

Skeletal muscle consists of a bundle of muscle cells, known as myofibres. Each myofibre is cylindrical, multinucleated and contains further bundles of filaments (myofibrils) that extend across the length of the cell. These myofibrils can also be further subdivided into alternating light and dark bands (when viewed under a conventional light microscope) giving them a striated appearance. The dark bands are called A bands (anisotropic) and the light bands are called I bands (isotropic). The A bands are bisected by a dark region termed the H zone, whilst the I bands, are bisected by a different dark line, the Z disk. The segment from one Z disk to the next is called a sarcomere.

The sarcomere

The sarcomere is the structural and the functional unit of skeletal muscle. During contraction, each sarcomere can become 30% shorter than its original uncontracted length. There are a number of important proteins that make up the sarcomere:

Actin: A polymeric thin filament protein that is composed of two twisted α-helices. Along with the actin another rope-like molecule called *tropomyosin* forms a chain along each actin filament and associated with the tropomyosin is another protein, *troponin*

Nebulin: Large filaments associated with actin

CapZ and *Tropomodulin* are associated with the Z-line and H-zone ends of actin, respectively

Myosin: Thick filaments that are the 'motor proteins' of the sarcomere. Contain numerous 'globular heads' that interact with the actin filaments

Titin: very large 'spring-like' filaments connecting the myosin to the Z-line

Excitation-contraction (E-C) coupling

Muscle contraction is initiated by an increase in the cytosolic calcium (Ca^{2+}) concentration. Skeletal muscle cells maintain a low cytosolic Ca^{2+} (below 100 nM) due to the actions of a Ca^{2+}-ATPase that continuously pumps Ca^{2+} from the cytosol into the sarcoplasmic reticulum (SR).
When a nerve impulse reaches a skeletal muscle cell, it causes a change in the membrane potential and initiates a cascade of events beginning with the action potential:

1. The action potential propagates along the myofibril membrane (sarcolemma) and the T-tubules
2. Depolarisation activates dihydropyridine receptors causing a conformational change in the channel protein
3. This conformational change is transmitted to ryanodine receptors (RyR) on the SR, resulting in their opening and Ca^{2+} release from intracellular stores
4. Thus depolarisation leads to an increase in intracellular Ca^{2+}

The sliding filament theory

The fact that thick and thin filaments do not change in length while the sarcomere shortens, led to the development of the sliding-filament model of muscle contraction. According to the model:

- In the presence of Ca^{2+}, there is a slight movement of the troponin molecule from the tropomyosin chain
- This movement exposes a myosin binding site on the surface of the tropomyosin chain
- The 'charged' myosin heads bind to the tropomyosin on the actin filament
- The binding and subsequent discharge of ADP causes the myosin head to pivot (the 'power stroke'), pulling actin filament towards the centre of the sarcomere
- Binding of ATP then releases the myosin head from the actin filament
- Hydrolysis of the ATP molecule provides the energy to 'recharge' the myosin head

Contraction of muscle results from the activity of hundreds of myosin heads on a thick filament, interacting with each thin actin filament and this is amplified by the hundreds of thick filaments in a sarcomere and thousands of sarcomeres in a muscle fibre.

Cardiac muscle

The heart is a muscular organ located in the chest that is responsible for pumping blood around the body. The walls of the heart (myocardium) are primarily made up cardiac muscle cells but also contain pacemaker cells and conducting fibres.
The pacemaker cells situated within the sinoatrial (SA) node are excitable cells that depolarise and generate action potentials in a regular rhythmic pattern. The discharge rate of these cells determines the heart beat and the action potential spreads from the pacemaker cells to the cardiomyocytes.
Individual cardiomyocytes are connected to each other at specialised regions known as intercalated disks, where many gap junctions allow action potentials to spread rapidly from cell to cell.
The cardiomyocytes also contain a regular arrangement of actin and myosin filaments, as with skeletal muscle and are therefore also known as striated muscle. The contractile units of cardiomyocytes are also sarcomeres and the mechanism of contraction is the same as skeletal muscle.

Cardiac E-C coupling

As with skeletal muscle, contraction is initiated by an increase in the cytosolic Ca^{2+} concentration. However, there are subtle differences in the way the intracellular Ca^{2+} levels rise within these two cell types:

- The action potential propagates along the cardiomyocyte membrane and the T-tubules
- Depolarisation opens voltage-gated Ca2+ channels (VGCCs) allowing Ca2+ influx
- Within the cardiomyocyte the Ca2+ has three main effects:
  i. Causes further depolarisation
  ii. Initiates contraction by binding to troponin
  iii. Causes Ca2+ induced Ca2+ release (CICR) by binding to ryanodine receptors (RyR) on the SR
- Thus depolarisation leads to increase intracellular Ca^{2+} and muscle contraction

Smooth muscle

The third type of muscle, which is found within the walls of blood vessels, the gastrointestinal tract, the trachea and other hollow organs, does not contain a regular arrangement of actin and myosin

filaments. Hence it is termed smooth muscle, as opposed to the 'striated' skeletal and cardiac muscle.
Also, unlike striated muscle, smooth muscle cells do not express voltage-gated Na^+ channels and therefore a smooth muscle action potential is entirely dependent on depolarisation resulting from Ca^{2+} entry through VGCCs. Therefore the process of smooth muscle contraction is a far slower affair.

Smooth muscle E-C coupling

The process of E-C coupling in smooth muscle is very different to that seen in striated muscle:

1. Depolarisation activates VGCCs which allows the influx of Ca^{2+} into the cells
2. The Ca^{2+} binds to the intracellular protein calmodulin (CaM) forming a complex
3. This Ca^{2+}-CaM complex activates myosin light chain kinase (MLCK)
4. MLCK phosphorylates the myosin light chains allowing them to form cross-bridges with the actin filaments resulting in contraction

Learning objectives revisited

5. Understand the ultrastructure of skeletal muscle and general functional characteristics

Skeletal muscles connect the bones and antagonist muscle pairs consist of a flexor and an extensor. They consist of a bundle of cylindrical, multinucleated myofibres. Further bundles of filaments (myofibrils) extend across the length of the cell. Myofibrils can also be further subdivided into alternating light and dark bands

6. Understand the process of excitation-contraction coupling in skeletal muscle

An impulse causes a change in the membrane potential and the action potential propagates along the myofibril membrane (sarcolemma) and the T-tubules. The depolarisation activates dihydropyridine (DHP) receptors. The DHP receptors open ryanodine receptors (RyR) on the SR causing Ca^{2+} release from intracellular stores.

3. Be able to name all the major structural features of a sarcomere

Actin: thin filaments associated with tropomyosin, troponin, nebulin, CapZ and tropomodulin
Myosin: thick filaments that contain numerous 'globular heads' and associated with Titin:

4. Understand the 'sliding filament' theory of muscle contraction

In the presence of Ca^{2+}, there is a slight movement of the troponin molecule from the tropomyosin chain. This movement exposes a myosin binding site allowing the 'charged' myosin heads bind. The binding and subsequent discharge of ADP causes the myosin head to pivot (the 'power stroke'), pulling actin filament towards the centre of the sarcomere.

Binding of ATP then releases the myosin head and hydrolysis of the ATP provides the energy to 'recharge' the myosin head

5. Understand the basics of cardiac muscle and how it differs from skeletal muscle

Cardiomyocytes are connected to each other at specialised regions known as intercalated disks, containing numerous gap junctions.
Depolarisation activates voltage-gated Ca^{2+} channels (VGCCs) which allows the influx of Ca^{2+}. The Ca^{2+} binds to ryanodine receptors (RyR) on the SR, causing CICR

6. Be aware of the characteristics that distinguish smooth muscle from striated muscle

Irregular arrangement of actin and myosin filament; no VGSCs; contraction involves Ca^{2+}, CaM & MLCK

TISSUES 8
Signalling between cells I

Dr Sohag Saleh NB: Please see corresponding lecture slides for diagrammatic representations

Learning objectives

- Give examples of why cells in a multi-cellular organism need to communicate with each other.
- Provide specific examples of communication between tissues and within a tissue.
- Explain with examples the modes of intercellular signalling: endocrine, paracrine, autocrine and signalling by membrane attached proteins.
- Explain how an extracellular signal is transmitted within the cell, either by direct access of the cell cytoplasm or through an external signal triggering an internal cascade of events.

Why do cells need to communicate with each other?

1. Process information

The human body receives several million inputs from a variety of different sources each day and it must be able to detect these inputs, process the information that it receives and manufacture an appropriate response. For example a solitary eyeball or the auditory apparatus within the ears are of little use unless they can send the 'data' that they receive to a 'central processing unit' that can both understand and interpret this information. The majority of the time there is no 'output' resulting from the 'input' information and this in itself is of paramount importance, but when an 'output' is required the brain must be able to communicate with a different subset of organs to produce an appropriate response.

2. Self-preservation

The most innate response resulting from an external stimulus is for the purpose of self-preservation and this can often circumnavigate the brain for the purpose of speed. The simplest example of this involves the spinal reflex arc, where the sensory receptors on the periphery detect a painful input (e.g. excessive heat or a pin prick) and communicate this information to the skeletal muscle via the spinal cord resulting in withdrawal of the affected area from the painful stimulus.
However, the threatening stimulus may not be simply associated with a small area of the body and may require the coordinated movement of a number of different areas i.e. fleeing from danger and this involves communication between a brain and a number of different areas.

3. Voluntary movement

Although movement is occasionally required for the purposes of self-preservation, for the average individual voluntary movement is mostly required to perform daily tasks such as getting from A to B. The process of voluntary movement involves a complex interplay between a variety of different sensory and motor organs all coordinated by different areas of the brain.

4. Homeostasis

Voluntary movements are not the only reason that an organ such as the brain needs to communicate with other organs. There are numerous involuntary processes that are continuously checked and regulated in order to preserve the cellular environment within the parameters that are required for the body to function properly. It is of vital importance that this homeostasis is maintained and therefore the brain must be able to somehow communicate with all the organs, tissues and cells within the human body.
Although the brain acts as the 'central processing unit' it does occasionally 'outsource' certain tasks that can be overseen by other organs. For example the parathyroid glands are responsible for regulating calcium (Ca^{2+}) levels within the body and the pancreas plays an essential role in regulating blood glucose levels. Therefore it is imperative that lines of communication also exist connecting other organs and tissues within the body.

Communication methods

The two main systems within the body that provide these lines of communication are:

1) Nerve fibres of the central and peripheral nervous system
2) The blood vessels of the cardiovascular system

These two systems provide very different means of communication. The nervous system provides a rapid almost instantaneous response, whilst the blood vessels provide a slower more versatile regulation.
The nervous system is analogous to communication via e-mail, where the message is sent and received almost immediately, whereas the endocrine system can be likened to the postal delivery of a letter. Where despatch and receipt take a lot longer but a larger variety of packages can be sent through the post.
The type of messages sent and the mechanisms involved are substantially different and will therefore be discussed separately.

Modes of intercellular signalling

1) Nerve fibres of the central and peripheral nervous system

The synaptic junction

See notes on tissues 6: Nerve

2) The blood vessels of the cardiovascular system

The blood vessels provide a transportation route not only for blood cells but for numerous other chemical messengers too. The chemicals that utilise the circulatory system for transportation are generally referred to as hormones. Hormones are produced by most of the major organs of the body however there are a few organs that play a more prominent role than others, such as the hypothalamus and the pituitary gland.
Hormones act as the message and the recipient of the message is termed a receptor. Receptors can be located either on the surface of cells as membrane spanning proteins or within the intracellular compartment. The latter type of receptor are only available to hormones that have the ability to traverse the cell membrane i.e. membrane permeable hormones.
Another method of communication utilised primarily by the immune system involves receptor-receptor interaction. One prerequisite for this type of communication is that one of the participating cells is mobile (see table 1).

Table 1: Different types of signalling

Type of signalling	Description	Physiological examples
Endocrine	Hormone travels within blood vessels to act on distant target cell	- Glucagon produced in the pancreas acts on the liver - Insulin produced in the pancreas acts on the liver, muscle cells and adipose tissue - Adrenaline produced in the adrenal glands acting on the trachea
Paracrine	Hormone acts on an adjacent cell	- Insulin produced in β-cells acting α-cells - Endothelin-1 produced by the endothelial cells within blood vessels - Osteoclast activating factors produced by adjacent osteoblasts
Membrane-attached proteins	Interaction between membrane proteins from two different cells	- T-cell receptor interacting with MHC class II molecule - The HIV GP120 glycoprotein interacting with the CD4 receptor - Bacterial cell wall components binding to toll-like receptors on haematopoietic cells
Autocrine	Hormone is produced by and acts on the same cell	- Interleukin-2 acting on T-lymphocytes - Acetylcholine acting on presynaptic M_2 receptors - Endothelin-1 produced by the endothelial cells within blood vessels

Extracellular to intracellular

As mentioned previously a signal needs to be transmitted to the intracellular compartment of a cell to have its desired effect. A large proportion of chemical messengers are unable to traverse the cell membrane and are therefore reliant on membrane spanning proteins, known as receptors, to transmit the message from outside the cell (extracellular) to inside the cell (intracellular). Even those hormones that are able to cross to cell membrane require intracellular receptors to transmit their message.
The different receptor types can be broadly separated into four different categories based on their physical properties and mechanism of action.

PLEASE NOTE: Only a brief description will be provided at this stage. This will be expanded in the next section.

- Ligand-gated ion channel receptors (ionotropic receptors)

These transmembrane receptors have a central pore incorporated within them. When the ligand attaches to the 'ligand-binding domain' on the external surface of the protein the pore will open. This pore is basically a hole within the membrane, which allows certain charged ions to enter or exit the cell depending on the type of receptor.
NB: The voltage-gated ion channels that alter the membrane potential do not fall within this category since they are not activated by ligands.

- G-protein linked receptors

These are also transmembrane receptors, with characteristic seven transmembrane domains (they are alternatively referred to 7-TM (**T**rans**M**embrane or serpentine receptors). Once again the ligand-binding domain is located on the external surface of the receptor and the message is transduced by a specialised G-protein complex bound to the receptor within the intracellular compartment. Ligand binding results in activation of the G-protein complex, which triggers an intracellular signalling cascade.

- Enzyme-linked receptors

These transmembrane receptors ordinarily only consist of one transmembrane domain, which has the ligand-binding domain on the outside and specialised enzymes (usually tyrosine kinase enzymes) on the inside. These receptors do not ordinarily work alone and require clustering of more than one receptor protein to activate the intracellular enzyme. Once activated the intracellular enzymes trigger a signalling cascade within the cell.

- Intracellular receptors

As the name suggests these receptors are located within the intracellular compartment and are only activated by chemical messengers that are able to cross the cell membrane (e.g. steroid hormones). Intracellular receptors are usually transcription factors, meaning that once they are activated they bind to DNA within the nucleus and alter protein synthesis.

Learning objectives revisited

- Give examples of why cells in a multi-cellular organism need to communicate with each other.

Process information, self-preservation, voluntary movement and homeostasis

- Provide specific examples of communication between tissues and within a tissue.

The pancreas communicating with the liver in glucose homeostasis. The pancreatic cells communicating with each other

- Explain with examples the modes of intercellular signalling: endocrine, paracrine, autocrine and signalling by membrane attached proteins.

Endocrine: hormone acting on distant target e.g. Glucagon
Paracrine: hormone acting on adjacent cell e.g. Insulin
Autocrine: hormone acting on the same cell that it is produced by e.g. IL-2
Membrane-attached proteins: cellular receptors communicating with each other e.g. MHC & TCR

- Explain how an extracellular signal is transmitted within the cell, either by direct access of the cell cytoplasm or through an external signal triggering an internal cascade of events.

Ionotropic receptors, G-protein coupled receptors & enzyme-linked receptors trigger an internal cascade following an external signal. Intracellular receptors transmit a response from an internal ligand

IMMUNOLOGY 4
Antibodies

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Learning objectives:

- Describe with the aid of a simple diagram the immunoglobulin molecule, identifying the antigen-binding site (Fab) and Fc portions of the molecule.
- Briefly describe the properties of the antigen-binding site.
- Distinguish between antibody affinity and avidity.
- List the immunoglobulin classes and sub-classes in man. Describe their functions and relate these to their individual structure.

IMPORTANT DEFINITIONS:

An **antibody** is a protein that is produced in response to a foreign molecule (**antigen**) and has the property of binding specifically to the antigen that induced its formation. Antibodies constitute the class of proteins known as **immunoglobulins**.

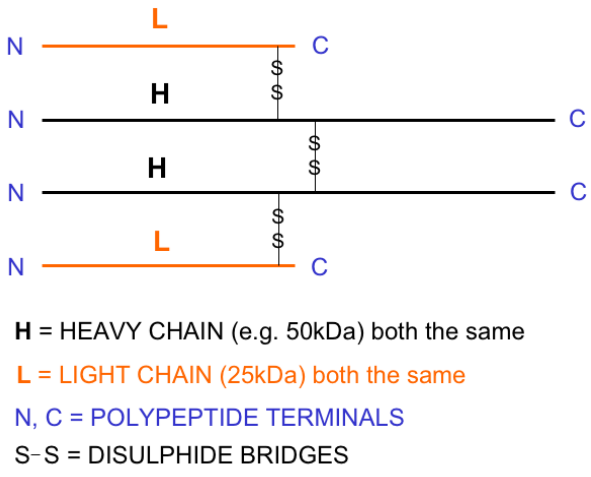
The term **isotype** refers to immunoglobulin structures that are present in all members of a species (e.g., the kappa and lamda light chains, the different heavy chains, the immunoglobulin classes and sub-classes).

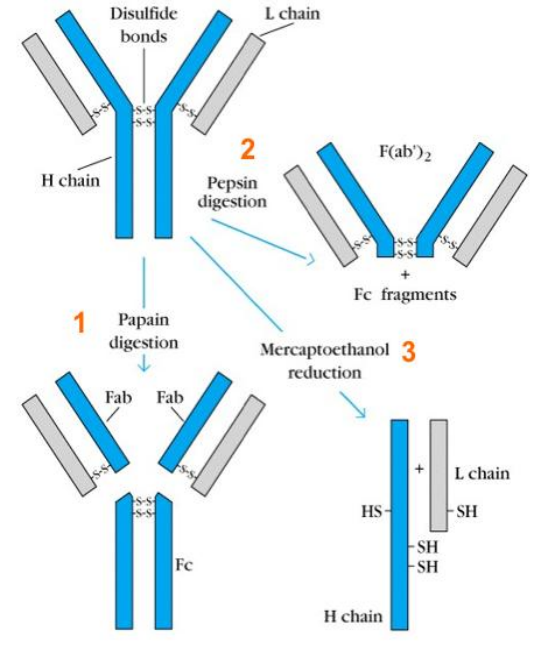
An **allotype** is an allelic polymorphism of an immunoglobulin molecule and is therefore not present in all members of the species. Allotypes are usually detected by antibodies.

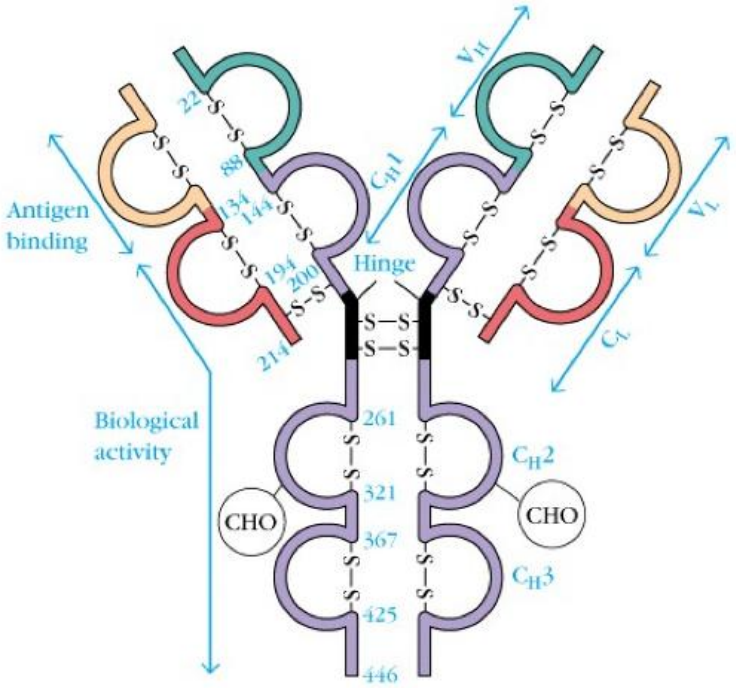
Affinity is a measure of the strength of binding between a single binding site of an antibody and its antigen.

Avidity is the overall strength of binding between antibody and antigen, taking into account the number of binding sites on the antibody and the number of sites on the antigen (**antigenic determinants or epitopes**) that can be bound.

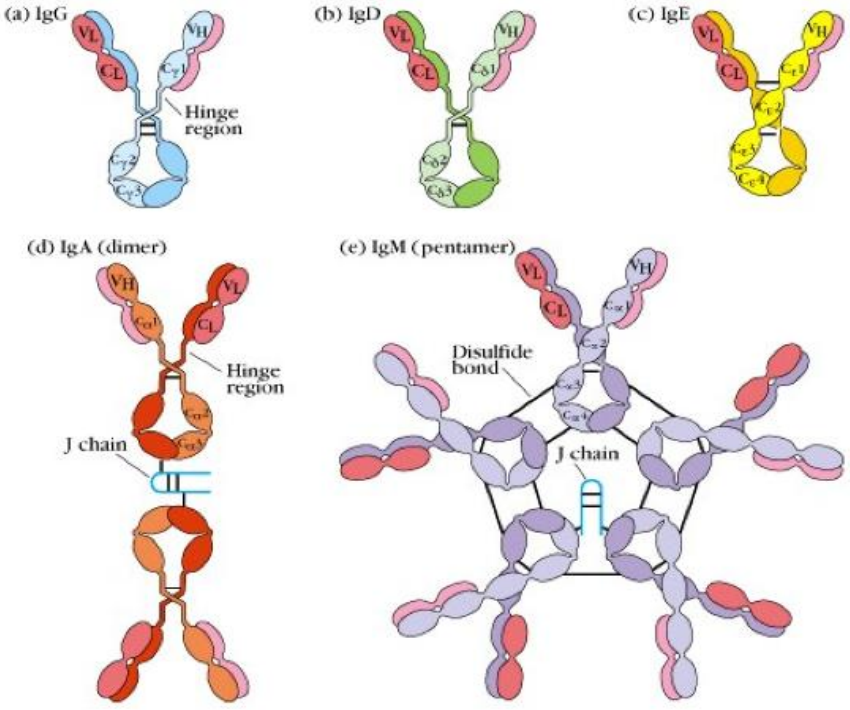
BASIC ANTIBODY STRUCTURE







The antigen binding site is a relatively large, approximately flat surface with undulations, and is made from the 3 hypervariable regions of V domains that form the complementarity determining regions (CDR's). The affinity for antigen binding comes from many non-covalent interactions, each of which individually is relatively weak. These interactions are made up of hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions.



Outline structure of the different antibody classes

ANTIBODY FUNCTIONS

IgG This is the most abundant Ig in blood and tissue spaces and is the most important in defence generally - the other Ig classes have a more specialised role. IgG neutralises toxins and viruses. Further, it performs a number of functions that are dependent on the nature of its Fc. When IgG binds to a bacterium or other infectious agent, macrophages or neutrophils recognise the Fc (they possess Fc receptors) and this leads to phagocytosis (opsonisation). In the same situation complement activation can take place promoting phagocytosis or lysis of the bacterium. IgG crosses the placenta and, because of its comparatively long half-life, is able to provide protection in the new born child for at least 3 months.

There are 4 sub-classes of IgG: IgG1(70% of total IgG), IgG2(20%), IgG3(7%), IgG4(3%). For most of these functions, IgG1 and 3 are more active than IgG2 and 4. Antibodies to bacterial carbohydrates are often IgG2.

IgA The data given in the table below with an asterisk refers to IgA in the blood where it occurs in monomer form, but its function there is not well understood. IgA has an important role in seromucous secretions where it occurs as secretory IgA - a dimer together with J chain and secretory component (SC). SC is added to the molecule during its passage through epithelial cells out into the secretions, and it helps to protect the molecule from degradative enzymes that may be present there (e.g., in the gut). Secretory IgA can neutralise toxins. Moreover, by binding to infectious agents it can block their infectivity - often by preventing adherence of the agents to the epithelial cells.

There are two sub-classes of IgA: IgA1 and IgA2. While IgA1 predominates in the blood, IgA2 is strongly represented in the secretions.

IgM IgM is the first Ig to be made following contact with an antigen, and it is active in the blood. Its five Fab pairs allow it to bind strongly to the surface of bacteria causing agglutination. Like IgG, it is adept at activating complement.

IgE Mast cells and basophils have a high affinity receptor for the Fc of IgE (FcεR1). When IgE that is bound in this way is cross-linked by antigen, degranulation of the cells occurs with release of inflammatory mediators such as histamine. This process is important in protection against certain parasitic infections, but it can be a nuisance giving rise to allergic diseases such as hay fever or life-threatening anaphylaxis.

IgD The least well characterised Ig class, present at extremely low concentrations in the blood. Surface IgD is expressed early in B cell development, and is involved in signalling during B cell development and activation.

	IgG	**IgA**	**IgM**	**IgE**
Mol. wt.	150,000	160,000 *monomer 400,000	900,000	190,000
Serum (mg/ml)	13	3.5*	1.5	0.0001
% Intravascular	45	44*	80	
Half-life (days)	23	6*	5	0 2
Opsonisation	+	-	-	-
Complement activation	+	-	+	-
Placental transfer	+	-	-	-
Seromucous secretions	-	+	-	-
Mast cell/basophil Attachment	-	-	-	+

IMMUNOLOGY 5
B-Lymphocytes

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Learning objectives:

- Describe the origin and maturation of B lymphocytes.
- Briefly outline the principles of immunoglobulin gene rearrangement in the generation of diversity.
- Describe the process of activation of B lymphocytes to divide and differentiate into antibody-secreting and memory cells.
- Explain the differences in antibody production in primary and secondary immune responses.

The process of Immunoglobulin gene rearrangement (see below) ensures that when any foreign antigen enters the body there will be a few B lymphocytes with immunoglobulin (Ig) molecules on their surface able to bind that antigen. After binding the antigen, such a lymphocyte will divide to form a clone of identical cells with the capacity to produce antibody of this specificity. Remember that each B lymphocyte in the body has the capacity to make antibody of only one specificity. The antigen thus 'selects' a clone which has the capacity to produce antibody able to bind that antigen.

An important aspect of the process of B cell stimulation to produce antibody-secreting plasma cells and memory B cells is the co-operation with T lymphocytes, and you will need to combine information from this lecture with that from Immunology Lectures 6 and 7.

Generation of diversity
Read this section and study a simple diagram in a textbook.

Three chromosomes are involved in coding for Ig chains. One is responsible for kappa light chains, one for lamda light chains and one for all the heavy chains. The principles of re-arrangement are the same for all three.

The genes coding for the variable regions are stored together up-stream of the genes for the constant region. In this way it is not necessary to repeat the constant region DNA for each different variable region. To make matters more complicated the variable genes exist in segmented form before re-arrangement. That is to say for kappa light chains one has a series of V segments which code for most of the variable region followed by a series of J segments which code for the rest of the variable region. During re-arrangement, which will convert a progenitor B cell into a functional B cell, one of the V segments comes together with one of the J segments. Unwanted DNA is looped out by a special mechanism. It is then possible to produce a primary RNA transcript consisting of VJ linked to the constant region(C). Unwanted RNA between J and C is spliced out to give mRNA for VJC. This can then be translated into the light chain. The process is similar for lamda light chains and the heavy chains, but for the heavy chains there is a series of D segments between the V and J segments and the mRNA thus represents VDJC.

Diversity is further extended by variation in the precise joining points of the V, D and J segments, somatic mutation of the re-arranged genes and the random pairing of H and L

chains to make the Ig molecule. The number of different antibody specificities that can be produced by these processes is enormous.

The heavy chain constant genes are sited one after the other, starting with Cμ, following the variable region genes. Under the influence of T cells, a process of class switching can occur in which the already re-arranged VDJ can switch its attachment from Cμ to Cγ, Cα or Cε This produces the different classes of antibody but does not affect antibody specificity.

Each lymphocyte (B and T) is specific for a particular antigen and the specificity of binding resides in the receptor for antigen (B cells: BCR = B cell antigen receptor; T cell: TCR = T cell antigen receptor). T cells use a similar process than B cells to generate diversity. Note: the mechanism used to generate antigen-specific receptors on B and T cells seems to be unique in the entire body, no other genes use the rearrangement strategies.

Polyclonal *vs* monoclonal antibodies
When an antigen enters the body the antibody response that occurs is normally **polyclonal**. That is to say a heterogeneous mixture of antibodies are produced. There will be antibodies directed to several different determinants on the antigen, and the antibodies to a particular determinant will be of variable affinity. So these are polyclonal antibodies: they derive from many different clones of B cells.

Monoclonal antibodies are homogeneous being derived from a single B cell clone. They are normally made artificially by fusing one antibody-producing cell, making a particular molecule of antibody, with a tumour cell to form a clone of cells which will divide and produce this antibody for a long time. Monoclonal antibodies are useful reagents for detecting cell markers such as CD4 or CD8.

Myeloma proteins are the monoclonal Ig products of a plasma cell tumour. Such tumours may arise spontaneously causing major clinical problems. They are helpful in studying Ig structure, but the antigen to which they bind is generally unknown.

MICROBIOLOGY 1
Bacterial properties

Professor David Holden

Learning objectives: NB these supersede those printed in the Course Guide

- Give examples of intracellular and extracellular bacterial pathogens
- Describe the differences between Gram positive and Gram negative bacteria
- Describe how some bacteria interfere with host cell processes to enable invasion and intracellular movement
- Outline the three main ways bacteria use to exchange genetic material

Bacteria can be broadly divided into two groups on the basis of the Gram stain. Gram positive bacteria retain a violet dye in the ……..of their cell wall. The stain is excluded from Gram negative bacteria by the presence of an outer ……

Most bacteria are harmless or beneficial but a few are pathogenic. *Salmonella typhi* causes……*Shigella* causes……. Two species of *Neisseria* cause two very different diseases, …… and…… Pneumonia can be caused by……, and tuberculosis is caused by……..

To be a pathogen, a bacterium must be able to colonize, persist, replicate and disseminate. Bacterial pathogens can be extracellular (e.g……) facultative intracellular (e.g……..), or obligate intracellular (e.g…….).

Salmonella is motile and invasive. Motility is due to…….and bacteria invade host cells using a ……., through which they transfer virulence proteins into the host cell. These cause actin polymerisation, membrane ruffling and uptake of bacteria.

Listeria invades host cells and then breaks out of the membrane-bound……. Once in the cytoplasm of host cells, they move around and spread from cell to cell by polymerising……. at one pole of the bacterial cell.

Variation in vertically transmitted DNA can occur as a result of…….. DNA can also be acquired by horizontal transfer.

The three basic mechanisms of horizontal gene transfer are……, ……., and……..

Genomes of bacterial pathogens can encode between 1000 and 5000 proteins, depending on the species. Only a small proportion of these are related to pathogenicity. These 'virulence genes' are frequently found on '…….. ……..' Distinguishing features of these elements are……..., ……., and ……

Because the doubling time of bacteria can be very short (as low as 20 mins) they can achieve vast numbers in short time-frames.

Further reading: Bacterial Pathogenesis: A molecular approach. Salyers and Whitt. ASM press (2nd Ed); Cellular Microbiology. Cossart, Boquet, Normak, Rappuoli. ASM press.

Properties of bacterial pathogens: self test

Questions

1. What distinguishes Gram-positive from Gram-negative bacteria?

2. What attributes of bacterial pathogens distinguish them from non-pathogens?

3. Give examples of pathogens that replicate mainly extracellularly

4. Give examples of pathogens that replicate mainly intracellularly

5. What multi-protein machine enables *Salmonella* and many other bacteria to move?

6. What multi-protein machine enables *Salmonella* to invade host cells?

7. How does *Listeria* move inside host cells?

8. What are ‘core’ and ‘accessory’ genes?

9. What are the main means by which bacteria exchange genetic material?

10. What is a ‘Pathogenicity Island’?

IMMUNOLOGY 6
T-Lymphocytes and Antigen Recognition

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Learning objectives:

- Outline the origins and functions of T lymphocyte subsets
- Briefly describe the structure and distribution of major histocompatibility complex (MHC) class I and class II molecules
- Outline the mechanisms by which antigen presenting cells (APCs) process and present endogenous antigens
- Compare and contrast antigen recognition by B and T lymphocytes and by $CD4^+$ and $CD8^+$ T lymphocytes

T LYMPHOCYTES

Destroy intracellular pathogens; recognise infected cells using a specific receptor on their cell surface, the ***T cell receptor (TCR).*** The TCR resembles a membrane-bound form of an antibody Fab fragment in structure; that is it consists of two polypeptide chains each containing a variable domain and a constant domain. There is a very large number of different TCR's present in each individual, providing a diverse ***T cell repertoire****.*

The antigens recognised by T cells are ***processed***; that is they are small peptide fragments derived from larger proteins (some specialised T cells are able to recognise non-peptide antigens). Processed antigen is ***presented*** to T cells on cell surfaces by specialised molecules called ***MHC molecules*** (see below).

There are 2 major populations of T cells:

- use ***CD4*** co-receptor, see peptide antigen presented by MHC class II ("class II restricted")
- use ***CD8*** co-receptor, see peptide antigen presented by MHC class I ("class I restricted")

Co-receptor molecules bind to the appropriate type of MHC molecule, increase the avidity of T cell-target cell interaction, and are important in signaling.

$CD8^+$ T cells (also known as CTL or Tc) are cytotoxic and kill target cells; they also secrete cytokines *e.g.* interferon-γ.

$CD4^+$ T cells (also known as T helper cells or Th) secrete cytokines which may recruit cells of innate immunity, help activate macrophages, and amplify and help CTL and B cell responses. $CD4^+$ T cells may be subdivided into the Th1 type or Th2 type, depending on the cytokines they produce.

T LYMPHOCYTE DEVELOPMENT

T lymphocytes develop in the ***thymus*** from bone marrow-derived precursors. Initially, they do not express a TCR or CD4 or CD8 (double negative stage). As they develop they move from the thymus outer cortex towards the inner medulla. The first stage is the formation of a TCR, using gene segment recombination similar in principle to antibody gene rearrangement (although different gene segments are used). Cells which express a TCR then express both CD4 and CD8 (double positive stage). These cells then undergo selection for those which express a useful TCR: ***positive selection*** allows the survival of cells whose TCR recognises self MHC, ***negative selection*** removes cells whose TCR recognises self MHC very strongly (to avoid dangerous reaction to self and ***autoimmunity***). Only about 5% of developing T lymphocytes (thymocytes) survive these selection processes and move into the circulation as mature single positive either $CD4^+$ or $CD8^+$ cells.

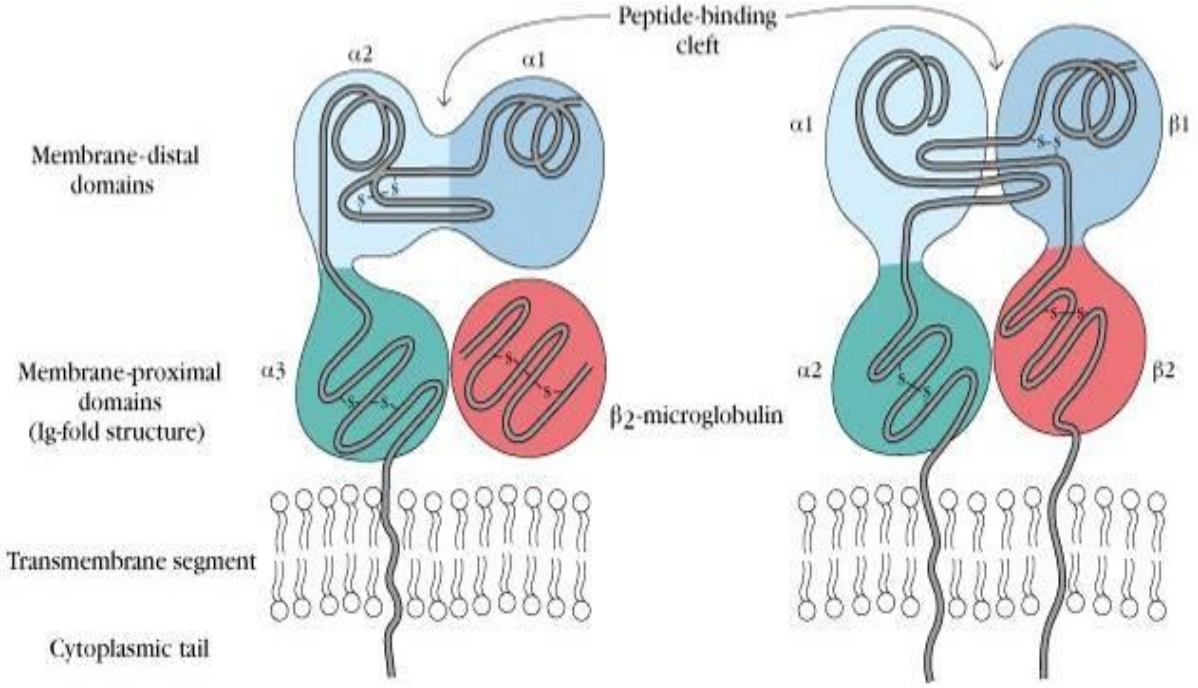
THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

A group of tightly linked genes important in specific immune responses. Discovered by experiments looking at the genetic basis of transplant rejection in mice; 2 classes: MHC class I are the classical transplantation antigens, MHC class II are regulatory, controlling the ability to mount immune responses. Nearly all cells express MHC class I (at various levels), but MHC class II is only normally expressed on “professional” antigen presenting cells (APC), i.e. dendritic cells, macrophages and B lymphocytes.

Outline structures:

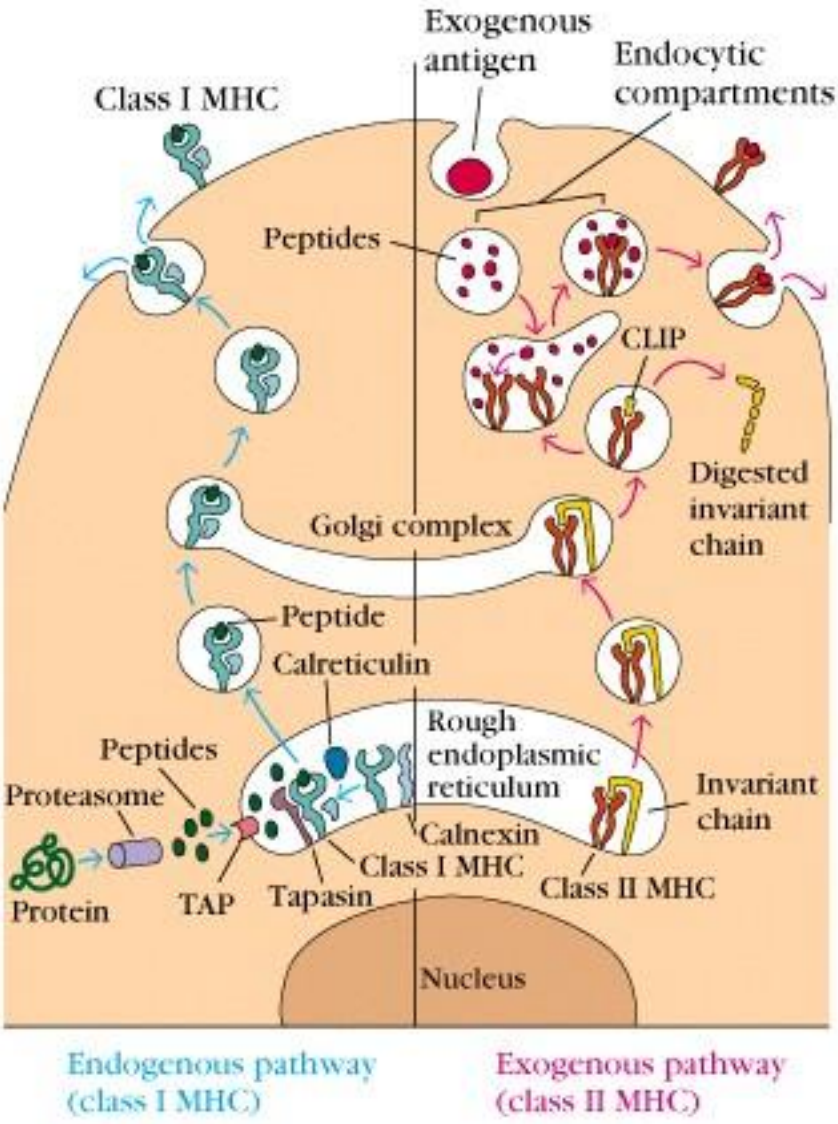
MHC CLASS I **MHC CLASS II**

The human MHC is known as the HLA region: **H**uman **L**eukocyte **A**ntigens.



Human MHC genes are highly ***polymorphic*** (variable in the population). A group of MHC genes linked together on one chromosome is termed ***MHC haplotype***. Diversity in MHC molecules exists at the population level, not within an individual, unlike T cell receptors.

THE TWO MAIN PATHWAYS OF ANTIGEN PRESENTATION



Antigens in different locations require different responses. Endogenous antigens (cytoplasm) are presented by MHC class I to CD8 T cells.

Exogenous antigens (external to the cell) are presented by MHC class II to CD4 T cells.

TAP=**t**ransporter for **a**ntigen **p**rocessing

CLIP=**cl**ass II associated **i**nvariant chain **p**eptide

SUMMARY: ANTIGEN RECOGNITION BY B and T CELLS

B cells: surface Ig receptor, intact extracellular antigen

T cells: surface T cell receptor, processed antigen presented by MHC molecules.

TISSUES 9:

Signalling Between Cells II

Dr Sohag Saleh

Learning objectives

- Be able to provide examples of physiological processes regulated by ionotropic receptors
- Explain the mechanisms of G-protein action in signal transduction and provide examples of physiological processes regulated by G-protein coupled receptors
- Explain the mechanisms of enzyme-linked receptor actions and provide examples of physiological processes regulated by enzyme-linked receptors
- Explain the mechanisms of intracellular receptor actions and provide examples of physiological processes regulated by intracellular receptors

Introduction

Thus far we have explored the general physiological principles involved in transmitting a message from A to B. The body can utilise fast, predominantly electrical messages involving nerve fibres or slower chemical messages involving hormones in blood vessels.
These chemical messages or molecules are often referred to as ligands since they exert their effects through binding to receptors. Receptors are usually proteins that bind the chemical mediators known as ligands and upon activation they elicit an effect within a cell. The intracellular effect evoked by an occupied receptor usually arises due to any chemical messenger that is a separate entity from the receptor or the ligand. This entity is known as a second messenger.
The properties of ligands, receptors and second messengers will now be explored in greater detail.

Receptors

As discussed previously, receptors can be separated into four distinct categories:

1. Ligand-gated ion channel receptors
2. G-protein linked receptors
3. Enzyme-linked receptors
4. Intracellular receptors

1. Ligand-gated ion channel receptors (ionotropic receptors)

These transmembrane receptors have a central pore incorporated within their quaternary structure. When the appropriate ligand attaches to the 'ligand-binding domain' on the external surface of the protein the pore will open.

The process of ligand-gated ion channel activation can be summarised step-wise.

1. The ligand binds to the receptor protein
2. A change in conformation of the channel protein results in the opening of a pore, which spans the cell membrane.
3. The pore allows ions to move in or out of the cell according to their respective concentration gradients. For example there is a higher extracellular concentration of Na^+ ions in the so upon opening of Na^+ channels, Na^+ ions move into the cell.

Table 1: Ligand-gated ion channel receptors

Receptor	Structure & Ion selectivity	Physiological effects of activation
Nicotinic ACh	Heteromeric pentamer $Na^+>K^+>Ca^{2+}$	Muscle contraction, cognitive enhancement
$GABA_A$	Heteromeric pentamer Cl^-	Inhibition of neuronal activity
NMDA	Heteromeric tetramer $Ca^{2+}>Na^+>K^+$	Synaptic plasticity & memory formation
$5\text{-}HT_3$	Homo/heteromeric pentamer $Na^+>K^+>Ca^{2+}$	Anxiety, emesis

Abbreviations used: ACh- acetylcholine; GABA-γ-amino butyric acid; NMDA- N-methyl-D-aspartic acid; 5-HT- 5-hydroxy tryptamine; Na^+- sodium, K^+-potassium; Ca^{2+}-calcium; Cl^-chloride.

2. G-protein linked receptors

G-protein linked receptors are also commonly known as 7-transmembrane receptors because the channel protein crosses the cell membrane 7 times. They are linked to an intracellular G-protein complex, which consists of an alpha (α) subunit, a beta-gamma (βγ) subunit and an associated GDP molecule.
There is significant variation between the Gα subunits and these can be broadly separated in to three categories (see table 2), which are associated with alternative signal transduction pathways. The Gβγ subunit is also physiologically active.

Table 2: G-proteins

G-protein	2nd messenger	Downstream effect 1	Downstream effect 2
$G\alpha_{q/11}$	Activates PLC	PIP_2 hydrolysed into IP_3 & DAG	IP_3: Promotes Ca^{2+} release from intracellular stores DAG: Activates non-selective ion channels
$G\alpha_s$	Activates AC	Converts ATP to cAMP	Activates PKA
$G\alpha_{i/o}$	Inhibits AC	Reduces cAMP formation	Inhibits PKA

Abbreviations used: PLC- phospholipase C; PIP_2-phosphoinositol bisphosphate; IP_3- Inositol triphosphate; DAG- diacyl glycerol; AC- adenylyl cyclise; ATP- adenosine diphosphate; cAMP- cyclic adenosine monophosphate; PKA- protein kinase A

The process involved in G-protein activation is summarised below in a step-wise process:

1. In the resting state the G-protein complex consists of a Gα subunit, a Gβγ subunit and an associated GDP molecule, which are in close proximity to the receptor
2. Ligand binding causes the G-protein complex to associate with the receptor resulting in the GDP molecule being phosporylated to a GTP molecule
3. The Gα subunit dissociates from the Gβγ subunit
4. Both Gα and Gβγ can act as second messengers

5. When the ligand dissociates from the receptor, internal GTPase on the Gα subunit hydolyses GTP to GDP

6. The Gα and Gβγ subunits re-associate and are once again available to the receptor

Around 800 different G-protein linked receptors have been identified using molecular techniques. Some of the more prominent and well characterized examples are tabulated below (see table 3).

Table 3: Examples of G-protein linked receptors

Receptor	Gα subunit	Ligand	Physiological effects of activation
AT-1	Gαq	Angiotensin II	Blood vessels: Vasoconstriction- ↑ Blood pressure
M_3 muscarinic	Gαq	Acetylcholine	Bronchi: Bronchoconstriction- ↓ Airflow
β_1 adrenergic	Gαs	Noradrenaline	Heart: ↑ Heart rate,↑ Force of contraction
D_1 dopaminergic	Gαs	Dopamine	Neurones: ↑ Neuronal growth
α_2 adrenergic	Gαi	Noradrenaline	Blood vessels: Vasodilation- ↓ Blood pressure
M_2 muscarinic	Gαi	Acetylcholine	Heart: ↓ heart rate

3. Enzyme-linked receptors

These transmembrane receptors ordinarily only consist of one transmembrane domain, which has the ligand-binding domain on the outside and specialised enzymes (usually tyrosine kinase enzymes) on the inside. These receptors do not ordinarily work alone and require clustering of more than one receptor protein to activate the intracellular enzyme. Once activated the intracellular enzymes trigger a signalling cascade within the cell.

The process involved in enzyme-linked receptor activation is summarised below:

1. Ligand binding results in receptors clustering

2. Receptor clustering activates enzyme activity within the cytoplasmic domain

3. The enzymes phosphorylate the receptor

4. This phosphorylation leads to the binding of signalling proteins to the cytoplasmic domain

5. These signalling proteins recruit other signalling proteins and a signal is generated within the cell

6. The signal is terminated when a phosphatase dephosphorylates the receptor

***Phosphorylate**: The addition of a PO_4 group. This simple chemical reaction activates a number of protein enzymes and is carried out by kinases*

***Phosphatase**: An enzyme that remove a phosphate group from its substrate*

***Dephosphorylate**: The removal of a PO_4 group. This hydrolysis reaction inactivates a number of protein enzymes and is carried out by phosphatases*

Receptor (R)	Enzyme	Ligand	Physiological effects of activation
Insulin (CD220)	Tyrosine kinase	Insulin	Glucose uptake, lipid metabolism
NPR1	GC	ANP & BNP	Vasodilation- ↓ Blood pressure
TGF β R1	Ser/thr kinase	TGF β	Apoptosis

Some notable physiological mediators that exert their effects through enzyme-linked receptors are detailed in table 4.

Table 4: Examples of enzyme-linked receptors
Abbreviations used: GC- Guanylyl cyclase Ser/thr- Serine/threonine *ANP- Atrial natriuretic peptide; BNP- Brain natriuretic peptide; TGF β- Transforming growth factor β*

Intracellular receptors

Steroid hormones are membrane permeable (i.e. hydrophobic, lipophilic) and therefore exert their actions on *intracellular receptors*. Intracellular receptors are essentially transcription factors and therefore regulate mRNA and protein synthesis. These receptors can also be subdivided into two classes:

- Type I receptors are located within the cytosolic compartment and are associated with heat shock proteins (hsp). Once the hormone binds to the receptor the hsp dissociates allowing the monomeric receptor protein to bind to other identical receptor monomers. These homomeric proteins subsequently translocate to the nucleus where it binds directly to DNA.

- Type II receptors are located within the nucleus of a cell and are often already bound to DNA. Binding of the hormone ligand to the receptor usually results in direct transcriptional regulation by the activated receptor-hormone complex.

A multitude of important functions are associated with ligands binding to intracellular receptors. Some of the best characterised are tabulated below (see table 5).

Table 5: Examples of intracellular receptors

Receptor (R)	Ligand	Intracellular location	Physiological effects of activation
GC-R	Cortisol	Cytosolic	Stress, immunosuppression, gluconeogenesis
ERα	Estradiol	Cytosolic	Female sexual development
TRα	Thyroxine	Nucleus	General physical development, ↑ metabolism

Abbreviations used: GC- Glucocorticoid; ER- estradiol; TR- thyroxine

Learning objectives revisited

- Be able to provide examples of physiological processes regulated by ionotropic receptors

Muscle contraction (nACh receptors), neuronal activity ($GABA_A$ receptor)

- Explain the mechanisms of G-protein action in signal transduction and provide examples of physiological processes regulated by G-protein coupled receptors (GPCRs)

Ligand binding→ G-protein activation & uncoupling→ target protein activation→ G-protein dephosphorylation. Physiological processes regulated by GPCRs include heart rate and blood pressure

- Explain the mechanisms of enzyme-linked receptor actions and provide examples of physiological processes regulated by enzyme-linked receptors

Ligand binding→ receptor clustering→ intracellular phosphorylation. Physiological processes regulated by enzyme-linked receptors include glucose uptake and apoptosis

- Explain the mechanisms of intracellular receptor actions and provide examples of physiological processes regulated by enzyme-linked receptors

Entry into cell→ Ligand binding→ receptor translocation/ activation→ DNA transcription. Physiological processes regulated by intracellular receptors include immunosuppression and physical development

IMMUNOLOGY 7
Effector T-lymphocytes

Dr Ingrid Müller

- Outline the importance of antigen presenting cells in the induction of T lymphocyte responses
- Describe effector functions of T lymphocytes
- Briefly outline the function of T helper subsets and regulatory T cells

CELL-MEDIATED IMMUNITY:

Mediated by effector T cells and their secreted products (cytokines, perforins, granzymes)
Naïve T lymphocytes **do not** have effector functions

T CELL EFFECTOR FUNCTIONS:

$CD8^+$ effector T cells kill target cells that present peptides of cytosolic pathogens (viruses) in context with MHC class I molecules on their cell surface. Effector CTLs secrete granules.

$CD4^+$ Th1 effector cells activate infected macrophages which present peptides in context with MHC class II molecules on their cell surface.
Th1 effectors produce IFN-γ, IL-2, TNF-β

$CD4^+$ Th2 effector cells are also MHC class II restricted and help B cells to differentiate into antibody secreting plasma cells.
Th2 effector cells produce IL-4, IL-5, IL-6, IL-10, IL-13

Th-17 cells : a functional subset of CD4+ T cells, produce a particular set of inflammatory cytokines, including IL-17. Th17 cells are protective against some bacterial infections, and also mediate pathogenic responses in autoimmune diseases

Effector T cells differ from naïve T cells

Two signals, antigen-specific recognition (TCR-antigen) and a co-stimulatory signal (CD28-B7) are required for activation of naïve T cells.
Effector T cells do not require the co-stimulatory molecule to act

Cell mediated cytotoxicity
Cytotoxic T cells (**CTL**) kill their targets by programmed cell death i.e. **apopotosis** which is characterised by fragmentation of nuclear DNA. CTLs store **perforin** and **granzymes** in their granules. After recognition of infected targets, granules are released, perforin molecules polymerise and form pores in the target cell membrane.
Fas-FasL interaction also leads to killing of target cells

Macrophage activation
Inflammatory Th1 effector cells activate macrophages to promote killing of intracellular pathogens (mycobacteria, leishmania, etc.) Activated macrophages express increased levels of CD40 and TNF-α receptors, and secrete TNF-α which synergises with IFN-γ in the induction of antimicrobial effector mechanisms.

Delayed type hypersensitivity (DTH) reaction

Mediated by pre-existing antigen-specific T cells, mainly by inflammatory Th1 cells. $CD4^+$ Th1 cells release inflammatory cytokines that affect blood vessels (TNF-β), recruit (chemokines) and activate (IFN-γ) macrophages.
DTH inducers: intracellular parasites (*Leishmania*), intracellular bacteria (*Mycobacteria*), intracellular fungi (*Candida*), intracellular viruses (Herpes simplex). Local swelling with cellular infiltrates occur 24-72 hours after antigen exposure.

T-B cell collaboration
Immunoglobulin $(Ig)^+$ B cells bind specific antigen. The Ig-antigen complex is internalised, processed and antigenic peptides are presented on the B cell surface in context with MHC class II molecules. T helper cells with specific TcR recognise antigen-MHC complex on the cell surface. The T-B interactions trigger expression of CD40 ligand (CD40L) on T cells. CD40 L will interact with CD 40 expressed by B cells; T cells secrete cytokines and B cells express cytokine receptors. The activated B cell will differentiate into immunoglobulin (antibody) secreting plasma cells.

Function of T helper cells in relation to their cytokines:

Th1 cells and their soluble effector molecules (IFN-γ, IL-2, TNF-α) are involved in cell-mediated immunity. Th2 cells and their soluble effector molecules (IL-4, IL-5, IL-6, IL-10, IL-13) function in humoral immunity.

Th1 associated functions:	**Cytokines involved**
Macrophage activation	IFN-γ, TNF-α
Delayed type hypersensititvy reaction	IL-2, IFN-γ, TNF-α, IL-3, GM-CSF
Help for CD8 cells	IL-2
Downregulation of Th2 responses	IFN- γ

Th2 associated functions	**Cytokines involved**
B cell proliferation	IL-2, IL-4, IL-5
B cell differentiation and immunoglobulin class switch	IL-2, IL-4, IL-5, IFN- γ, TGF-β
Downregulation of Th1 responses	IL-4, TGF-□, IL-10

Function of regulatory T cells

Some T cells may differentiate into regulatory cells in the thymus or in peripheral tissue; regulatory T cells inhibit the activation of naïve and effector T cells by contact-dependent mechanisms or by secreted cytokines

Immunological memory
- Is one of the hallmarks of adaptive immune responses.
- Memory responses are faster and greater in magnitude than primary immune responses.
- Immunological memory can confer life-long protection to many infections, is basis of vaccination.
- Memory cells show qualitatively different and quantitatively enhanced responses upon re-exposure.

IMMUNOLOGY 8
Regulation of lymphocyte responses

Dr Ingrid Müller

Learning objectives:

- Outline T and B lymphocyte collaboration and the mechanisms governing the generation of antibody classes.
- Briefly describe how cytokines produced by T lymphocytes regulate other cells of the immune system.
- Describe how lymphocyte responses can be regulated: the roles of antigen concentration, innate immunity, cytokine cross-regulation, immune suppression, and regulatory T lymphocytes.
- Appreciate the importance of regulation of lymphocyte responses: to prevent responses against self (tolerance), to avoid tissue damage and excessive lymphocyte activation during immune responses.

MICROBIOLOGY 2
Bacterial Diseases

Professor Shiranee Sriskandan

Infectious Diseases are the leading cause of mortality world-wide, with bacterial disease resulting in more death than viral and parasitic infections combined.

Not every bacterium can cause disease. Some possess particular **v…….. d……..** that enable them to infect and afflict humans.

A bacterium that can cause disease is called a p**……..**. Typically the term p…… refers to bacteria that cause disease in otherwise healthy individuals (e.g. causes of diarrhoea such as *Salmonella* spp. and *Shigella* spp., causes of pneumonia such as *Streptococcus pneumoniae* and tuberculosis, causes of septicaemia such as *E. coli* and *Neisseria meningitidis*). However, some organisms can cause disease in immunocompromised hosts (e.g. *Aspergillus* in neutropeanic patients, *Pneumocystis carinii* in patients with AIDS), and are called **o……. p…….**.

The *extent* of damage a bacterium causes its host is called **v……..……**.

V…… d……...s act in several ways. They can enhance a bacterium's:

1) **……..**: ability to infect certain tissues. This can be by expressing certain adhesins which recognise and bind to host cells, or by allowing bacteria access to and mechanisms for survival within a particular site in the body (e.g. cerebrospinal fluid in bacterial meningitis).

2) **…….**: through acquisition of vital nutrients (such as iron, carbon sources) and evasion of the immune response (*Neisseria meningitidis*).

3) **……**: through the action of toxins such as lipopolysaccharide on the bacterium surface.

Microbes must also be able to effectively colonise a host, and be spread from person to person (a process called **t…….**). These attributes, although important, are not well understood.

Vi…… d……..s may be encoded in regions of DNA which are only found in a pathogen. Clusters of genes which are required for pathogenesis and found exclusively in pathogenic bacteria are referred to as **p…… i……..**, and were usually acquired in a single genetic event**. p…… i……** have had a major effect on the emergence and evolution of bacterial pathogens, by providing previously **…….. bacteria** (harmless members of the flora) with novel attributes that allow them to infect a new niche or cause damage.

There is still a need for vaccines to prevent bacterial infection and drugs to combat multi-resistant strains. This will be achieved through greater understanding of the molecular basis of the disease process.

Extended reading:

Smith H. What happens to bacterial pathogens *in vivo*? Trends Microbiol. 1998 **6:**239-43.

MICROBIOLOGY 3
Hospital acquired infection and antibiotic resistance
Dr Andrew Edwards

- *Learning objectives*

1. Understand the scale of the problem
• Antibiotic resistance is an emerging problem in healthcare settings and the community.
• Resistance typically emerges soon after the arrival of a new antibiotic.
• Antibiotic therapy provides a selection pressure for the spread of antibiotic resistance.
• Resistance has been reported to all major antibiotics. Many serious pathogens are resistant to multiple antibiotics.
• Associated with Increased morbidity, mortality, length of hospital stay and cost.

2. Name the important bacterial pathogens that are multi-drug resistant
Gram-negative: *P. aeruginosa*, *E. coli* (ESBL, NDM-1), *Salmonella spp*, *A. baumanii.*
Gram-positive: *S. aureus*, *S. pneumoniae*, *C. difficile*. *Enterococcus spp*.
Also, *M. tuberculosis*.

3. Outline reasons for the high rate of hospital acquired infections
High density of susceptible people, presence of pathogen, staff vectors, open wounds, inserted medical devices, e.g. IV catheters, disruption of normal flora due to antibiotic prophylaxis/therapy.

4. Describe the mechanism of action of some important antibiotics
Selective toxicity – key differences between host and bacterium. Beta-lactams, vancomycin-cell wall; Quinolones-DNA replication; Erythromycin, chloramphenicol & tetracycline-protein synthesis.

5. Outline mechanisms of antibiotic resistance
Four main mechanisms: Altered target site, Drug inactivation, efflux, altered metabolism. Genes spread via mobile elements e.g. plasmids. Conjugation, transduction, transformation.

6. Describe some of the approaches used to prevent the emergence of drug-resistant bacteria and nosocomial infections
Better prescribing practices. Infection control. Combination therapy. Narrow vs broad spectrum antibiotic therapy.

Revision quiz, fill in the blanks!

Gram negative
…….Hospital acquired pneumonia, burn wounds, particularly affects immunocompromised hosts (e.g. chemotherapy, individuals with cystic fibrosis). Survives on abiotic surfaces.
……..Extended spectrum beta-lactam resistant *E. coli*.
…….. ITU infections, survives on abiotic surfaces.

Gram positive
…… colonises nasopharynx, causes bloodstream infections, disseminated spread e.g. osteomyelitis & infective endocarditis.
……commensal of gastrointestinal tract. Causes bloodstream and urinary tract infection.
…….major cause of antibiotic associated diarrhea and mortality.

Insert:
Enterococcus spp, Methicillin Resistant *Staphylococcus aureus*, ESBL, *Pseudomonas aeruginosa, Acinetobacter baumanii*, *Clostridium difficle.*

CELL PATHOLOGY 1
Cell injury
Rob Goldin r.goldin@imperial.ac.uk

Learning Objectives

- List the causes of cell injury
- List the mechanisms of cell injury
- Define (and give examples of) hyperplasia, hypertrophy, atrophy, metaplasia and dysplasia
- Describe the morphological changes associated with reversible and irreversible injury
- Describe the differences between apoptosis and necrosis
- Define the terms necrosis, ulcer, degenerative, sublethal injury

Causes of Cell Injury:

1. **Oxygen deprivation**
2. **Chemical agents**
3. **Infectious agents**
4. **Immunological reactions**
5. **Genetic defects**
6. **Nutritional imbalances**
7. **Physical agents**
8. **Aging**

The cellular response to injurious stimuli depends on:

1. **the type of injury,**
2. **its duration and**
3. **its severity**

The consequences of an injurious stimulus depend on:

1. **on the type of cell,**
2. **its status,**
3. **its adaptability and**
4. **its genetic makeup**

Four intracellular systems are particularly vulnerable:

1. **cell membrane integrity,**
2. **ATP generation,**
3. **protein synthesis and**
4. **the integrity of the genetic apparatus**

Mechanisms of Cell Injury

The structural and biochemical components of a cell are so integrally related that multiple secondary effects rapidly occur. Cellular function is lost before cell death occurs which in turn occurs before the morphological changes are seen

Cellular Adaptation to Injury:

1. Atrophy

Shrinkage in the size of the cell (or organ) by the loss of cell substance

2. Hypertrophy

Increase in the size of cells and consequently an increase in the size of the organ
Can be physiological or pathological
It is caused either by increased functional demand or specific hormonal stimulation

3. Hyperplasia

An increase in the number of cells in an organ

1. **Can be physiological or pathological**
2. **Physiological hyperplasia can be either hormonal or compensatory**
3. **Pathological hyperplasia is usually due to excessive hormonal or growth factor stimulation**

4. Metaplasia

A *reversible* change in which one adult cell type is replaced by another

5. Dysplasia

Precancerous cells which show the genetic and cytological features or malignancy but not invading the underlying tissue

The Light Microscopic Changes Associated with Reversible Injury

1. **Fatty change**
2. **Cellular swelling**

The Light Microscopic Changes Associated with Irreversible Injury

1. **Coagulative necrosis**
2. **Liquefactive necrosis**
3. **Caseous necrosis**
4. **Fat Necrosis**

Apoptosis

Causes:

1. **Embryogenesis**
2. **Deletion of auto-reactive T cells in the thymus**
3. **Hormone-dependent physiological involution**
4. **Cell deletion in proliferating populations**
5. **A variety of mild injurious stimuli that cause irreparable DNA damage that triggers cell suicide pathways**

The differences between apoptosis and necrosis:

1. **Apoptosis may be physiological**
2. **Apoptosis is an active energy dependent process**
3. **Not associated with inflammation**

IMMUNOLOGY 9
Host defence overview

Professor Peter Openshaw (p.openshaw@imperial.ac.uk)

Learning objectives:

- Name the main components of the immune system
- Understand the sequence and timing of events during infection
- Summarise and give examples of the roles taken by:
  (i) physical, chemical and mechanical barriers; (ii) antigen presenting cells; (iii) immune regulatory mechanisms
- Define explain and contrast: Innate versus adaptive/acquired immunity: Humoral versus cellular immunity: Defences against viruses and bacteria: Defence at mucosa versus the skin

Why do we have an immune system?

It can be argued that the immune system has developed to provide us with a survival advantage against infection, and that all other functions are a by-product. Our internal and external surfaces are bathed in microbes. We inhale potentially lethal microbes with every breath that we take, and our cells are outnumbered by our bacteria by 10:1, which form about 3% of our body mass. The essential challenge of the immune system is to remain indifferent to non-pathogenic microbes, while responding rapidly and appropriately to the constant microbial onslaught.

The physical and chemical barriers: innate defence

A vital barrier to the entry of pathogens is the so-called 'wall of death', made up of surface layers of skin that are dead or dying and constantly being shed. Unless the skin is broken by trauma or biting insects, it is very unlikely that infection can gain access except through the lung or gut. The lung and gut are organs specialised to provide a large area of contact with the environment, necessary for gas exchange and absorption of food and water. The mucosal surfaces turn over at a very fast rate, with all the superficial cells being sloughed within a few hours or days. Any microbe that attaches to these cells is soon lost along with the dead and dying cells. The mucocilliary system in the lungs clears microbes from the lung; Cystic fibrosis patients cannot form mucus normally and suffer from recurrent respiratory infections. Mechanical defence should not be underestimated.

There are also chemicals (fatty acids, enzymes etc.) that bathe the skin and other body surfaces: Lysozyme in our tears digests bacterial cell walls and the acid in our stomachs kill many of the microbes we ingest. The normal flora in our gut prevents other bacteria from gaining a foothold, so affecting susceptibility to gut infections following antibiotic treatment.

Detection of Pathogens by the Innate Immune System

The innate immune system is our first line of defence against infection. Its components are generally innate, i.e. pre-formed, and rapidly react to pathogen invasion. Classically, the innate system does not 'adapt' and therefore shows no memory response.

Recognition is based on the sensing of common molecular patterns on the surface of pathogens, a signal that is contingent on whether or not that particular foreign component is normally present at the site concerned. Therefore, a molecular pattern may be sensed at the surface and lead to no response, whereas the same molecular pattern sensed in the cytosol may induce a vigorous reaction. The toll-like receptors (TLR) are an excellent example of this pathogen sensing system.

The complement system is a pre-formed protein cascade which can rapidly punch holes in the outer membrane of microbes, coat them for phagocytosis ('opsonisation') and produces chemoattractants which recruit cellular components of the immune system.

Chemical signals: interferons, chemokines and cytokines

The production of interferons is also crucial to host defence. Interferons are soluble low molecular weight mediators released by cells in response to infection, that act both on the cell that releases them (autocrine action) and on other neighbouring cells (paracrine) to induce an antiviral state and increase defence. The type 1 interferons activate natural killer (NK) cells and increase the expression of molecules involved in processing and presenting viral proteins on the cell surface. The importance of the interferon system to viruses is shown by the very large number of viruses that have evolved mechanisms to block the synthesis and actions of interferons.

Low molecular weight mediators are also very important in recruiting other cells to the site of information. Cells that circulate in the blood and lymph migrate out into the tissues in response to infection, particular combinations of mediators attracting particular cells (by secretion of chemoattractants called 'chemokines'). Neutrophils, for example, are attracted by a chemokine called interleukin 8 (IL-8). The eosinophils, on the other hand respond to eotaxin or RANTES.

Cytokines are chemical signals used for communication by the immune system. They may have local and systemic effects and direct the extent and the nature of the immune response. For example, Interferon-gamma can be produced by T cells to enhance activation of macrophages. The cytokine TNF-alpha has many systemic effects associated with infection, including fever and weight loss.

Innate cellular defences

Once in the tissues, the inflammatory cells produce additional chemoattractive or activating mediators, and may themselves be phagocytic (e.g. they take up particles that are degraded by vesicles within the cells). Macrophages are important phagocytes which may be tissue resident or be recruited during infection. Neutrophils, which make up the majority of circulating leukocytes are rapidly recruited to sites of infection. Phagocytes can use their surface receptors to directly recognise the outer surface of microbes or to recognise other components of the immune system, including complement and antibody, that have coated or opsonised the microbe surface. The phagocyte's defence mechanisms include toxic enzymes, reactive radicals and defensins that are produced in the phagosome once the pathogen has been taken up.

Natural killer (NK) cells are regulated by a combination of inhibitory and stimulatory receptors. Surface receptors on NK cells that recognise a normal cell (e.g. one displaying class I major histocompatibility complexes or 'MHC I') stops the NK cell from becoming active. Lack of MHC on the surface of the cell may indicate that a virus is trying to hide in the cell. On the other hand, a stimulatory receptor may be triggered by recognition of cell surface proteins on other cells that signify an abnormal state of infection or transformation, leading to NK activity.

NK cells form a bridge between the innate and the acquired immune system. They kill abnormal or infected cells; if they are defective (e.g. in rare inherited deficiency states), common virus infections tend to be severe and can be fatal. They are an important source of some of the mediators produced by classic T-cells (see below). By producing different combinations of T-cell cytokines, they can help to shape the adaptive immune response.

T and B cells (the acquired immune system)

Acquired immune responses are highly specific to each antigen and result in memory of that antigen.

The acquired immune system can be divided into T-cells and B-cells. Both of these originate from the bone marrow and circulate in the blood, but T-cells need to pass through the thymus to mature. T and B cells recognise specific antigens using their surface receptors; each T or B cell will recognise only one antigen. When these cells mature cells with a huge diversity of receptors are generated by random reassortment of the genes encoding the receptors. Because this process is random, there is a risk that 'autoreactive' T and B cells are produced. These cells are usually eliminated or regulated but autoimmune disease can result, if these processes fail.

B-cells have antibody on the surface as their receptor, and secrete soluble antibody that is able to bind to an almost infinite variety of protein or non-protein 'antigens'. Each B-cell represents a clone, able to produce only one exact variety of antibody. The antibody can bind directly to the surface of pathogens so helping the pathogen to be engulfed by a phagocyte or punch holes in the membrane of the pathogen using the complement system. Alternatively, antibodies may 'neutralise' a pathogen, blocking its surface receptors and preventing it from attaching to or infecting host cells.

T-cells are quite different. They do not recognise the molecular surface, shape and charge of antigen, but instead recognise sequences of peptide from digested antigens presented by antigen presenting cells. The T-cell receptor locks on to MHC surface proteins which are of two types. MHC I is present on all nucleated cells, under normal circumstances. There is a cleft on the external tip of this protein that holds a short peptide 'signature', representing a digest fragment of internally synthesised proteins. If this is a normal host protein, T-cells detect the presence but are selected not to respond strongly. If it is a novel sequence, the T-cells recognise it as foreign and respond strongly. On the other hand, the MHC II has an external cleft that bears a digestion fragment of protein that has been picked up from outside a professional antigen presenting cell. These 'professionals' include dendritic cells, macrophages and some B-cells. MHC II is not present on ordinary cells.

T-cells with a helper function (those recognising peptide presented by MHC II) are often subdivided according to the soluble mediators that they produce. 'Th1' cells classically make interferon gamma and tumour necrosis factor (TNF). On the other hand 'Th2' cells make IL-5, IL-4, IL-9 and IL-13. These are mostly involved in allergic responses and lead to eosinophil recruitment. However, the situation is getting ever more complex; it has recently been shown that there are cells specialised to produce IL-17 ('Th17') and various types of regulatory T-cell that make combinations of inhibitory cytokines. Regulatory T cells can also dampen immune responses by depriving other cells of the immune system of vital factors (like IL-2) or by acting on dendritic cells to inhibit activation.

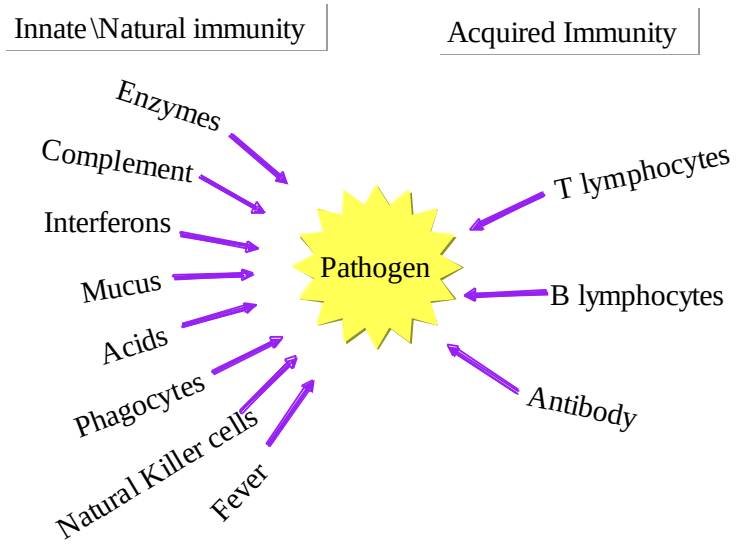
Protection against specific microbes

The key defences against bacteria are the intact surfaces of the body, antibody, complement, phagocytosis and the acute phase proteins (which are opsonins, and bind in a relatively non-specific way to bacteria). On the other hand, defences against viruses include the surface defences, interferons, inflammatory mediators, NK-cells, antibody and T-cells.

We can exploit the ability of the immune system to remember previous exposure to specific antigens in developing protective vaccines. If the immune system encounters a foreign substance for the first time, it is required to recognise the material as foreign and then to expand the number of cells that recognise that specific antigen before it can mount a full T and B-cell response. However, on second encounter the response is very much more rapid and vigorous (more antibody is produced) and better (antibody is of higher affinity). Indeed,

some vaccines lead to very long lasting antibody that may be enough alone to protect against infection.
Since vaccination was popularised by Edward Jenner, many different vaccines have been introduced to protect against the majority of the severe life threatening infections that were so prevalent only a century ago. It is very rare to see cases of tetanus, diphtheria, typhus, anthrax or measles, except in people who have not received the benefit of vaccination. However, these medical marvels are only available to people living in well-resourced parts of the world, and about 3 million children die every year because they have not been given standard vaccines that would have otherwise have saved their lives. Perhaps good, stable political systems should be regarded as a crucial component of our defence against the microbial world.

Major challenges in the future are to control the immune response when it causes disease (in allergy, autoimmunity, in toxic shock and transplantation, for example). We also need a better understanding of why the immune system fails to protect us against some infections (such as HIV) and in cancer, and design novel strategies for enhancing protective immune responses.



MICROBIOLOGY 4
Viral properties

Professor Wendy Barclay
Section of Virology, St Mary's campus

History of early virology:
In 1892 Ivanovsky working on a plant disease, tobacco mosaic disease, suggested there must be a pathogenic organism smaller than a bacteria because it passed through a filter. The same year, Beijerink concluded this must be a distinctive entity and called such things *contagium vivum fluidum*, or 'ultrafilterable viruses'. Virus means poison in Latin.
The first animal virus to be discovered, in 1898, was Foot and Mouth Disease Virus, also shown to be filterable by Loeffler and Frosch. This and the plant agent TMV replicated ONLY in a susceptible host, not in broths that would support amplification of bacteria.
The first human virus to be described was Yellow Fever Virus. In 1901 Colonel Walter Reed of the US Army was intensely researching this disease because it caused havoc during the building of the Panama canal. There was a clear link with mosquitoes. Reed injected filtered serum from a patient into non-immune individuals and reproduced the disease, thus fulfilling **Koch's postulates**.

- **Describe the nature of viruses: their small size, dependence on a host and their structural and genetic diversity**

Two key features of viruses are:

1. **Structural simplicity**: Virus particles are structurally simple. They have highly repetitive units that are so regular they can be crystallized. TMV was crystaliized in 1932. This achievement fed the debate as to whether viruses were alive or not. Their capacity to form crystals made them seem more like an inorganic substance.

2. **Intracellular parasitism**.

A usable definition of a virus would include:

1. Viruses are infectious OBLIGATE intracellular PARASITES.
2. A virus has a genome that comprises DNA or RNA.
3. Within an appropriate cell, the viral genome is replicated and directs the synthesis, by cellular systems, of more viral components and genomes.
4. The components effect the transport of replicated viral genomes through the environment to new host cells.

- **Define the following terms as used in the description and classification of viruses:**
  DNA virus, RNA virus, capsid, enveloped, non-enveloped

Classification. Virus names are random. Viruses have been historically named after:
associated disease, e.g. poliovirus, rabies,
type of disease e.g. murine leukemia,
place in the body where isolated, e.g. rhinovirus
geographical location where first found e.g. Sendai, Coxsackie,
person who discovered it e.g. Epstein Barr,
way imagined to be spread e.g. dengue means evil spirit, influenza means influence of bad air
It is more sensible to use common features to assign viruses to taxonomical groups, such as;

1. Nature of nucleic acid in the virion
2. Symmetry of the protein shell
3. Presence or absence of a membrane
4. Dimensions

Commonly used nowadays is the **Baltimore Classification system** which places viruses into groups depending on the pathway they use to make their genomes into mRNAs

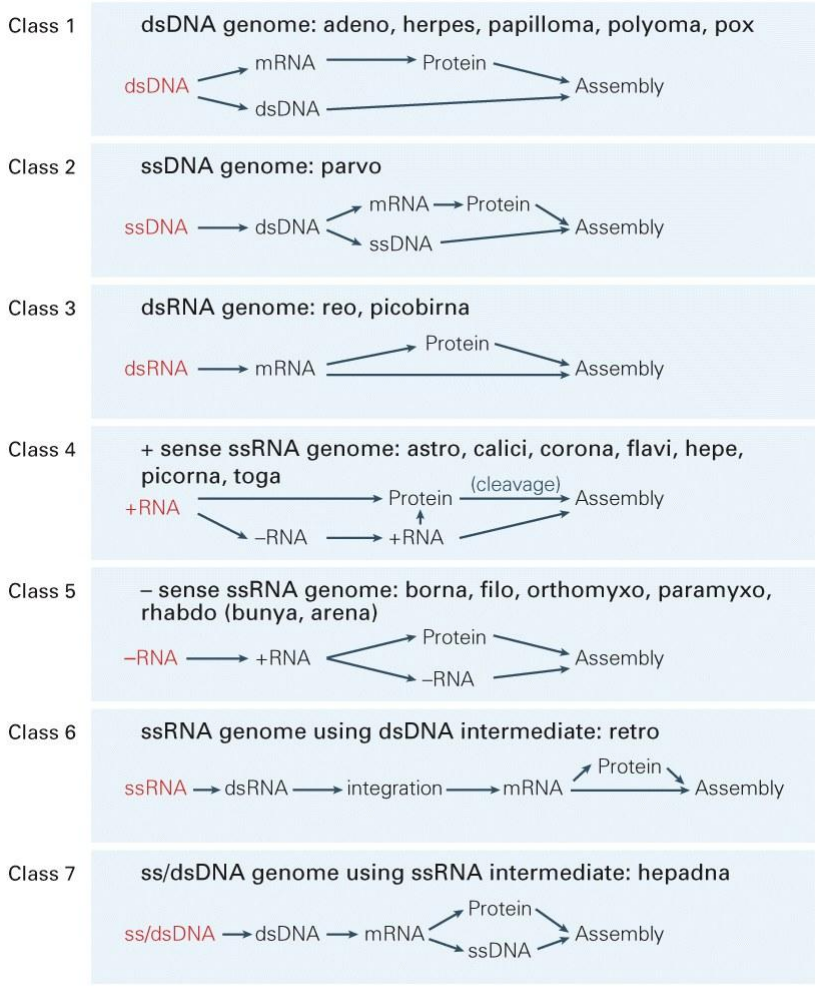
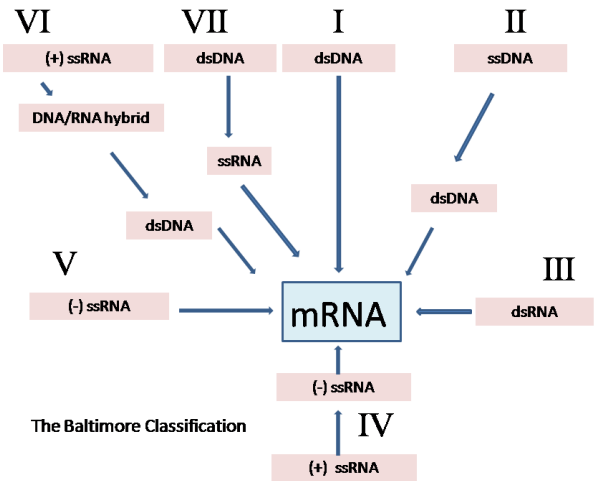


Figure 3.5 Viruses: Biology Applications Control (©2012 Garland Science)



- **Describe a generic virus replication cycle**

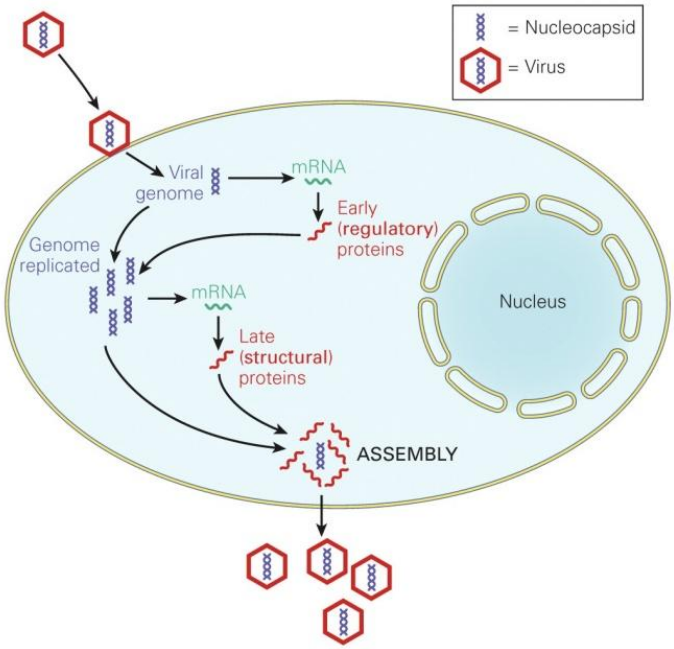


Figure 1.1 Viruses: Biology Applications Control (©2012 Garland Science)

The various sequential steps of the replication cycle of a typical virus are:

1. **Attachment** to the host cell by specific interaction between the virus attachment protein and a host cell receptor (a molecule on the surface of a host cell that has a completely different role but that the virus has evolved to use as its key for entry to the interior of the cell).
2. **Entry** of the virus genome to the host cell and synthesis of viral mRNA (primary transcription).
3. **Translation** of viral mRNAs into viral proteins by host cell ribosomes in the cytoplasm.
4. **Replication** of the viral genome, usually by making a small number of complementary copies that are then themselves copied at high numbers into new genomes (asymmetric replication).
5. **Assembly** of the virus proteins into new virions, association and encapsidation of the new genomes.
6. **Exit** from the infected cells and dissemination to new hosts.

The kinetics of virus replication is described in the one step growth curve:

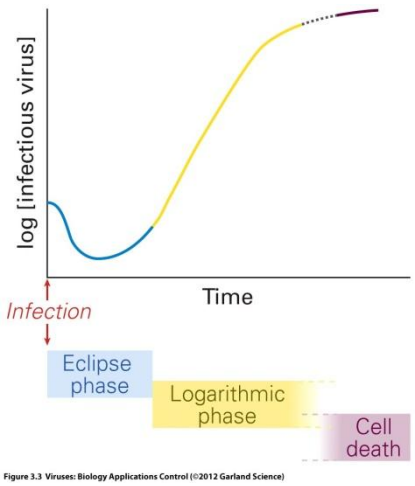


Figure 3.3 Viruses: Biology Applications Control (©2012 Garland Science)

- **Describe how viruses are detected, cultivated and manipulated**

  Viruses are very small (10-500 nm diameter) and are visualized with the **electron microscope**.
  We also 'see' viruses by their effects on their hosts. This can be a plant or animal (*in vivo*) or cells cultured in the laboratory (*in vitro*).
  Primary cell cultures may include several different cell types and may retain aspects of the original tissue.
  Continuous cell lines are monocultures propagated indefinitely due to their transformation. They may grow as monolayers on a plastic support or in suspension.
  Viruses may produce effects on the cells as they replicate such as **cytopathic effect, cpe**, or syncytia, inclusion bodies, membrane blebbing.
  Dilutions of virus can be assayed to find the end point at which they no longer produce such effects. This allows their quantification.
  A specific example is the **plaque assay** in which foci of infection can be counted.

  Some viruses produce little visible change on the cells but can be detected using antibodies to their proteins that will be expressed during replication. **Immune fluorescence** or immunohistochemistry allow the antibody binding to be seen.
  Techniques for 'seeing' viruses that do not use cell cultures include detection of the particles or the viral proteins, or the virally encoded nucleic acid.
  Viruses that attach to red blood cells can be visualized using the **haemagglutination assay**.
  Cells infected by viruses can be lysed and antibodies to viral antigens used in **Western Blot** or **ELISA**.
  Nucleic acid detection is usually by **PCR**.

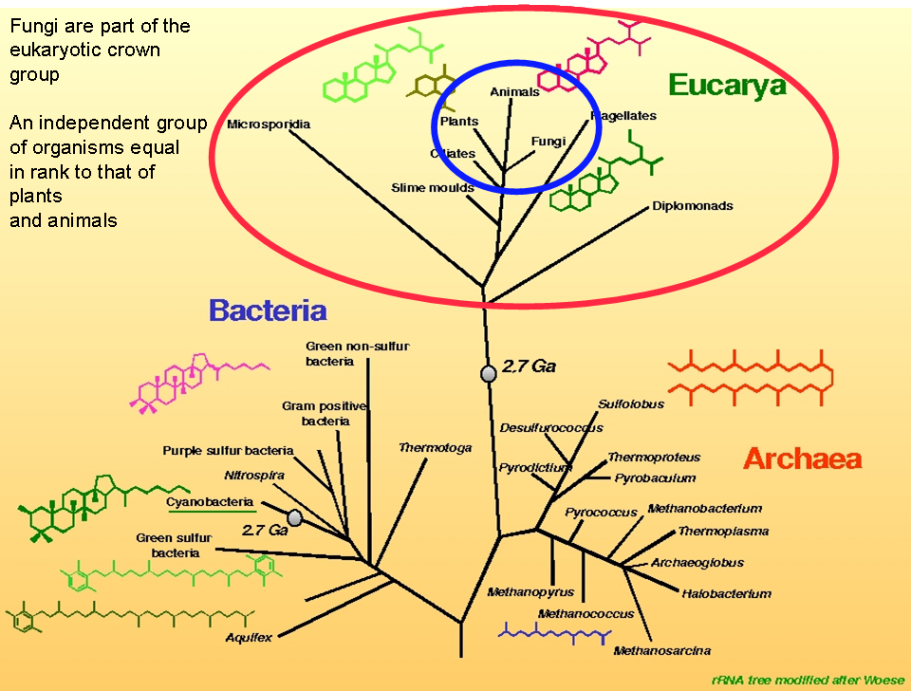
  Viruses can be manipulated in the laboratory in order to study them.
  Classical genetic techniques involve growing the virus under different conditions, for example temperature or in the presence of a drug, that may induce changes in the genome and studying their effects.
  Reverse genetics techniques involve engineering cDNA that represents the sequence of the virus genome, introducing it into cells and recovering an altered virus.

MICROBIOLOGY 5
Fungal Infections
Dr Elaine Bignell

After this lecture you should know:

The various types of fungal infection and the major fungal pathogens
The principals of diagnosis of fungal infection
The major classes of antifungal drugs and their mechanism of action
The mechanisms by which fungal pathogens cause disease

Fungi have been called “The Fifth Kingdom” and are the largest group of organisms on the planet.

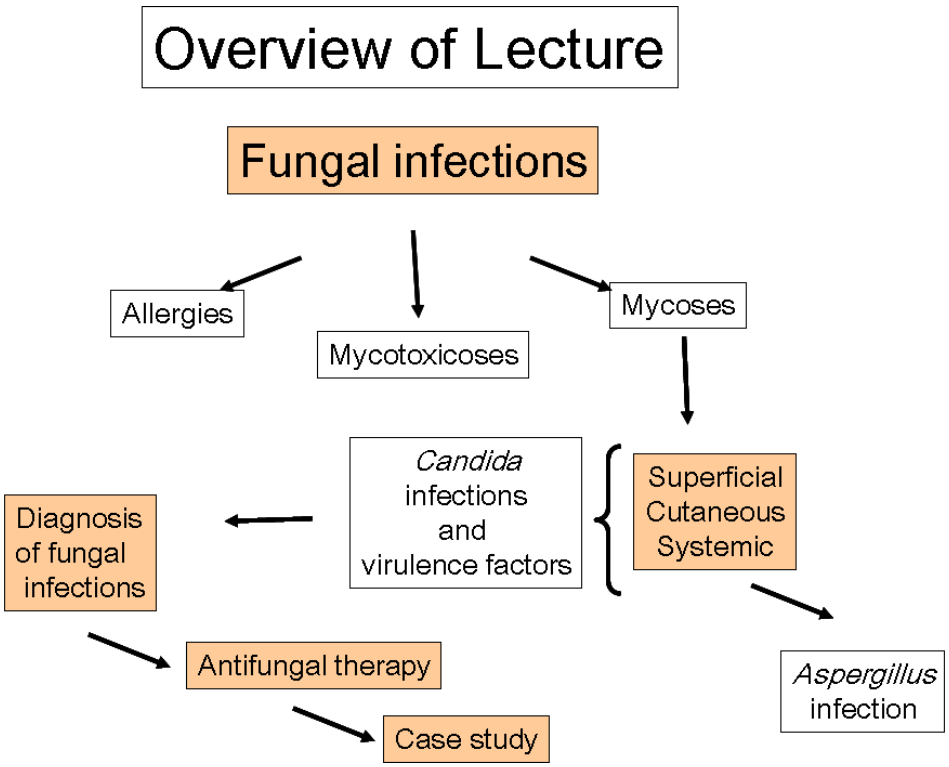


How many fungal species are known to science?

How many fungal species exist?

Fungi are eukaryotic – what implications does this have for therapy?

Fungi digest their food outside of the cell by secreting hydrolytic enzymes which can break down biopolymers to be absorbed for nutrition.

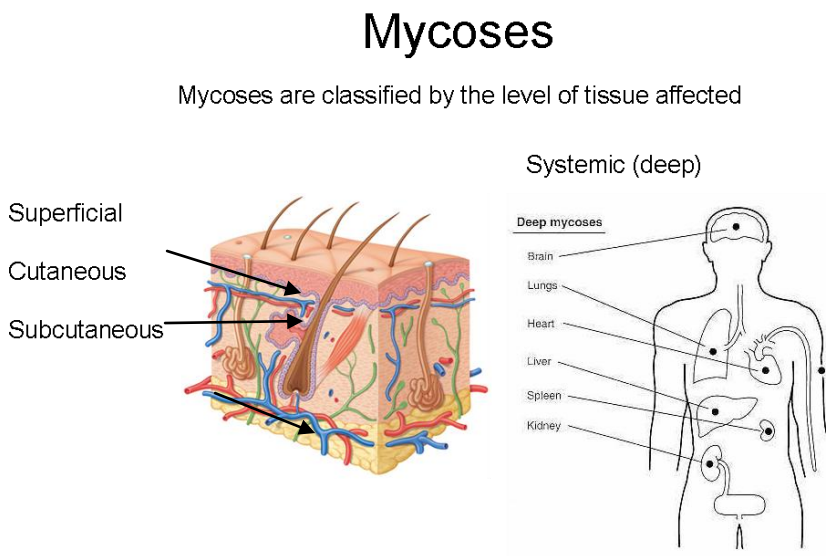


Types of fungal Infection

1) **Allergy** – allergic reactions to fungal products e.g. allergic bronchopulmonary aspergillosis (ABPA).

2) **Mycotoxicoses** – ingestion of fungi and their toxic products e. g. aflatoxin.

3) **Mycoses –** superficial, subcutaneous or systemic colonisation, invasion and destruction of human tissue.



Name one example for each of superficial, cutaneous and subcutaneous fungal infection.

Define the following terms:

Pathogen

True pathogen

Opportunistic pathogen

Can you give fungal examples of each?

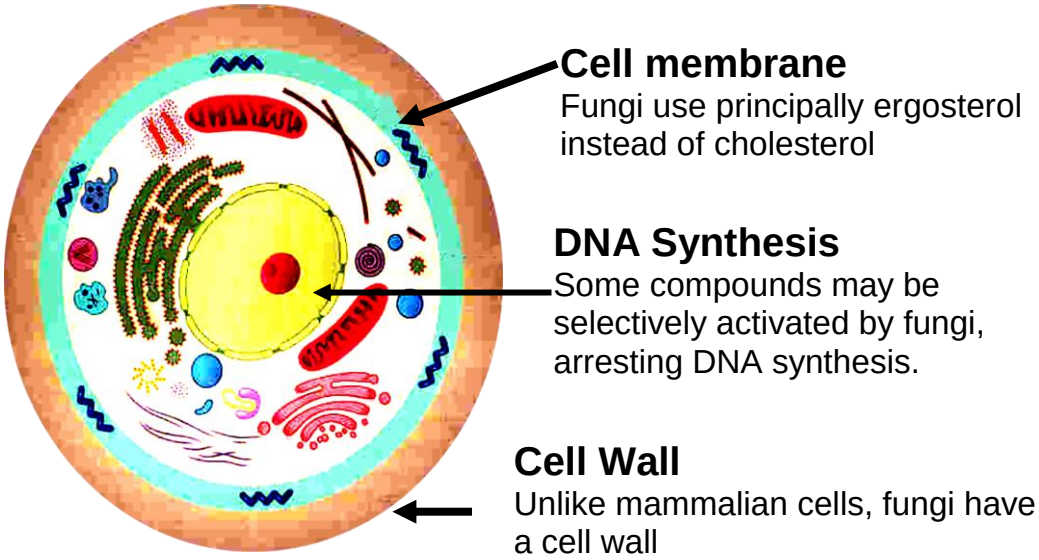
Why have incidences of hospital-acquired fungal infection risen in recent decades?

How are fungal infections diagnosed?

What problems are associated with diagnosis of fungal infection?

How can diagnosis affect outcome?

What are the targets for antifungal therapy?



Most antifungal drugs target fungal components that have no human homologue.

What attributes should an antifungal drug target have?

MICROBIOLOGY 6
Patterns of viral infection
Professor Wendy Barclay

- **Describe different routes of infection by viruses: define the term tropism and understand what defines the tropism of a virus**

Viruses can enter the body
1. Through the epithelial layers; respiratory tract, GI tract, genital tract
2. Directly into the blood through a bite or needle
3. Through the skin, often following abrasion

From the site of entry the virus may travel, often in the blood (primary **viraemia**), to another organ where amplification via replication takes place. There may be secondary viraemia to the main organ site for replication.
The **tropism** of the virus is the place where it replicates.
Tropism may be determined by the expression of the **host cell receptor**. HIV enters cells through the CD4 molecule found on T cells.
Tropism may also be limited by the ability of the virus to replicate inside a particular cell type due to abundance or paucity of **essential intracellular host cell components**.
Polioviruses with mutations in their 5' noncoding regions cannot utilize neuronal host cell factors to translate their mRNAs.
Tropism may also depend on **extracellular factors** required for activation of virus infectivity. Influenza virus HA protein requires to be cleaved by a host encoded protease expressed in respiratory secretions.

- **Outline, with named examples, the different modes of transmission of viruses**

Transmission of a virus from one host to another usually requires it to be shed into the environment.
Rarely viruses are transmitted through the germline, acquired through cannibalism (prion diseases like Kuru) or through nosocomial blood contamination (HIV or hepatitis B or C).

Respiratory transmitted viruses are carried in aerosols (influenza, rhinovirus).
Viruses may be shed into the oral cavity and transmitted in saliva (human cytomegalovirus, EBV, mumps).
Enteric viruses are transmitted through the fecal oral route (poliovirus, norovirus, hepatitis A virus)
Viraemic viruses are transmitted through blood (dengue virus when bitten by an arthropod, Ebola virus)
Virus can be present in urine of animals (hantaviruses in rodents) but urine is rarely a source of human to human transmission.
Viruses in skin can be transmitted by direct skin contact, poxvirus, papillomavirus.

- **Describe different outcomes of infection by viruses: acute infection, persistent infection, latent re-activating infection, slow infection, oncogenesis**

To be a human pathogen, a virus will need to have strategies to counteract host defences.
The capacity of the virus to cause disease (pathogenesis) will depend on
1. the effects of its replication
2. the strength of the host's defence system
3. the ability of the virus to spread in and amongst its hosts

Acute infection is the typical expected outcome for **influenza**.
Rapid production of infectious virus, rapid resolution and elimination of virus by host immune system.
The outcome is determined by intrinsic and innate immunity.
Acquired immunity stimulated after several days mediates final clearance from the host.
Memory provides defense against subsequent exposure.
Acute infections frequently cause epidemics. Transmission occurs before symptoms.
Inapparent infections (asymptomatic) are common.

Persistent infections also have to overcome innate defense at the start of infection. They are not cleared by the adaptive immune response. They may be **chronic** or lifelong (**latent, slow**).
75-85% people infected by **Hepatitis C virus** will not clear the virus with their CTL response. This may be because the virus rapidly mutates to escape the response by changing its T cell epitopes. Of these chronically infected people, 1-5 % will develop hepatocellular carcinoma. Since more than 170 million people are infected, this accounts for up to 3 million hep carcinoma cases. Chronically infected hepatocytes are destroyed by the immune system leading to fibrous scars (cirrhosis).

The classic example of a **latent virus infection** is **herpes simplex virus**. The virus first replicates in mucosal or epidermal cells. Perpiheral ganglia become infected and produce a large burst of virus that disappears after 1-2 weeks. The virus establishes a latent infection in **terminally differentiated non-dividing neurons** of the peripheral nervous system. Since neurons do not replicate their DNA nor divide, the HSV genome survives inside these host cells. The only evidence of the virus is the expression of RNAs known at latency associated transcripts LATs. By this time the infected host is 'immune', they have antibodies to their latent virus. Some people reactivate their virus every 2-3 weeks, others experience few or no reactivation events. Stress signals can trigger reactivation. **Reacitvation** can also be by drugs like glucocorticoids that stimulate transcription but suppress immune responses. Transient production of virions allows spread of the virus across innervated mucosal surfaces to a new host. Then the infected host's immune response curtails virus production.

- **Give examples of different viruses associated with infectious disease in humans and describe their replication cycles and the way in which they cause disease**

HIV (human immunodeficiency virus) is a lentivirus and a member of the retrovirus family. HIV infects and destroys helper T cells of the immune system causing a marked reduction in their numbers. Loss of CD4 cells leads to generalized failure of the immune system and susceptibility to life threatening opportunistic infections.

gp120 – an HIV glycoprotein having a molecular weight of 120 that protrudes from the outer surface of the virion. This glycoprotein binds to a CD4 receptor on a T cell to facilitate entry of viral nucleic acid and proteins into the cell.

CD4 – a large glycoprotein that is found on the surface of helper T cells, regulatory T cells, monocytes, and dendritic cells. Its natural function is as a co–receptor that assists the T cell receptor (TCR) to activate its T cell following an interaction with an antigen presenting cell. CD4 is a primary receptor used by HIV–1 to gain entry into host T cells.

Co–receptor (CCR5 or CXCR4) – protein molecules on the surface of lymphocytes or monocytes that bind to the gp120 protein of HIV and facilitate, usually with CD4, entry of viral nucleic acid and proteins into the cell.

Fusion of virus and cell membranes – a merging of cell and virus membranes that permits HIV proteins and nucleic acids to enter the host cell.

Preintegration complex (PIC) – It is composed of viral RNA and proteins (nucleocapsid, p6, Vpr, integrase, and matrix) as well as some host proteins. It functions to reverse transcribe genomic RNA into double stranded DNA prior to integration into the host genomic DNA.

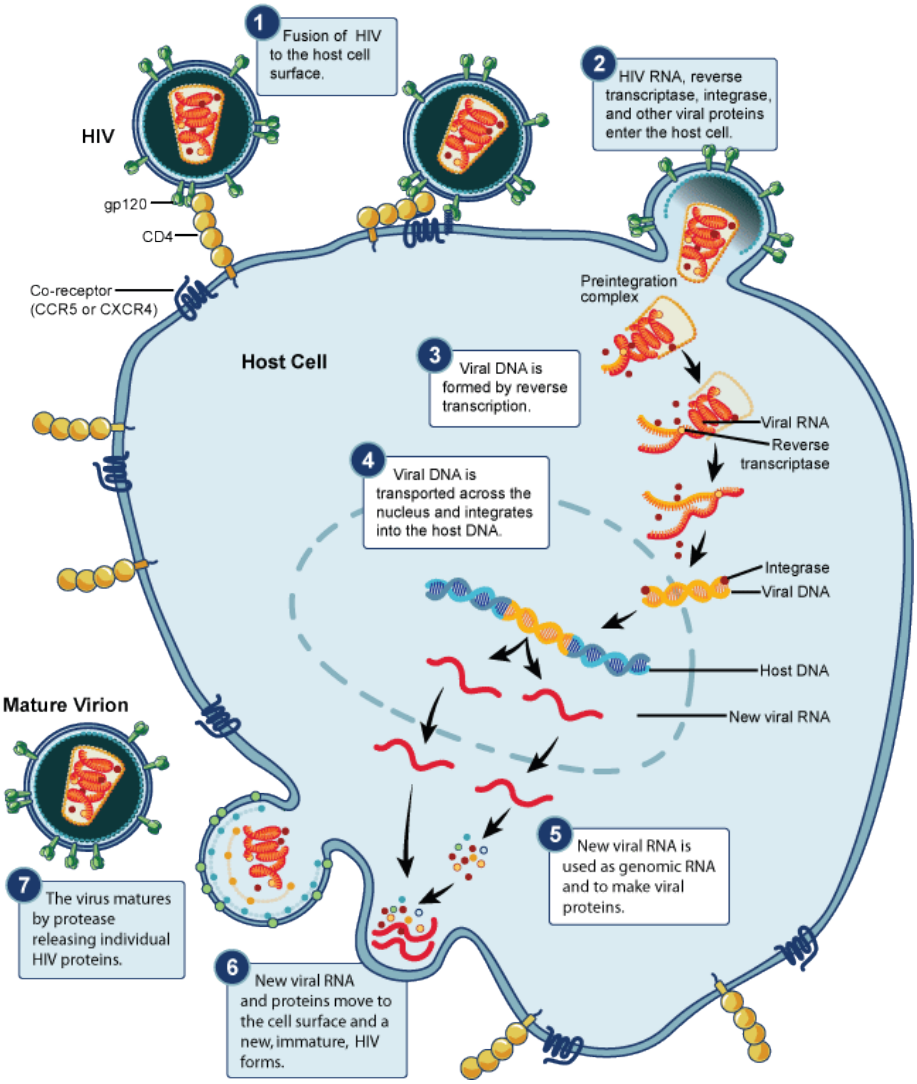
Reverse transcriptase – an enzyme found in HIV that creates double stranded DNA using viral RNA as a template and host tRNA as primers.

Integrase – An enzyme found in retroviruses including HIV that permits the viral DNA to be integrated into the DNA of the infected cell.

Protease – an enzyme that hydrolyzes or cuts proteins and is important in the final steps of HIV maturation.

Steps in the HIV Replication Cycle

1. Fusion of the HIV cell to the host cell surface.
2. HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.
3. Viral DNA is formed by reverse transcription.
4. Viral DNA is transported across the nucleus and integrates into the host DNA.
5. New viral RNA is used as genomic RNA and to make viral proteins.
6. New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms.
7. The virus matures by protease releasing individual HIV proteins.



CELL PATHOLOGY 2
Haemodynamic Disorders

Dr James Carton

Learning objectives:
- Describe the causes and consequences of oedema at different sites
- Define thrombosis & give causes and potential consequences of such an event.
- Define embolism & know the importance of pulmonary embolism in clinical practice.
- Describe possible causes of haemorrhage and potential outcomes.
- Define shock and identify the possible causes and mechanisms.
- Define infarction and describe possible causes, including atherosclerosis.

Oedema

- Abnormal increase in fluid in the interstitial space.
- May result from a raised hydrostatic pressure, a reduced osmotic pressure, or disruption to the capillary bed.
- May be localized (e.g. pulmonary oedema, cerebral oedema) or generalized (affects subcutaneous tissues and serous cavities).
- Pulmonary oedema is usually the result of raised pulmonary capillary hydrostatic pressure due to pulmonary venous congestion. The most common cause of this is left ventricular failure. Consequences include breathlessness and susceptibility to pneumonia.
- Cerebral oedema is usually the result of breakdown of the normal capillary barrier and typically occurs in brain tissue surrounding lesions such as cerebral contusions, haemorrhages, infarcts, and tumours. Cerebral oedema contributes to a rise in intracranial pressure which can be fatal.
- Generalized oedema causes pitting peripheral oedema, pleural effusions and ascites. The pathogenesis of generalized oedema is complex and multifactorial. A key factor is thought to be activation of the renin-angiotensin-aldosterone system which stimulates renal sodium retention. Common causes of generalized oedema include left ventricular failure, hepatic failure and nephrotic syndrome.

Thrombosis

- Pathological clot formation in a blood vessel.
- Caused by abnormal activation of the haemostatic system.
- Three broad factors predispose to thrombosis: changes in the vessel wall; changes in blood flow; changes in blood coagulability (Virchow's triad).
- Thrombi can form in arteries, veins and the heart.
- Venous thrombosis is usually related to stasis of blood and an increase in blood coagulability. They commonly form in deep leg veins (deep venous thrombosis).
- Arterial thrombosis is usually related to changes in the vessel wall due to unstable atherosclerotic plaques.
- Cardiac thrombosis is usually related to stasis of blood in a cardiac chamber, most commonly the left atrium in association with atrial fibrillation or the left ventricle in association with a myocardial infarct.
- Thrombi can completely resolve or undergo organisation and recanalisation, in which case they may remain clinically silent.
- **Thrombi become significant if they occlude a vessel or embolize.**

Embolism

- An embolus is a detached mass within the circulatory system that is carried in the blood to a site distant from its point of origin.
- Emboli are important because they can lodge in small vessels and block them off.
- Most emboli are fragment of dislodged thrombus (thromboemboli).
- Venous thromboemboli travel via the heart into the pulmonary arteries causing pulmonary embolism.
- Arterial thromboemboli may impact in cerebral arteries (causing stroke), the mesenteric arteries (causing small bowel infarction) or the lower limbs (causing acute lower limb ischaemia).
- Fat emboli, septic emboli, and amniotic fluid emboli are rare types of emboli.

Pulmonary embolism

- Occlusion of a pulmonary artery by embolic thrombus.
- Very common and often underdiagnosed.
- Emboli lodging in a major pulmonary artery cause instantaneous death.
- Emboli lodging in medium sized arteries present with breathlessness.
- Emboli lodging in small arteries cause subtle symptoms of breathlessness, chest pain, and dizziness – these are the hardest to diagnose.
- About 30% of all patients die from pulmonary embolism.
- The risk of death is much higher if the diagnosis is made late.

Haemorrhage

- Extravasation of blood due to vessel rupture.
- May be due to traumatic rupture or an intrinsic disease of the vessel.
- Rupture of a major vessel causes acute haemorrhage with risk of hypovolaemia, shock and death. Examples include ruptured abdominal aortic aneurysm or ruptured thoracic aortic dissection.
- Even a small bleed at a vital site can be fatal e.g. brainstem haemorrhage.
- Formation of a solid haematoma within the enclosed cranial cavity can also be fatal by causing a rise in intracranial pressure and tonsillar herniation.
- Chronic low grade haemorrhage may present with iron deficiency anaemia e.g. bleeding from a colonic carcinoma.

Shock

- A generalised failure of tissue perfusion.
- Caused by pump failure (e.g. acute myocardial infarction) or peripheral circulation failure (e.g. hypovolaemia, sepsis, anaphylaxis) leading to circulatory collapse.
- Patient looks grey and feels clammy. There is tachycardia and hypotension.
- Untreated shock causes ischaemia of the heart, lungs, gut, kidneys and brain.
- **Rapid treatment is necessary to prevent multiple organ failure and death.**

Infarction

- Tissue necrosis due to ischaemia.
- Most infarcts are due to obstruction of an artery, either by thrombosis overlying a complicated atherosclerotic plaque or a thromboembolus e.g. acute myocardial infarction (‘heart attack’) and cerebral infarction (‘stroke’).
- Infarction may also occur due to venous obstruction. The tissue becomes massively suffused with blood and appears dark purple or black e.g. testicular torsion, sigmoid volvulus.
- Infarcts heal by repair i.e. laying down of granulation tissue which is replaced by a fibrous scar. Although structural integrity is maintained, there is permanent loss of functional tissue.

Atherosclerosis

- An inflammatory disease of large and medium sized arteries characterised by formation of lipid-rich plaques in the vessel wall.
- Very common, particularly in developed countries.
- Important risk factors include older age, male gender, obesity, diabetes mellitus, hypertension and smoking.
- Endothelial injury leads to an inflammatory and fibroproliferative response culminating in atherosclerosis.
- Oxidised LDL is a particularly potent driver of atherogenesis.
- Stable plaques cause symptoms of reversible ischaemia in the supplied organ e.g. angina pectoris, chronic lower limb ischaemia.
- Unstable (vulnerable) plaques can cause acute ischaemic events due to thrombosis overlying them e.g. acute coronary syndromes and cerebral infarction.

CELL PATHOLOGY 3
Inflammation
Dr Mary Thompson

Learning Objectives
- Understand basic pathology of acute, chronic and granulomatous inflammation
- Recognise the histological features of these
- Understand the long term sequelae of inflammation, including wound healing

"Inflammation"
- Essential for human survival
- Role in many human diseases

What is inflammation?
- *"Reaction of living vascularised tissue to sub-lethal cellular injury."*
- Function - Remove cause of injury and initiate repair.
  - acute (hours/days)
  - chronic (weeks/months)
  - Different cell types and mechanisms
- BUT NOTE – inflammation can also have harmful effects
  - Can cause tissue destruction

What causes inflammation?
- Infection….BUT inflammation is NOT JUST caused by infectious organisms….
- Foreign body
- Tissue destruction – mechanical trauma, chemical injury, radiation injury, endogenous (autoimmune reaction, crystal deposition)

The inflammatory reaction
- Inflammatory cells
  - Neutrophils
  - Macrophages
  - Lymphocytes
- **Vascular changes**
  - Immediate supply cells and soluble factors
  - Remodelling in chronic inflammation
- **Soluble factors**
  - Antibodies
  - Cytokines
  - Complement
- **ECM/Repair**

Neutrophils – The "foot soldier"

- Produced in bone marrow
- Circulate in blood
- Contain cytoplasmic granules
- Main role:-
  - Phagocytosis (gobble up) of organisms, debris.
  - Degranulation (release contents of granules)
    - Enzymes
    - Free radicals
    - Soluble mediators

Monocyte/Macrophages – The "commander"

- Monocytes in blood
- Once in tissue give rise to macrophages
- Role:
  - Phagocytosis
  - Control many other inflammatory cells
  - Release cytokines

Other

- Eosinophils
  - Seen in allergic and parasitic causes of inflammation
- Mast cells
  - Also seen in allergic diseases

Acute inflammation
The Clinical Features

- LOCAL
  - Described as early as 3000BC by Egyptian
  - Celsus described four cardinal signs in 1st century AD.
    - RUBOR – redness
    - TUMOUR – swelling (oedema)
    - CALOR – heat
    - DOLOR – pain
- SYSTEMIC
    - Fever, shock

Acute
Inflammation
Exudates

- Purulent
- Serous
- Haemorrhagic
- Fibrinous

Vascular changes

- Vascular calibre and flow increased REDNESS & HEAT
  - Dilatation and increased blood flow to injured area enables rapid delivery of inflammatory cells and mediators
- Adhesion molecules expressed on endothelium
  - Inflammatory cells stick to vessel wall
- Vascular permeability -> SWELLING
  - Leaky capillaries allow cells and mediators to enter tissue – EXUDATE

White cell changes

- Leave blood and enter tissue
- Migrate through tissue to injured site (chemotaxis)
- Become activated
- Ingest organisms/cell debris (phagocytosis)
- Secrete soluble mediators that aid inflammatory process

How do cells get to sites of injury?
Chemotaxis

- Chemotaxis – move towards injured area
  - Exogenous and endogenous compounds attract cells.

Activation
Phagocytosis
Inside the phagosome
Oxygen Independent cell killing

- Bactericidal permeability increasing protein – “does what is says on the tin”
- Lysozyme
- Lactoferrin
- Major basic protein
- Defensins

Soluble Factors in Exudate

- Antibodies
- Factors produced locally by cells
- Circulating plasma proteins

Vasoactive amines

- Released from mast cells, basophils and platelets
- Histamine
- Causes increased vascular permeability
  - E.g. Acute asthmatic reactions

Complement System
Lipid mediators
Other Factors

- Platelet Activating Factor
- Clotting factors
- Nitric oxide
- CYTOKINES – proteins produced by many cell types (lymphocytes, macrophages, endothelial cells, etc) that modulate function of other cells
  - IL1 and Tumour necrosis factor (TNF).

Case 1

- 38-year-old overweight woman
- C/o right upper quadrant pain
- O/e febrile, loss of appetite, tender RUQ
- Fbc – Raised neutrophil count
- CRP ESR both elevated

Diagnosis - Acute cholecystitis

Harmful effects of Acute Inflammation

- OEDEMA (swelling)
- MULTI ORGAN FAILURE

Summary of Acute inflammation

- Acute co-ordinated response vessels, cells and soluble mediators
- Neutrophil is main cell
- Exudate formed
- Variety of outcomes

Outcome of acute inflammation

Resolution

- Tissue returns to normal
- Only occurs if :-
  - Tissue contains cells that can regenerate to replace lost cells
  - Little structural damage done – cells need a framework to build on.

Classic example – Pneumococcal pneumonia

Abscess

Chronic inflammation

- **Causes**
  - Persistent damage
    - Persistent infection
    - Prolonged exposure to toxic agent
    - Autoimmunity
    - Foreign body e.g. splinter

- **Cells different**
    - MACROPHAGES
    - LYMPHOCYTES
    - NO exudate

- **Special types**
  - GRANULOMATOUS

Case 2

- 45-year-old man
- Repeated episodes of bloating and upper abdominal pain, especially after eating fatty food.

Diagnosis - Chronic cholecystitis

Macrophage is key cell

Granulomatous inflammation

- What is a granuloma?

- **What causes granulomatous inflammation?**
  - Infection – TB
  - Foreign material
  - Reaction to tumours
  - Immune diseases (sarcoid)

Harmful effects of Chronic inflammation

- **Amyloidosis**
  - In response to chronic inflammation anywhere in body, liver produces and releases increased amounts of serum amyloid A protein into the blood.
  - In some cases this is deposited in tissue as dense protein (amyloid).

Summary of Chronic inflammation

- Usually follows acute, but occasionally develops straight off
- Main cells are lymphocytes and macrophages
- Causes include viruses, autoimmune disease, chemicals,
- Special type = granulomatous
  - Variety of causes – TB good e.g.

Repair

- Tissue loss too great, and cells unable to regenerate
- Replace normal tissue with fibrous scar tissue

- **Fibroblasts –** produce collagen
- **Collagen –** strong “scar” type collagen
- **Remodelling –** reorientation of collagen fibres for maximal tensile strength

What hinders repair?

- **GENERAL**
  - **POOR NUTRITION -** Protein needed for collagen production, and energy needed for cell function.
  - **VITAMIN DEFICIENCY**
    - Vitamin C – needed by fibroblasts to make collagen
    - Vitamin A - required for epithelial regeneration
  - **MINERAL DEFICIENCY**
    - e.g.. ZINC
  - **SUPPRESSED INFLAMMATION**
    - e.g.. By Steroids
    - Old age
    - Diabetes

- **LOCAL**
  - POOR BLOOD SUPPLY
  - PERSISTENT FOREIGN BODY
  - MOVEMENT
    - E.g across a fracture site, hence the need for a cast.

Complications of Repair

- **Keloid formation**
  - Excess collagen deposition
- **Contractures**
  - Fibrous scar tissue contracts as it matures. If scarring occures across a joint can cause poor joint mobility.
- **Impaired function**
  - E.g fibrous scars in the myocardium after a hear attack.

- **Review the handout and lecture notes…**
- **Open a text book and annotate the lecture…**
  - Robbins Pathologic Basis of Disease (or “mini Robbins” but no pictures)
  - Woolf Basic and Systemic Pathology
- **Have a look online**
  www.pathguy.com/lectures
  www.pathmax.com
- **Cross reference –** the more links you make the more it’ll stick.

CELL PATHOLOGY 4
The Autopsy
Dr Michael Osborn

LEARNING OBJECTIVES
- List four types of death that must be reported to the Coroner
- List two reasons for conducting Hospital Autopsies
- Explain how the need for consent from the deceased's relatives differs between a Coroners' and a Hospital Autopsy
- List four causes of sudden unexpected death in the community
- What is a bruise? Give an example of a mechanism of injury that would lead to a bruise

TYPES OF AUTOPSY, THE CORONER, DEATH CERTIFICATES
Who is the Coroner?
An independent judicial officer of the crown who has a statutory duty to investigate the circumstance of certain categories of death for the protection of the public.

Cases that must be reported to the Coroner
- The cause of death is unknown
- The deceased has not been seen by the certifying doctor either after death or within the 14 days before death
- The death was violent, unnatural or suspicious
- The death may be due to an accident (whenever it occurred)
- The death may be due to neglect by self or others
- The death may be due to an industrial disease or due to the deceased persons employment
- The death may be due to an abortion
- The death occurred during an operation or before recovery from the effects of an anaesthetic
- The death may be a suicide
- The death occurred during or shortly after detention in police or prison custody
- The death may be related to poisoning
- If in any doubt the case must be discussed with the Coroner's office.

Coroners autopsy
Conducted to establish the cause of death
Once Coroner has the cause of death his remit is over

Reasons for hospital autopsy
Allows a very thorough examination of the deceased, the extent of their disease, their treatment and its effects
For:
- Audit – Major discrepancies between stated cause of death and actual cause of death (main diagnosis missed in 15% of cases subsequently autopsied Cameron et al 1980.)
- Monitoring effectiveness of new treatments e.g. complex congenital heart disease.
- Teaching e.g. unrivalled clinic pathological correlation.
- Research e.g. knowledge of variant CJD relies heavily on study of post mortem brain tissue.

So What?
Death certificate data used for epidemiology
i.e. Accurate morbidity and mortality data is needed to monitor the nations health, to direct the allocation of scanty resources and to detect environmental risks.

Two types of Autopsy
1. Hospital
Consent must be obtained from the relatives
With the relevant consent, any material can be taken

Coroners
No consent of family needed (but their wishes should be considered)
Material can only be taken if it bears upon the cause of death (with Coroner's permission)

The Death Certificates
Filled in for any death
Taken to Registrar (of Births, Deaths & Marriages) by family
Scrutinised and must be correct before registration of death possible

1a Immediate cause of death (must be filled in)
1b Predisposing factor
1c Predisposing factor
2 Other factors contributing to but not directly leading to death

E.G. – 1a Gun shot wound to head
or
1a Haemopericardium
1b Myocardial infarction
1c Ischaemic heart disease
2 Hypertension

Natural causes of sudden unexpected death (in the community)
Cardiovascular disease
Coronary artery disease
75% (approximately) of deaths handled by medical examiners in USA
50% die suddenly
25% die without any preceding history or warning
Cardiac Arrhythmia is usual mode of death
Severe coronary artery atherosclerosis is most common anatomical finding
Usually in 2 or more major vessels
Usually 75% or greater stenosis to cause death

Other findings include:
- Myocardial scarring
- Coronary artery thrombosis
- Acute or subacute MI (myocardial infarction)

If arrhythmia is the mechanism of death the diagnosis is one of exclusion, full autopsy must be conducted and severe coronary atherosclerosis must be the major finding.
(In such cases cause of death usually stated as1a Ischaemic heart disease)

Hypertensive heart disease
Usually accompanied by coronary artery atherosclerosis
Cardiomegaly with symmetrical left ventricular hypertrophy
Acute cardiac arrhythmia is usual cause of death

Other Cardiac Causes of Sudden Unexpected Death
Cardiomyopathy
Myocarditis
Structural anomalies (e.g. bridging)
Floppy mitral valve
Aortic stenosis (usually calcific)
Conduction abnormalities (e.g. long QT syndrome)

Vascular System
Ruptured aortic aneurysm associated with atherosclerosis & hypertension

Central Nervous System
Non traumatic subarachnoid haemorrhage:
(Usually due to Berry aneurisms, 2-4% adults, 90% silent until rupture 2/3 symptomatic between 40 & 65 years old)

Intracerebral haemorrhage:
10-30% of all strokes most common cause is hypertension.

Epilepsy

Respiratory System
Pulmonary embolus
Asthma

Gastro Intestinal Tract
(Not usually unexpected or sudden)
Bleeding Oesophageal Varices
Bleeding Ulcers
Pancreatitis

Other Causes of Sudden Unexpected Death (in the community)- (Not Natural)

DRUGS
Alcohol
Not usually a cause of sudden unexpected death (but can be in alcoholics).
Often associated with GI problems.
Often alcohol related damage goes with drug use so think of drugs when you see it.

TRAUMA
Self induced
Caused by others

TYPES OF INJURY
What is a Bruise? Or contusion
Bruise
A blunt trauma injury. Occurs alone (skin intact) or is associated with other injuries.
An extraversated collection of blood which has leaked from damaged small arteries, venules and veins but not capillaries
Occur more easily where skin is lax. Fragility of vessels, coagulation state etc all effect bruising.
May take hours or days to form. May get patterned bruises (can see better with special light sources). Deep bruising may never be seen on the surface.
You can bruise after death (but usually small and lie on dependant parts).
Not everything is a bruise: e.g. Pink areas may be due to hypothermia.

What is an abrasion ?

Abrasion

A graze or scratch. The most superficial of blunt trauma injuries.
Confined to the epidermis (strict definition) but may actually extend into the superficial dermis due to skin anatomy).
Can occur before and after death
Due tangential force – may have a distal skin tag eg Friction burn, or Vertical force eg Stamp – no distal skin tag.
Abrasion examples - Friction burn, Car radiator, Flooring, Whip, Stamp.

What is a Laceration?

Laceration

A split to the skin. The result of blunt force overstretching the skin. Usually pass through the full thickness of the skin. They are deep and will bleed.
Margins ragged with crushing and bruising. “Bridging fibres” arch across the skin defect.
Common where skin can be compressed between the force and underlying bone e.g. Scalp, elbow, shin.
Rare over soft fleshy areas e.g. Buttocks, breasts. A laceration shows poor reproduction of the object causing it.
“Flaying” – tangentially applied force leading to a horizontal laceration.
examples – Fall, Punch, Stick, Hammer, Bomb, Wheel of car (flaying).

Cut & Stab wounds

Cut (or slash)

The length of the injury is longer than its depth

Stab(or penetrating injury)
The depth of the wound is greater than the width

Cut & Stab Wounds

Causes by an object with a sharp or cutting edge
Usually a knife but can be anything eg a broken glass or bottle or a piece of metal
Edges are clean and well demarcated. Minimal injury to surrounding tissue.
Information about weapon type can be gained from the wound but beware of over interpretation.

N.B “Incised Wounds”

Some discrepancy in terms used (Knight). To some (e.g. Rutty/Burton) includes cuts & stabs.
To others (e.g. Shepherd) is synonymous just with cuts.

When describing wounds either use the correct term or use a generic term such as INJURY.

Not everything is a Laceration

MICROBIOLOGY 7
Prevention and treatment of viral disease
Professor Wendy Barclay

- **Describe the difference between prophylactic and therapeutic approaches to virus control**

We use drugs and vaccines to combat viruses. Most vaccines are used **prophylactically**. Most antiviral drugs are given after the person is infected as a **therapeutic** agent, although in controlling diseases outbreaks prophylactic antiviral administration has been used.

- **Understand the difference between a live attenuated vaccine and an inactivated or subunit vaccine**

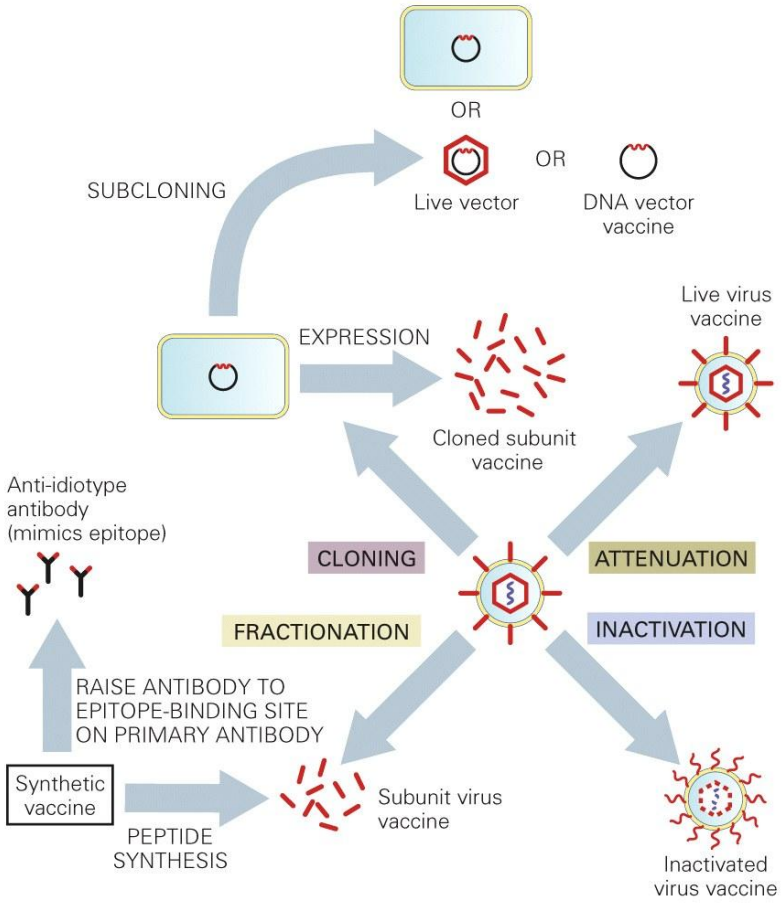


Figure 5.3 Viruses: Biology Applications Control (©2012 Garland Science)

Vaccine history:
The process of **scarification** to prevent smallpox had been practised in the Far East for centuries. Lady Mary Montague encouraged it after observing it in Turkey. Jenner noted that milkmaids exposed to cowpox rarely suffered smallpox. He infected subjects (James Phipps with cowpox (vacca = cow) and proved his theory by showing they did not get smallpox after deliberate inoculation with it.
This was **live vaccination** with a virus attenuated in human host.
Pasteur used dried spinal cords from rabbits infected by rabies to immunize against that disease. This was likely vaccination with **inactivated virus**. The antigen still induced a humoral immune response that was protective.
Today we often further purify the inactivated virus to make a **subunit** preparation of the relevant antigenic viral proteins. Most influenza vaccines contain purified HA and NA proteins. It is impossible to catch flu from this type of vaccine because it is an inert fragment of the virus. Similarly hepatitis B virus vaccine is a subunit vaccine that consists of the surface antigen protein of HBV that has been expressed in recombinant yeast.

- **Give examples of viral infections for which vaccination can be a successful strategy**

Table 5.1 Current vaccines

Live attenuated	Inactivated virus	Purified subunit	Cloned subunit
Adenovirus[1]	Hepatitis A	Influenza	Hepatitis B
Influenza	Japanese encephalitis		Human papillomavirus
Measles	Polio		
Mumps	Rabies		
Polio	Tick-borne encephalitis		
Rotavirus			
Rubella			
Smallpox			
Varicella			
Yellow fever			

[1] Not currently manufactured.

Table 5.1 Viruses: Biology Applications Control (©2012 Garland Science)

- **Describe the eradication of smallpox and similar efforts to control other viral diseases**

After Jenner's success in 1796 the cowpox used for vaccination was propagated by growth on people's arms. During the 20th century other strains of poxvirus (vaccinia) that could be grown in quantity on the skins of calves or other animals were used. Although vaccination was widely practised, epidemics flared sporadically across the world. In 1966 WHO invested $2.5 million for an immunization campaign designed to eradicate the disease. This aim was achieved in 1977 after the last case of natural smallpox was detected in Somalia. The end game involved intensive ring vaccination of all persons in the vicinity of the detected cases.
Eradication of smallpox was possible because there is **no animal reservoir** and thus once eliminated from man the disease cannot be reseeded. In addition the ability to detect infected cases due to the **obvious symptoms** and the fact that all infected individuals become symptomatic was crucial.

Today **variola major virus** exists in just two laboratories in the world where it is preserved. Newer versions of vaccinia virus exist that have been developed as safer live attenuated vaccines such as **MVA Ankara**. These can be genetically manipulated to express other antigens and may be used in the future as vaccines against other diseases.

WHO also aims to eliminate poliovirus and measles virus using a vaccination strategy. Poliovirus was largely controlled by two different types of vaccine, an inactivated vaccine known as the **Salk vaccine** and a live attenuated vaccine known as the **Sabin vaccine**.

The animal virus Rinderpest, a relative of measles, was successfully eradicated in 2008.

- **Explain why it is difficult to develop drugs which selectively act against viral infections**

Viruses are intracellular obligate parasites.
This makes them particularly difficult to combat with chemotherapeutic agents.
It is hard to find a stage of the virus replication cycle to attack with a drug that does not involve a host function. A drug that inhibited viral translation for example would knock out our own cells' ability to translate mRNAs.
Most antiviral drugs used today are very specific for the particular virus their work against. They usually target **viral enzymes** that have been found to differ from any enzymes used by our own cells. They are difficult to use because an **accurate diagnosis** is required to inform the correct drug choice. Viruses also often develop **resistance** to the drugs particularly if they are used individually.

- **Give examples of classes of drugs which have been used successfully in antiviral therapy**

The most successful antiviral drug in use today is acyclovir, used to treat herpes virus infections.

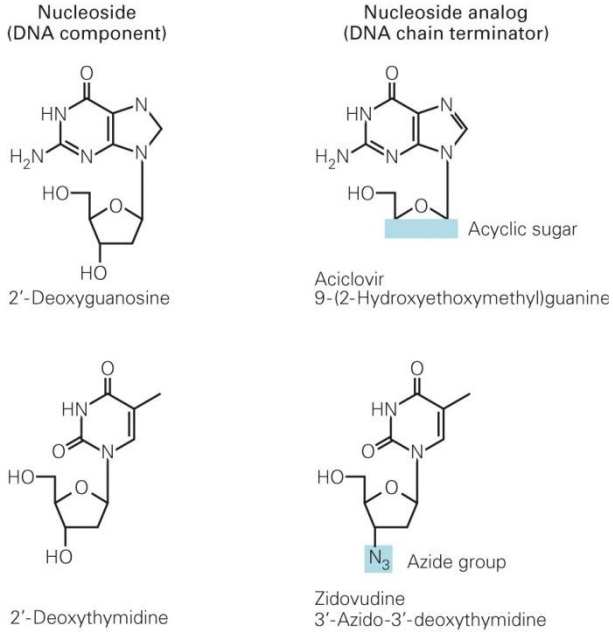


Figure 6.9 Viruses: Biology Applications Control (©2012 Garland Science)

Acyclovir is a nucleoside analogue. It is given to the patient in an unphosphorylated (inactive) form, a **prodrug**, that means it cannot yet be used as a substrate in DNA replication. Its specificity comes from the fact that it is only converted into its active form inside a cell that is already infected by herpes virus. This is because the virus encodes an enzyme **thymidine kinase** that can phosphorylate the drug. Once acyclovir triphosphate is incorporated into the growing DNA chain in the herpes virus genome, it **terminates** the reaction because it lacks the OH groups by which the next nucleoside would normally be attached.
Zidovudine AZT is also a nucleoside analogue. It was the first anti HIV drug but resistance quickly emerged. Current HIV therapy uses three or four different drugs in combination known as **HAART** highly active antiretroviral therapy. This prevents the virus from being able to generate resistance mutants but does lead to difficult drug regimens and is associated with significant side effects.
Other types of antiviral drugs are protease inhibitors (HCV and HIV), ion channel blockers (influenza) neuraminidase inhibitors (influenza). Interferon is also currently used in combination with ribavirin to treat HCV.

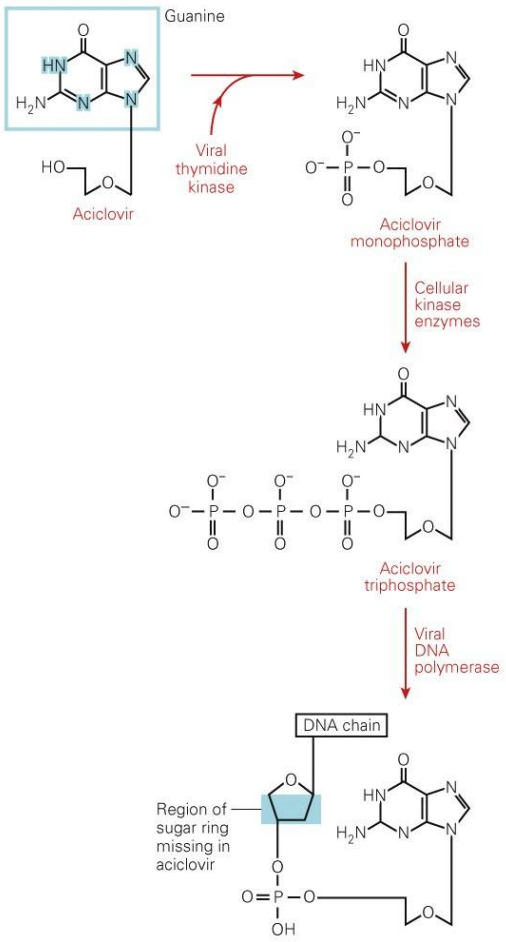


Figure 6.10 Viruses: Biology Applications Control (©2012 Garland Science)

- **Describe the strategies underlying the search for novel antiviral agents**

A new era of antiviral drug discovery has been sparked by the genomics revolution. We can now identify many host cell genes that viruses need for their replication The hope is that we can target some of them because our genome has some redundancy. A good example of this is the CCR5 protein used by HIV to enter cells and the fact that a group of exposed uninfected individuals remain resistant to HIV because they have a 32bp deletion in CCR5 but are otherwise completely healthy.
There is hope that more broadly acting antivirals may be discovered. New breakthroughs include small molecules that freeze the lipids on enveloped viruses but seem not to affect the plasma membranes of our own host cells.

CELL PATHOLOGY 5
Cancer

Dr Rathi Ramakrishnan

Learning objectives:

- Define cancer, neoplasia, tumour, metastasis, carcinogen
- List features which distinguish benign from malignant tumours
- Give examples of cancers caused by infection, chemical and environmental agents
- Briefly outline the principals of cancer screening
- Describe features of pathology which predict the prognosis in cancer

What is Cancer?

- An important socioeconomic problem
- A significant cause of morbidity and mortality worldwide

Definitions

- **Cancer**- from the Latin for crab
- **Tumour**- swelling, originally for inflammation
- **Neoplasm**- new growth "abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after the cessation of the stimuli which may have evoked the change" Willis 1952

Tumour nomenclature

All tumours have two basic components

1. Proliferating neoplastic cells *parenchyma*
2. Supportive *stroma*

Names of tumours

Benign tumours given suffix *-oma* to cell of origin eg. Adenoma (epithelial derivative) Fibroma (mesenchymal derivative)

Malignant tumours of epithelial origin are called *carcinomas*, of mesenchymal origin are called *sarcomas*

Consider - lymphoma, melanoma, seminoma

dysplasia

Literally - disordered growth, limited to epithelium

- Loss of uniformity of individual cells
- Loss of their architectural orientation
- Mild to moderate dysplasia may revert to normal
- Severe dysplasia *aka* carcinoma in situ

metaplasia

- Substitution of one mature cell type for another mature cell type more suited to the environment
- The result of a chronic stimulus, when withdrawn may resolve to normal
- Is adaptive, not premalignant
- Eg. Smoking causes metaplasia of glandular bronchial epithelium to squamous epithelium

Neoplasia - nomenclature

Factors distinguishing benign and malignant tumours

1. differentiation and anaplasia
2. rate of growth
3. local invasion
4. metastasis

1. differentiation and anaplasia
   - Differentiation refers to the extent to which the parenchymal cells resemble there normal counterparts
   - Benign tumours are well differentiated
   - Malignant tumours show various levels of differentiation (well, moderately, poorly)
   - Anaplasia is lack of differentiation
     Characterised by marked pleomorphism, hyperchromasia, large nuclei, nucleolation, irregularity of nuclear membrane, mitotic activity (the cytological features of malignant cells)

2. rate of growth
   - Generally the rate of growth of tumours correlates with the level of differentiation
   - Benign tumours grow slowly
   - Malignant tumours grow more rapidly

3. local invasion
   - Most benign tumours grow as cohesive expansile masses that remain localised to their site of origin (encapsulated)
   - Malignant tumours infiltrate and destroy the surrounding tissue, poorly demarcated

4. metastasis
   - Unequivocal evidence of malignancy
   - Formation of discontinuous tumour implants at a distance from the main tumour mass
   - With 2 exceptions, all malignant tumours can metastasise (gliomas and basal cell carcinomas)
   - Approximately 30% patients present with metastasis

Mechanisms of invasion and metastases
- Studies in mice show that many tumour cells shed from primary site daily yet only a few metastases are produced
- Series of steps
- Cells must end up in favorable “soil”

Pathways of spread
1. direct seeding of body cavities and surfaces (peritoneal, pleural, pericardial, subarachnoid, joint)
2. lymphatic spread (most common route for carcinomas initially) along natural lymphatic drainage
3. hematogenous (typical of sarcomas also by carcinomas later)

Seeding of body cavities
Most commonly from ovarian carcinomas which may cake the peritoneal surface
Also spread of lung carcinoma into pleural cavity

Lymphatic spread
- Regional nodes drain tumours (i.e.. axillary then infraclavicular and supraclavicular from UOQ breast carcinomas)
- Nodes may contain the spread locally
- Evoke an immune response which causes nodal hyperplasia
- Not every enlarged node in the region of a tumour contains metastatic spread

Hematogenous spread

- Veins penetrated more frequently than arteries due to thickness of walls
- Liver and lungs are most common sites due to venous drainage
- Renal cell carcinoma can grow within the renal vein to the IVC and into the right atrium

Staging of cancer
Staging of cancers is

- CLINICAL e.g. TNM stage but also FIGO for ovarian cancer and Ann Arbour for Hodgkins lymphoma
- based on 3 factors

- Size of the primary lesion
- Spread to regional lymph nodes
- presence of metastases

TNM stage

T Primary **T**umour size (T1-T4)
N **N**odal status (N0 or N1,2,3)
M Presence of **M**etastasis (M0 or M1,2)

Grading of cancer

- Histological
- Based on the degree of differentiation and on the numbers of mitoses
- Less useful than staging

Cancer epidemiology

- AGE Incidence of cancer increasing overall as the world's population lives longer. In general, the incidence rises >55 years
- GEOGRAPHY(stomach cancer higher in Japan ct USA, melanoma higher in NZ ct Iceland)

Environmental agents
UV light, occupational agents, diet and weight, alcohol, smoking, infections notably viruses (HPV)

"it begins to appear that everything one does to gain a livelihood or for pleasure is fattening, immoral, illegal, or, even worse, oncogenic"

Genetic factors
For a large number of cancers there exist some hereditary predispositions (e.g. lung cancer mortality four times higher in non-smoking relatives of lung cancer patients ct controls)
Hereditary forms of cancer - 3 categories

Inherited autosomal dominant cancer syndromes
Familial retinoblastoma
Familial adenomatous polyposis coli
MEN
NF 1 and 2
Von Hippel Lindau syndrome
Familial cancers
Breast cancer
Ovarian cancer
Colonic cancer

Autosomal recessive syndromes of defective DNA repair

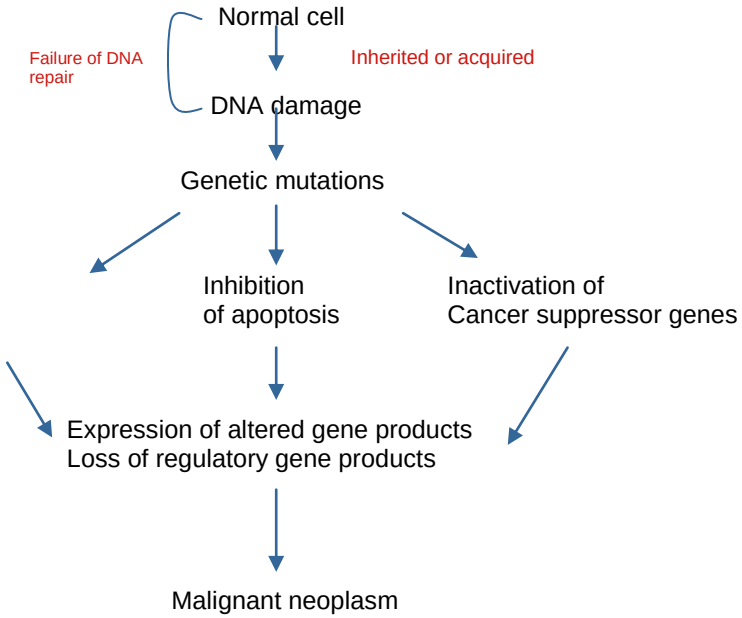
Xeroderma pigmentosa
Ataxia telangectasia
Bloom syndrome
Fanconi anaemia

Inherited cancer syndromes

- Inheritance of a single mutant gene greatly increases the risk of developing cancer
- Inherited retinoblastoma carriers have a 10,000 fold increased risk of developing retinoblastoma (usually bilateral)
- FAP 100% get colonic carcinoma by age 50

The molecular basis of cancer

- The genetic hypothesis of cancer implies that a tumour mass results from the monoclonal expansion of a single progenitor cell
- Subsequently additional mutations allow progression and account for heterogeneity



Targets of genetic damage

1. Growth promoting genes - *Oncogenes*
2. Growth inhibiting (tumour suppressor) - *Anti-oncogenes*
3. Genes regulating programmed cell death - *Apoptosis*
4. *DNA repair genes*

Oncogenes

- Derived from proto-oncogenes, genes regulating normal cellular growth
  Examples of oncogenes associated with tumours
- myc Burkitt lymphoma
- N-myc Neuroblastoma
- cyclinD1 Mantle cell lymphoma

Cancer (tumour) suppressor genes

- These genes usually regulate normal cell growth
  Classic example is Rb gene (13q)in genetically inherited retinoblastoma (40%)
  Development of this tumour requires “two hits”
  i.e. inheritance of a mutated copy of one Rb gene followed by acquisition of damage to the remaining copy, leading to cancer
- p53 (chromosome 17) 50% of all human tumours contain mutations in this gene. Its function is to prevent genetically damaged cells from replicating “housekeeper of the genome”
- BRCA-1 and BRCA-2 (chromosome 13q) 5-10% of breast cancer are familial and mutations in one of these genes is present in 80% of those cancers

Genes regulating apoptosis

- bcl-2 prevents programmed cell death
  overexpression of bcl-2 and prevention of apoptosis results in indolent growth of lymphocytes found in many low grade lymphomas

Carcinogens

Agents that cause genetic damage and induce neoplastic transformation of cells

3 categories
Chemicals
Radiation
Microbial agents (mainly viruses)

Chemical carcinogens

- Sir Percival Pott related the incidence of scrotal skin cancer in chimney sweeps to exposure to soot. His recommendation led to a measure which dramatically reduced the incidence (daily bathing!)

- Carcinogenesis is a multistep process
- Some chemicals are inducers (permanent DNA damage)
- Others are promoters (reversible DNA damage)
- Neither step alone is sufficient to cause cancer
- Direct acting agents (do not require metabolism for activation)
  Dimethyl sulphate
  Diepoxybutane
  Cyclophosphamide, chlorambucil, nitrosoureas and other anticancer drugs

- Procarcinogens which require metabolism for activation
  Beta napthylamine
  Benzidine
  Aflatoxin B1 (grains and peanuts)
  Betel nuts
  Vinyl chloride
  Insecticides, fungicides
- Promoters include
  Hormones (e.g. oestrogen)
  Drugs
  Phenols
  Bile salts
  Fat in diet?

Radiation

- Ultraviolet (SCC, BCC, MM)
- Ionising electromagnetic i.e. X-rays
  Cause an increase in leukaemia and solid tumours

Microbial carcinogens

- DNA oncogenic viruses HPV, EBV, HBV and HHSV8
- RNA viruses HTLV-1
- Bacterial carcinogens; Helicobacter Pylori

Cancer diagnosis

Laboratory methods

- Cytology FNA (freehand or USS guided)
- Histology (core biopsy, incisional or excisional biopsy)

Additional methods

- Tumour typing
    - Immunocyto/histochemistry
    - Flow cytometry
    - Molecular methods (PCR, FISH)

Clinical effects of tumours

- Both benign and malignant tumours affect the host
- Anxiety (breast lumps)
- General (pressure, ulceration, infection, bleeding, hormonal effects)
- Metabolic cancer cachexia (increased BMR, reduced fat and muscle bulk) TNF alpha

MICROBIOLOGY 8
Evolution and emergence of new viruses

Professor Wendy Barclay

- **Define the terms zoonosis and host range**

New viruses that infect humans often cross over from **animal reservoirs**. The crossing of an animal pathogen into humans is called **zoonosis**. This does not happen very often because there is a **host range barrier**. This means that most viruses that are adapted to infect animal hosts are compromised in their ability to replicate and spread in humans due to the genetic differences between host factors the virus needs. Because humans have no pre-existing immunity to animal viruses they can cause devastating outbreaks or **pandemics**.

- **Describe how viruses emerge and re-emerge; using named examples including influenza virus antigenic shift and drift, HIV, West Nile Virus, SARS and noroviruses**

Influenza viruses naturally infect waterfowl such as ducks and geese. Many different antigenic subtypes of influenza A virus exist in birds. Occasionally an avian virus acquires suitable mutations that adapt if for replication and transmission in humans. This results in a pandemic. The new virus spreads rapidly around the world and most often displaces any influenza virus previously circulating. This is known as **antigenic shift**. The most obvious way for the avian virus to jump the species barrier and adapt for replication in human hosts is by the process of **reassortment**. This is a special form of recombination between two different viruses that infect the same cell of the same host. New virions that result can contain mixtures of genes from each of the parental viruses. A virus containing genes that allow replication in a human cell but novel HA and NA viral antigens can result in a **pandemic**. Influenza pandemics occurred around every 4 decades through the 20th century.

After an influenza pandemic most people are immune to the new virus because they have made antibodies that can prevent its infectivity. However because of the **high mutation rate** of an RNA virus, mutants can be readily selected that escape the immunity by altering their epitopes. New viruses derived from the first one emerge. This is the process of **antigenic drift** and means that we have to update the influenza vaccine every year to keep pace with the evolution of the virus.

HIV emerged as a human pathogen in the mid 20th century. We can see from sequence analysis of related viruses of apes that HIV is derived from a simian retrovirus that was found in chimpanzees. It is believed that the close contact between man and monkey perhaps during the bushmeat trade enabled the virus to jump the species barrier.

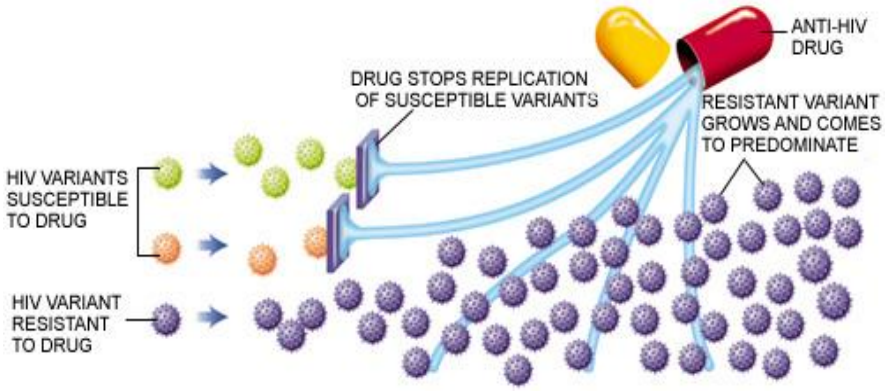
West Nile Virus is an arbovirus that was not seen before 1999 on the American continent. It is not know how the virus crossed from Asia to the Americas. The replication cycle involves infection of mosquitoes and birds are the natural host. The virus has spread across the USA in less than a decade.

Noroviruses are small RNA viruses that cause diarrhoea and vomiting. There has been an increase in the incidence of norovirus disease in recent years. A new genotype of virus has emerged that appears to use a new receptor for host cell entry that is particularly widespread in humans.

SARS coronavirus emerged in East Asia in 2003. The virus is normally found in bats. Farmed civet cats were an intermediate host in China for the evolution of a strain of the virus that could attach to a receptor on human cells.

- **Understand the principles of the evolution of drug resistant variants of viruses**

All viruses have the capacity to evolve rapidly. RNA viruses are particularly prone to generate many errors during their replication because their RNA dependent RNA polymerases lack proof reading activity. Coupled with the high numbers of progeny, this often results in the genomes existing as a **quasispecies**. It is likely that single nucleotide changes in the genome occur every time the virus undergoes a round of replication. And if such a mutation leads to a coding change in a target for a drug of antibody then that mutant virus will be fitter than its sister progeny under conditions where the drug is used.
Drug resistance thus occurs readily in the treated patient. The resistance mutation sometimes confers a fitness cost to the virus and this means the resistant virus is unlikely to spread beyond the treated patient. If there is no fitness cost then the drug resistant virus can predominate and render use of the drug redundant.



MICROBIOLOGY 9
Defence and vaccination against bacteria

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Learning objectives

1. List the major non-immune host mechanisms

2. List the components of the innate immune system and major antimicrobial mechanisms

3. Explain how an adaptive immune response is involve in defence against bacterial pathogens

4. Describe how infectious agents avoid host defences

5. Explain the difference between active and passive immunisation

6. Give examples of the different types of vaccine presently available and how they are used.

Non-Immune Mechanisms of Host Defence

- the skin provides a natural physical barrier to bacterial infection
- low pH and antibacterial secretions make it a difficult surface to colonise
- we can strengthen this significantly by routine application of soap and water
- mucosal surfaces are protected by mucous and ciliary clearance

Competition with Commensal Organisms

- bacteria make up 60% of the earth's biomass
- the vast majority of bacteria are harmless and often helpful
- "commensal" bacteria make up around 1% of our body mass
  [commensal = "eating at the same table as another"]

Innate Immune Response

- **bacteria that penetrate skin and mucosal barriers are met by the innate immune response**
- the complement system punches holes into bacteria
- neutrophils and macrophages phagocytose bacteria and expose them to toxic radicals and degradative enzymes

Adaptive Immune Response

- antibodies (produced by B cells) prevent adhesion, neutralise toxins, and enhance complement killing and phagocytosis
- T cells enhance B cell responses and activate macrophages
- adaptive immunity involves a memory response (works better on re-exposure to the same pathogen)

Pathogenic bacteria have evolved strategies to avoid host defences

strategy	*mechanism*	*example*
resist complement	thick cell wall	*Mycobacterium tuberculosis*
	capsule	*Neisseria meningitidis*
resist antibodies	IgA protease	*Streptococcus pneumoniae*
	antigenic variation	*Neisseria gonorrhoeae*
resist phagocytosis	polysaccharide capsule	*Neisseria meningitidis*
	debilitate phagocytes	*Yersinia pestis*
	hide inside other cells	*Chlamydia*
inhibit intracellular killing	escape from phagosome	*Listeria monocytogenes*
	block phagosome maturation	*Mycobacterium tuberculosis*

Vaccines enhance adaptive immune responses to infection
Passive versus *Active* immunization

Current routine bacterial vaccination in the UK

WHEN TO IMMUNISE	WHAT IS GIVEN	HOW IT IS GIVEN
2, 3 and 4 months old	Diphtheria, tetanus, pertussis (whooping cough), polio and Hib	One injection
	MenC	One injection
Around 13 months old	Measles, mumps and rubella (MMR)	One injection
3 years and 4 months to 5 years old	Diphtheria, tetanus, pertussis (whooping cough) and polio	One injection
	MMR	One injection
10 to 14 years old (sometimes neonatal)	BCG	Skin test, one injection, if needed
13 to 18 years old	Diphtheria, tetanus, polio	One injection

DTP: diphtheria, tetanus, pertussis (whooping cough)
- subunit vaccines
- antibodies neutralise toxins and block adhesion

Conjugate vaccines (carbohydrate)
- T cell recognition of protein carriers enhances B cell activation
- promotes efficient antibody response to polysaccharide capsule

Live attenuated vaccines
- BCG gives some protection against tuberculosis in children but is ineffective against adult pulmonary disease
- new vaccine strategies based on genome information
- very effective live attenuated vaccines are being developed for enteric pathogens (e.g. *Salmonella typhi*)

CELL PATHOLOGY 6
Cell pathology Case Studies

Professor Rob Goldin, St Mary's campus

- Using the example of *Helicobacter pylori* infection of the stomach, discuss the varied outcomes of infection and why these occur, and how inflammation can lead to cancer or lymphoma in this organ.
- Using the example of a case of atherosclerosis, list 3 major outcomes of this arterial disease.

Helicobacter pylori

Dyspepsia 1
Mr DU, aged 46, has had dyspepsia for three years, worse recently with abdominal pain after meals.
He had an episode of melaena
Blood tests Hb 9.0
Endoscopy

Biopsy of gastric antrum shows *Helicobacter* associated gastritis
chronic inflammation - lymphocytes
acute inflammation - neutrophils

Helicobacter pylori
20% adults in DCs by age 50, 80% adults in UDCs
Majority (70-80%) asx
Associated with 2x relative risk for gastric carcinoma

Gastric Ulcers
With age, infection spreads, Pangastritis, atrophy of body mucosa, acid decreases, gastric ulcers

Complications
Haemorrhage, Perforation

Dyspepsia 2
Mrs A, 76 years, complains of vague abdominal pain and nausea
Dyspepsia for years
Blood tests Hb 11
Endoscopy

Gastric Cancer
Japan: mass endoscopy programs led to 35% early gastric cancers vs. 10% in US
EGC and IM

Dyspepsia 3
Mr NG – 76 years
Dyspepsia
Early satiety
Blood tests Hb 8
Endoscopy

Investigation Mr NG
Liver function tests – abnormal, Liver CT scan multiple lesions

Gastric Cancer
High incidence in Japan, Chile, Italy, China, Portugal, Russia
2:3 men 90% of all malignant tumors in stomach are carcinomas

Gastric cancer
asymptomatic until late; weight loss, abdominal pain, nausea, vomiting, altered bowel habit, kills more people worldwide than lung cancer

Dyspepsia 4
Mr Ly 76 years
Dyspepsia
Early satiety
Blood tests Hb 8
Endoscopy

Lymphoma

Atherosclerosis
Occludes arteries slowly (angina, myocardial scarring, dementia, claudication,)
Occludes arteries suddenly plaque rupture (thrombosis, atheroembolization) or haemorrhages into plaques (MI, stroke, gangrene of the bowel)
Weakens artery walls (aneurysms)

Mr S
History of hypertension Sudden loss of consciousness
Died in A&E
PM

Mr A
Mr A died in an RTA
At PM an aneurysm like this was found
Cause of death natural/ unnatural?

Mr MI
Central chest pain, died 7 days post admission