W9L17 The Yin and Yang of Human Defense

Learning outcomes:

- The concept of a microbiome as a part of us and its implications for recognition of 'self'
- The microbiome trade-offs of nutritional benefit and infection risk
- Nutrient-based control of the gut microbiome
- Immune-based control of the gut microbiome
- Can give examples of how and why the host system encourages gut microbe activity, especially the colon
    - Why- benefits to host
    - How- by proving permissive growth environment (HMO in infants)
- Can explain with examples, how the host influence on microbial growth in the small and large intestines in different
    - Examples of nutrient availability (ex. amino acid, iron limitation)
    - Examples of antimicrobial stress (ex. bile)
    - Examples of death rate (ex. transit time)
- Can describe key factors in containing microbes within the gut
- Can explain the different roles of conserved and variable microbial molecules in recognition by the immune system with examples
    - MAMPs are for general recognition by PRRs (ex. LPS, Peptidoglycan)
    - Variable surface molecules are for specific recognition by immunoglobulins
- Can relate the concept of immune balance to the relative numbers of effector and regulatory cells
- Can be a simple example of how microbial signals can lead to change in immune balance (ex. role of SCFA as signals for Treg maturation)

Microbiome:

→ the *stable microbial community* of a defined habitat

- Small intestine: physico-chemically distinct from the large intestine (divided by the ileo-cecal valve)
    - A site of competition with microbes for nutrients
- The large intestine: has distinct conditions- far higher microbe cell density
    - A site of co-operation with microbes
- Most other internal organs are sites where presence of microbes is not tolerated

Nutrition-related chronic diseases connect diet, gut microbiome and immune functions:

Gut-Associated Lymphoid Tissue (GALT): present along virtually the entire length of the intestines → major site of cross-talk between host and microbiome

- Immune functions must be modulated at every level:
    - Immune functions must *allow* beneficial microbial activity in colon
    - Immune functions must *prevent* microbial overgrowth or escape
- Microbe growth in our gut is controlled however cells of our immune system are not the major source of control.

Diet changes our relationship with our microbiome

- Easily digestible carbs ex. sucrose ***doesn't support colonic microbes***
  → because it's absorbed to blood as glucose
- Partially digestive carbs ex. starch *support colonic microbes*
  → absorbed to blood as microbial metabolites (mainly SCFA)

Microbe growth in gut lumen

→ microbe growth is *controlled not prevented*

→ primary control being *environmental regulation*

Growth rate: the speed of new cells being produced *per unit time*

- Temperature
- pH
- Any stress
- Concentration of any nutrient

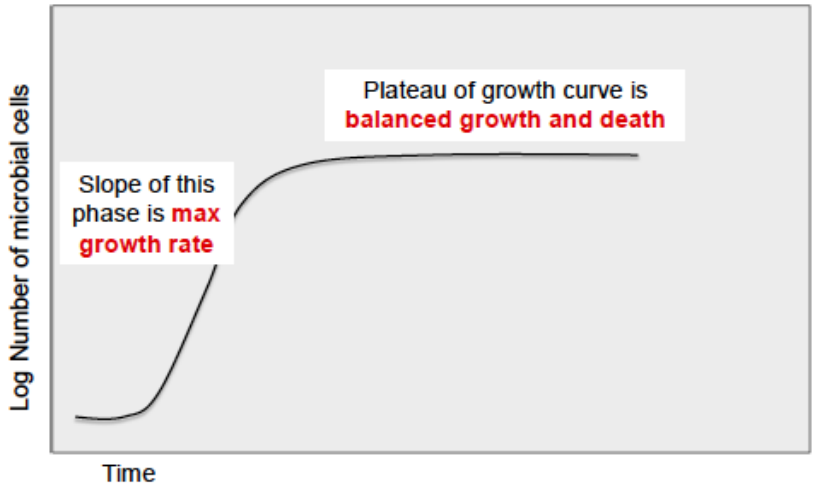
Growth yield: the number of new cells being produced *per volume or mass*

- Death rate
- Metabolic efficiency

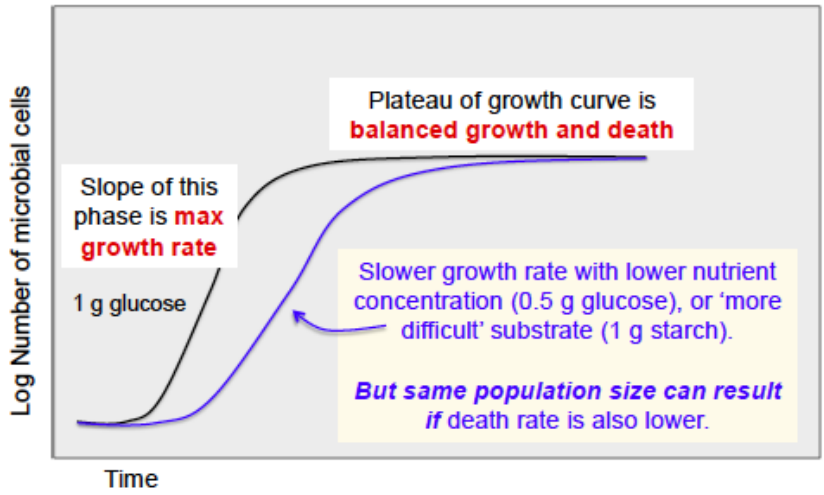
Cell density: the number of microbes in an *open system* is determined by *balance of cell production & cell loss*

- The rate at which food material moves through the gut dictates the rate at which *bacteria must keep growing* to maintain their population
- Sphincter= the growth conditions and transit time in gut regions differs
    - Underpins different cell densities
- A *chemostat* lets us experimentally model effect of growth conditions and dilution rate on bacteria

Hence,
The cell density of microbes in our gut is a product of balanced growth rate and death rate



The production of microbes in our gut is controlled by factors that limit growth rate



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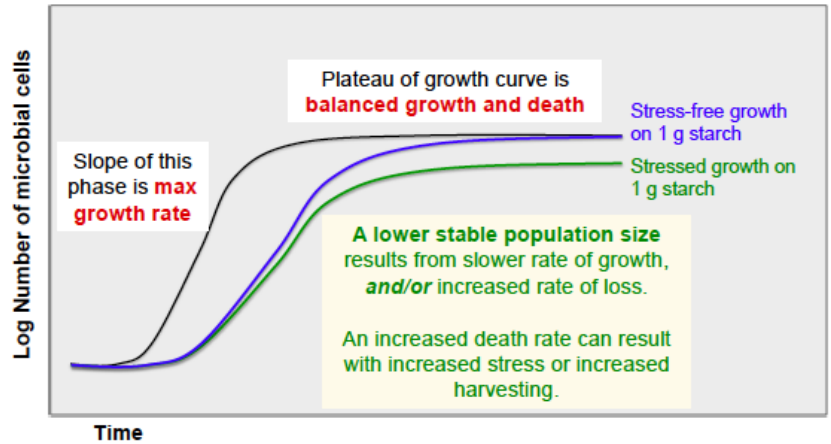


Cell numbers in the small intestine are controlled by **nutrient availability, antimicrobials and dilution rate:**

Microbes in SI (limited oxygen; high flux of nutrient substrates that depletes rapidly; low flux fibre) have to:

- Grow fast under stress and while competing for preferred substrates before getting washed out
  - Stress→ oxidative stress
  - Short residence time of chyme→ high death rate
  - High concentration of bile (anti-microbial)

Microbes in colon (No oxygen; very low substrates remain; most dietary fibre remains; input of waste N) can:

- Grow slowly under reduced stress, while using fibre substrates by fermentative metabolism
  - Long residence time of chyme→ low death rate
  - Low antimicrobials→ bile resorbed in ileum

Cell location by innate and adaptive immunity:

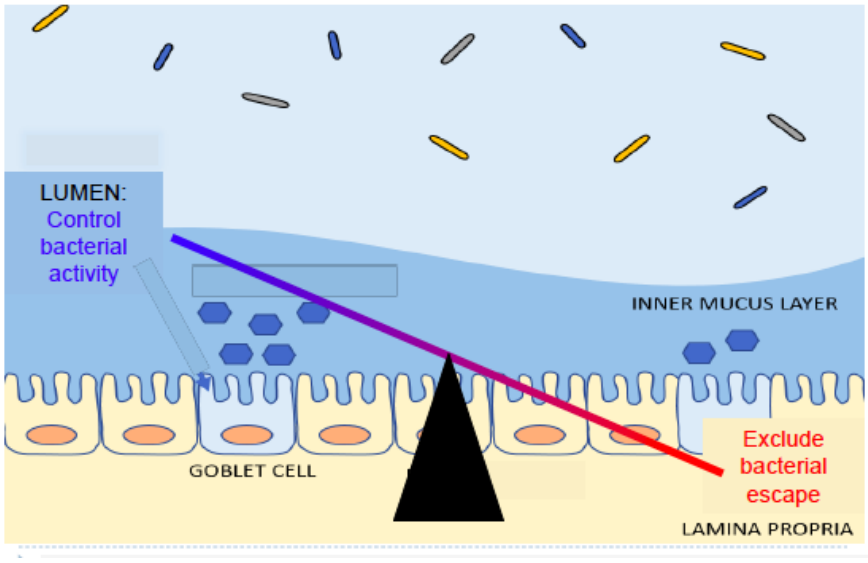
1. Nutritional immunity: determines population size and activity in the gut:
   - Body rapidly removes preferred nutrient sources→ directs bacterial growth
   - Body excretes waste N to gut→ directs bacterial growth
   - Body rapidly removes iron (lactoferrin)→ limits bacterial growth

→ *bacteria encouraged*

2. Other immune functions *contain* bacterial activity within the gut:
   - The intestinal mucosal surface has *much lower numbers* than the lumen
   - *The lamina propria →kept sterile*

→ *bacteria not tolerated*

Spatial structure of interactions with microbes within immune functions:



- Goblet cells in lumen (inner mucus layer) control bacterial activity by producing glycoprotein

Mucosal immune functions further limit bacteria-epithelium interaction:

1. A loose, diffusive outer layer- mucin layer and dense adherent inner layer provide a physical barrier
2. Antimicrobial peptides (AMPs) secretion provides a *biocide barrier*
3. ***IgA secreted into the lumen coats surface of bacteria***

→ Immune functions can non-specifically (AMPs) or specifically (IgA) impact microbes

Mucin (the diffusive) layer plays important roles in containment of microbes and spatial structure of immune response:

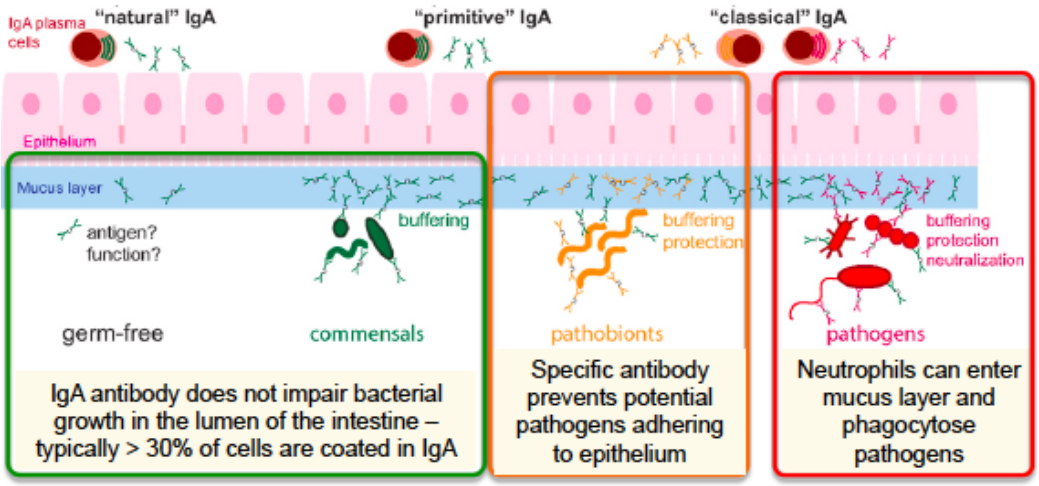
→ Stops *microbe cells and human cells* hurting each other

- Physical barrier to microbe cells = can't get to epithelium
- Diffusion barrier to macromolecules = antimicrobials don't diffuse to lumen

Glycoprotein *secreted by goblet cells:*

- Expression pattern of mucin genes influenced by microbial metabolites, notably butyrate
  - In colon, the diffuse outer layer supports growth of 'good' microbes
  - In colon, the dense inner layer limits microbe proximity/ contact with epithelium surface

The fundamental role of secreted IgA → to limit contact between microbes and epithelial cells



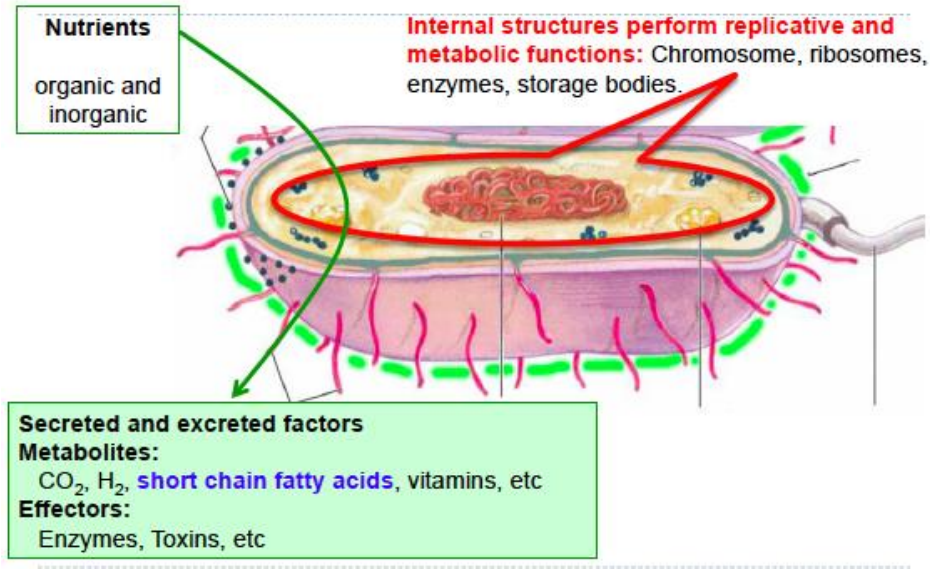
Natural IgA/ Primitive IgA:

- Doesn't impair bacterial growth in the lumen of the intestine

Classical IgA

- Specific antibody prevents potential pathogens adhering to epithelium
- Neutrophils can enter *mucus layer* and *phagocytose pathogens*

Bacteria:



Microbe-Associated Molecular Patterns (MAMPs) and antigens: same molecule in wide range of microbes

- Surface structure: the major structures of the cell envelop include distinctive molecules
  - Cell wall
  - Pili
  - Flagella
- Plasma membrane (all cells)
  - **Peptidoglycan:** a conserved cell wall molecule in most bacteria
- Cell wall (most bacteria)
- Other membrane (gram negative bacteria)
- Lipopolysaccharide (LPS): attached to outer membrane
  - Lipid A: a part of LPS that is conserved in **Gram negative bacteria**
  - **O antigen:** the polysaccharide part of LPS → it's highly variable and *is not a MAMP*

Healthy immune function depends on *recognition, interpretation and memory:*

1. Molecules that signal what type of agent is present:
- MAMPS, O-Antigens

2. Molecules that signal if a cell/tissue is damaged or working how it's supposed to.
- Microbe, Metabolites

3. Interpretation→ aim to avoid damage from pathogens *and* damage to self
- Action: engage immune effectors
- Preparation: stimulate cell development

When and where the specific receptors for these signals are expressed is important because it impacts 'where' and 'how' the response by appropriate immune cells occurs.

Immune balance:

Down regulation of immune responses: inability to regulate immune response
- Allergies
- Autoimmunity and inflammatory bowel disorders

Up-regulation of immune responses:
- Susceptibility to infections and cancer are outcomes of weak immune response or immunodeficiency

Immune balance→ the relative numbers of cells with distinct immune functions- predisposes to different outcomes
- The processes of development and differentiation of precursor immune cells aim to 'prepare' the body for a balanced response
- Interpretation of foreign cell presence *(graft outcome)* is strongly influenced by immune balance

Microbe recognition by PRRs, GPCRs and immunoglobulins in the gut *supports* healthy microbe interaction:

1. Pattern Recognition Receptors (PRRs)→ general response to *any* microbes
    - MAMPs
2. Immunoglobins→ target response to specific microbes
    - Variable-surface macromolecules
3. G protein-coupled receptors (CPCRs)→ respond to what microbes have been doing
    - Cell metabolites: useful as nutrient (ex. acetate, butyrate)

PRRs, GPCRs and immunoglobins as part of immune system:

MAMPs such as peptidoglycan *trigger* development of adaptive immune system → prepared to respond to diverse microbes

Infection by pathogens can *trigger* expansion of specific antibody-producing B cells and formation of memory → prepared for response to re-infection

Metabolites such as *acetate* can *promote* the development of T regulatory cells → prepared to balance immune response to *normal antigens*

Microbe signal trigger immune cell maturation:

→ most obvious in requirement for *postnatal immune development*

Present prenatally: Undeveloped lymphoid tissue
- Pattern Recognition Receptors (PRR)

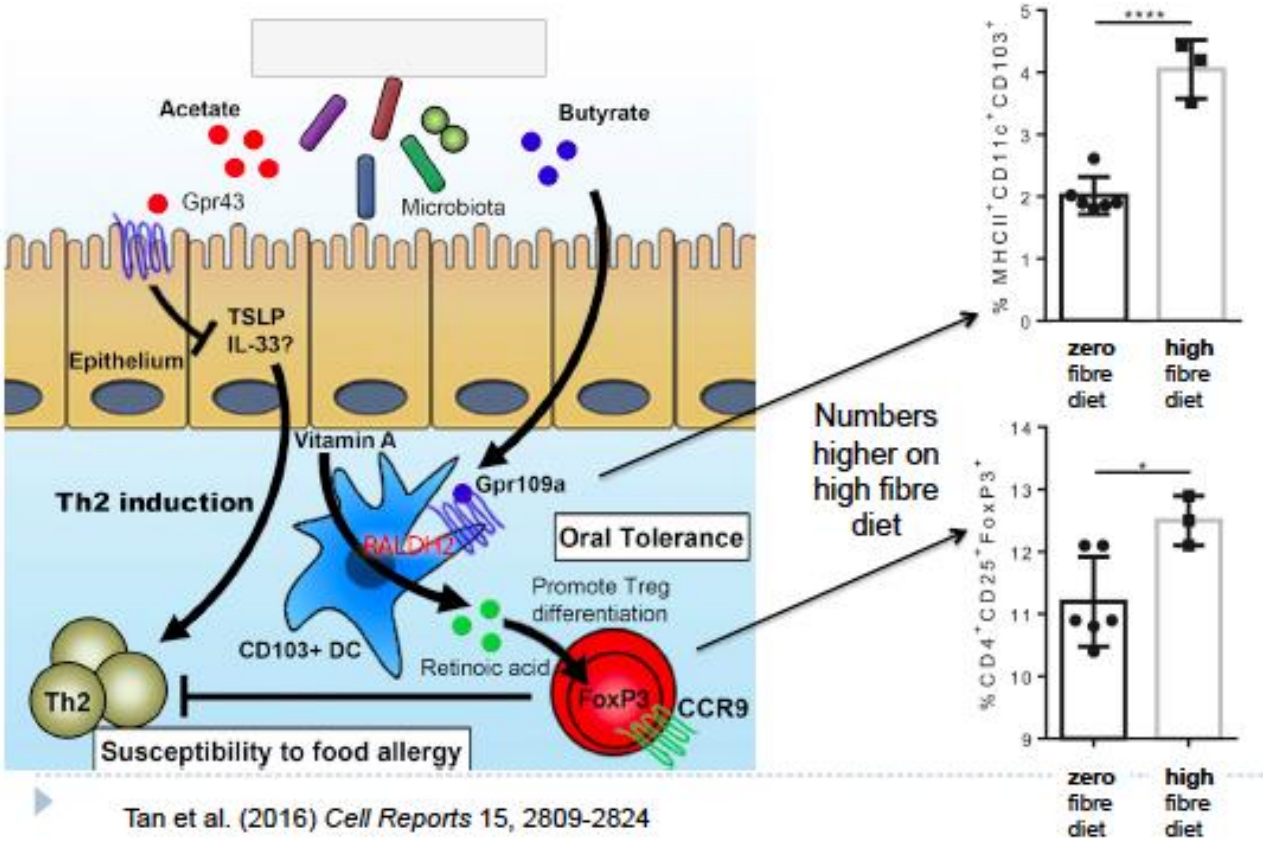
Only present postnatally: Mature lymphoid architecture, B cells, immunoglobins, antimicrobial peptides
- Ligand for PRRs (MAMPs)
- Antimicrobial peptides (defensins)
- Specific receptors (IgA)

<u>Throughout life, microbe-derived signals can influence the balance of effector and regulatory cells:</u>

With the recipient naïve T cell (APC):

- Food allergy:
  - Greater Teff than Treg
- Food tolerance:
  - Greater Treg than Teff

Hence, what we eat influences microbes, by *changing the signalling inputs* that determine immune balance



Tan et al. (2016) *Cell Reports* 15, 2809-2824

W9L20 Innate Immunity and Inflammation

Learning outcomes:

- Where is the immune system?
- What is meant by innate immunity?
- Inflammation, what causes it, and why we need it
- What happens if there's too much inflammation, or it persists
- How the different components of innate immunity function to combat different kinds of microbes
- How innate immune reactions stimulate and interact with adaptive immune response

The Immune System:

- A collection of cells, tissues, and molecules that *mediate* resistance to infections and eliminate tumours
- The function:
  - To prevent infections
  - To eradicate established infections
  - To detect and eliminate tumours and also tolerate them

Immunity:

→ resistance to disease

Infectious diseases caused by:

- Bacterial infections
- Viral infections
- Fungal infections
- Parasitic infections
- Tumour immunity

Location of the immune system:

→ Integrated with other systems such as gastrointestinal, cardiovascular etc.

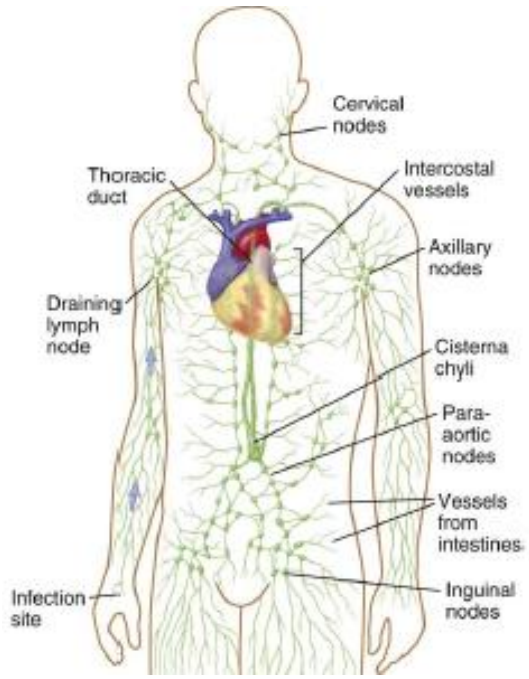
→ Immune cells are scattered in all parts of the body:

- Many of them migrate
- Others→ resident cells

→ Molecules of the immune system can act in an:

- Autocrine
- Paracrine, or
- Endocrine manner (when secreted)

The anatomy of the immune system



Physical and chemical barriers:

- Epithelial cells of skin, gut, respiratory tract
- Secretions incl. sweat, wax, and tears
- Mucus in the nose, trachea, gut
- Urine
- Proteolytic enzymes
- Low stomach pH
- Normal gut flora

The cells and molecules of the immune system access most organs via:

- The blood vessels
- The lymphatic vessels

Central sites:

- Lymphoid tissues or organs:
  - Primary lymphoid organs→ bone marrow, thymus
- Secondary lymphoid organs:
  - Spleen, lymph nodes, mucosal and cutaneous associated lymphoid tissues

Peripheral sites:
- All other tissues and systems: skin, liver, gut, CNS, etc.

Innate Immune System: common myeloid progenitor
- Neutrophil
- Basophil
- Eosinophil
- Monocyte

→ Speed: early; rapid
→ Duration: short-lived
→ Repetitive: responds the same each time
→ Interactive: with other cells of the innate & adaptive immune system
→ Non-reactive to the host

Components of innate immunity:
- Epithelial barriers
- Cells in circulation and tissues
    - Phagocytes:
        - **Neutrophils and macrophages**
        - **Scavengers that *ingest* microbes**
    - Exocytes:
        - **Eosinophils, mast cells, basophils**
        - **Release active mediators from granules**
- Molecules:
    - Tumour necrosis factor (TNF), interleukin-1 (IL-1)

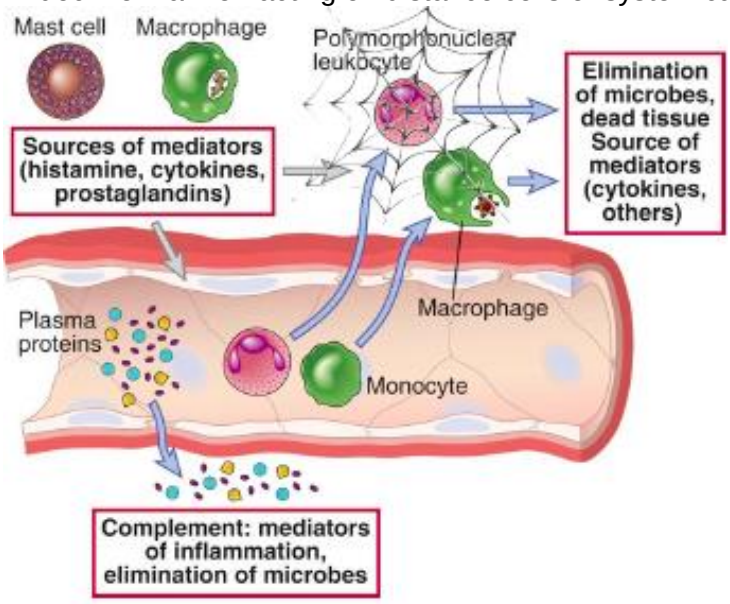
Cells Recognise and Respond to Patterns:
- Epithelial, endothelial, resident immune cells express receptors on their surface that allow them to sense danger
- Different microbes express different patterns
- Our own ells don't express these patterns → one way in which the innate immune system can tell the difference between self and non-self

Tissue Resident cells:
When danger is detected:
- Release of histamine and inflammatory cytokines
    - *Tumour necrosis factor (TNF) and interleukin-1 (IL-1)→causes inflammation*
- Dilated blood vessels allow for more blood flow to the area and fluid to come in
    - Carrying innate immune cells and plasma proteins
        - Complement and antibodies
- Prompts *the expression of adhesion molecules* on *endothelial cells lining the blood vessels*
    - Attracts innate immune cells→ neutrophils mainly
        - Perform phagocytosis
        - Secrete more inflammatory cytokines
        - Extend web-like extracellular traps for bacteria

Mast cells:
- Mast cells reside in *peripheral tissues exposed to the environment*
    - Skin, lung, gut
- Have receptors on their surface that allow them to sense danger
    - One of the first cells to respond to danger
- Perform antibacterial functions:
    - Degranulate the contents of their cytoplasm
        - Granules contain *histamine and other soluble factors*
        - *Increases vascular permeability* and promotes *inflammation*

Cytokines:
- Proteins produced and secreted by different cell types
- Modulate inflammatory and immune reactions
- Principle mediator of communication between cells

- Target cells in an:
    - Autocrine manner: acting on the cell that produced the cytokine
    - Paracrine manner: acting on neighbouring cells
    - Endocrine manner: acting on distance cells or systemically



<u>Chemokines: makes cell migration possible:</u>

- Chemo-tactic cytokines
- Different cells express *different chemokine receptors*
    - Allows the cell to respond to different chemokines

<u>Inflammation:</u>

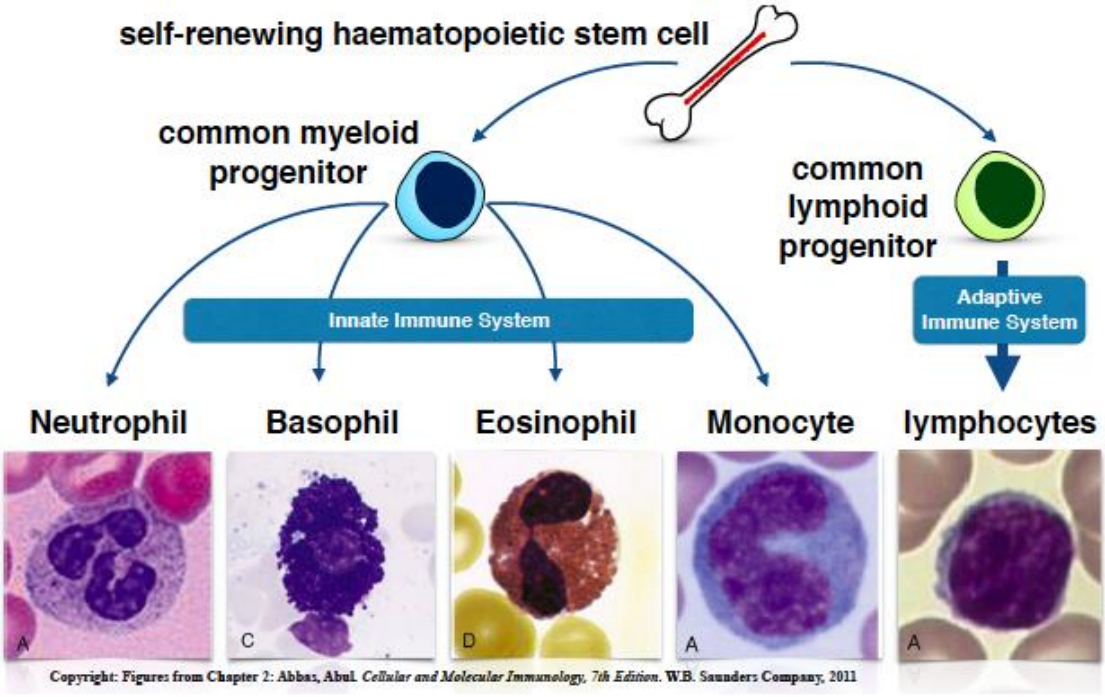
Too much inflammation:

- Septic shock
- Chronic inflammation → severe diseases such as cancer and diabetes

W9L21 Adaptive Immunity

Learning objectives:

- Some of the cells and molecules that make up the adaptive immune system
- How adaptive immune responses are generated and maintained
- Some of the hallmark features of adaptive immune responses



Lymphocytes:

- B lymphocytes (or B cells)
- T lymphocytes (or T cells)
  - T as they mature in the Thymus

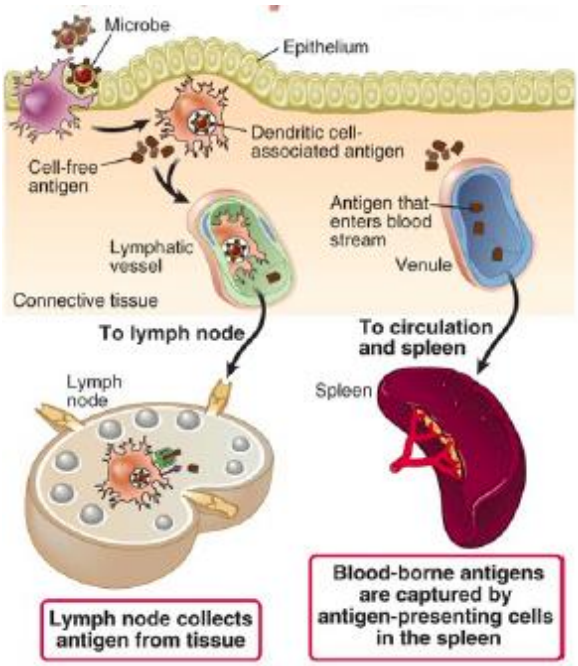
***Thymus** → primary lymphoid organ

Types of adaptive immunity:

- In ***humoral immunity,*** *B-lymphocytes secrete antibodies* that eliminate extracellular microbes.
- In ***cell-mediated immunity,*** different types of T-lymphocytes:
  - Help phagocytes to destroy ingested microbes
  - Kill infected cells

2 main types of T-cells in the periphery:

- Helper T-cells (Th cells)
  - Help other cells of the immune response
  - Different types exist with specialised functions
  - Some Th cells *suppress or regulate* the immune response rather than *activate* the immune response
- Cytotoxic T Lymphocytes (CTLs)
  - Kill their target cells in a highly specific way
  - Get help from Th cells
  - CTLs play a key role in:
    - Viral infections
    - Anti-tumour immunity



Dendritic cells (DC):

- Peripheral tissues contain DC
- Strategically located to maximise chance of 1st encounter
- DC detect microbes

- DC initiate adaptive immune responses
    - ***Bridge between innate and adaptive immunity***

Lymph System:

- Lymph constantly leaks out of blood vessels in all epithelia and connective tissues and most *parenchymal* organs
- Lymph is *drained by* lymphatic vessels ***from the tissues to the lymph nodes***
- Dendritic cells *pick up microbes from the periphery* and *transport these to the lymph nodes*
- *Lymph nodes provide a meeting place for T cells and DC*

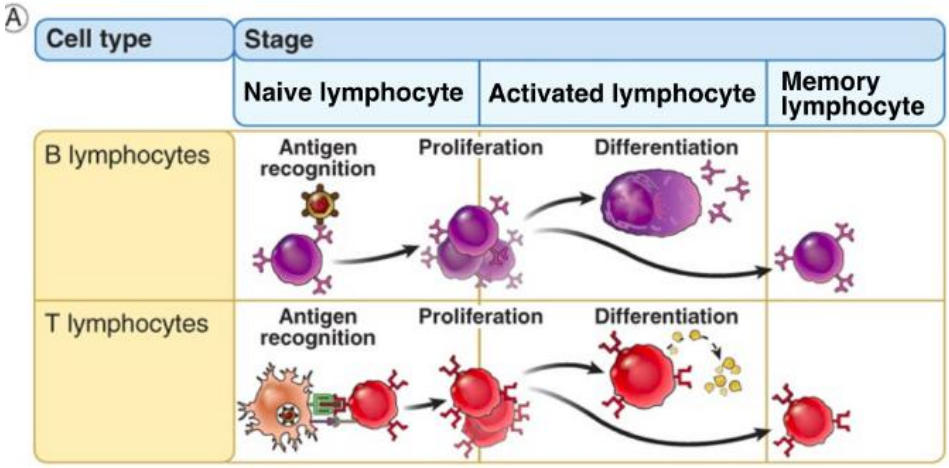
Antigens:

→ Substances (often foreign) that are recognised by the immune system
: antibody generator
→ Lymphocytes have receptors on their surface to respond to specific antigens

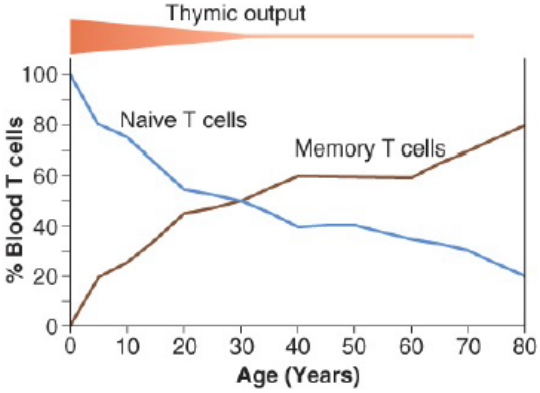
Native and activated lymphocytes

- Native lymphocytes:
    - Those that exist before antigen exposure
    - Never was in contact with their antigen
    - The number of native lymphocyte specific for any one antigen is very low
    - **DC will display antigens they have encountered on their surface for inspection by naïve T-cells**
        - **Once the right T-cell clone is found, the DC will *activate* the lymphocyte**
            - **Reason why DC are called "Antigen-presenting cells"**
        - **The newly activated T cell will undergo clonal expansion**

- Activated lymphocytes:
    - Those that have seen their antigen and been activated



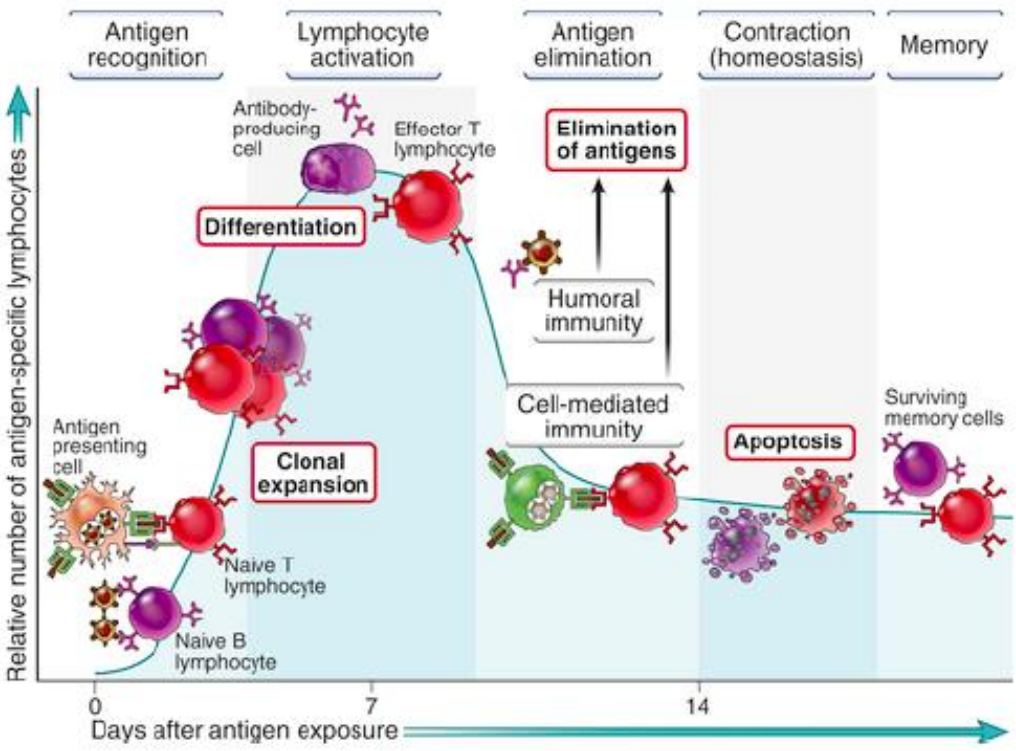
Memory lymphocytes survive for long periods in the absence of antigen:



W10L22 Immunology in Human Disease

Learning objectives:

- Link some common human diseases to the immune system
- Describe some of the ways in which targeting the immune system is helping us fight human disease



Immunological Tolerance

→ a system for determining which lymphocyte clones will be allowed to *survive*

There are 2 types of tolerance:

1. **Central**
   - Occurs in the primary lymphoid tissues
     - Bone marrow (for B cells)
     - Thymus (for T cells)
2. **Peripheral**
   - Mediated primarily by *regulatory cells*

*A breakdown in tolerance can lead to **autoimmune diseases**

Genetic factors

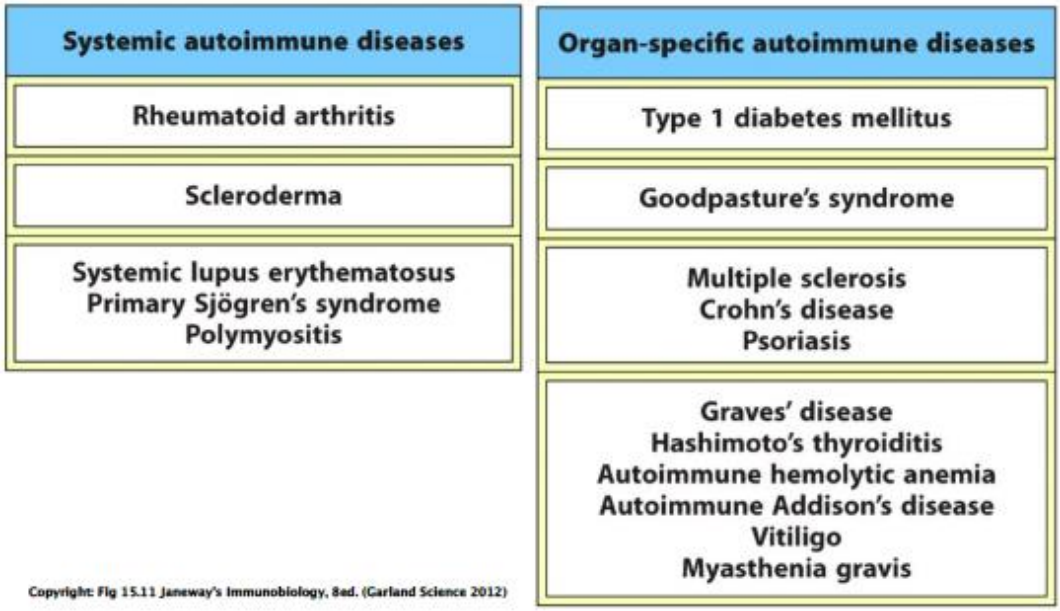
- Many autoimmune diseases are linked to the inherited genes responsible for:

1. T cell activation
2. Maintaining immunological tolerance
   - ➢ Finding and destroying self-reactive lymphocytes
   - ➢ Activity of regulatory cells

Environmental Factors

- Autoimmune disease is often preceded by an infection
- Other environmental/ host factors may contribute:
  - Many autoimmune diseases appear more commonly in women
  - Local trauma leading to an inflammatory reaction may release previously hidden antigens that our immune system responds to:
    - Exposure to sunlight → trigger the development of the autoimmune disease "systemic lupus erythematosus" (SLE) in which antibodies are produced *against* self-antigens.
      - It's postulated that these nuclear antigens may be released from cells that die *as a consequence* to the sun
      - In other contexts (ex. Multiple Sclerosis), *a lack of Ultraviolet Radiation from sunlight* is a contributing factor to autoimmunity

Autoimmune disease may by organ specific or systemic

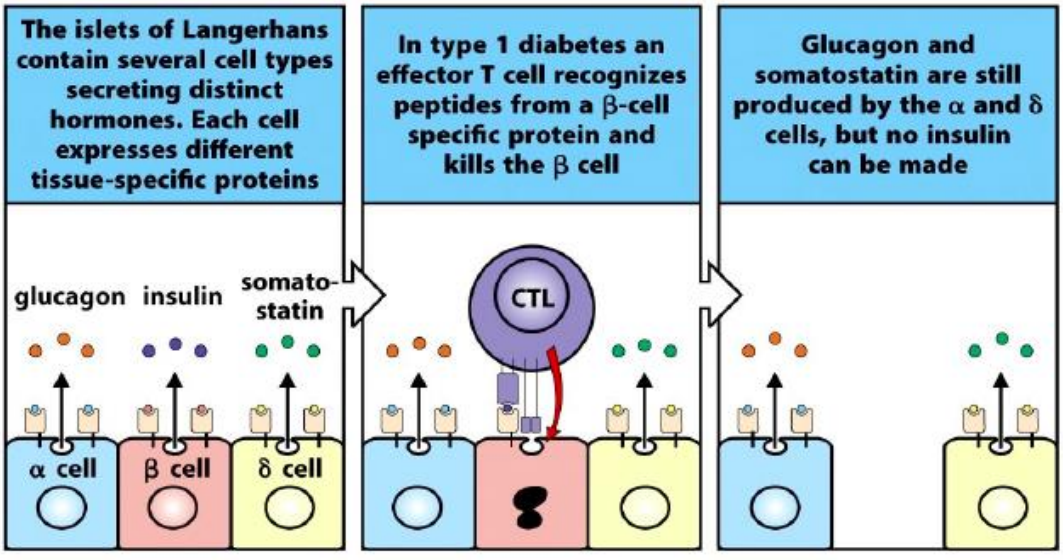


Autoimmune diseases involve all aspects of the adaptive immune response

	T cells	B cells	Antibody
Systemic lupus erythematosus	Pathogenic Helper T cells	Make auto-antibodies	Pathogenic
Type 1 diabetes	Pathogenic Cytotoxic T cells	Possibly pathogenic	Present but role unclear
Multiple Sclerosis	Pathogenic Helper T Cells	Pathogenic	Present but role unclear

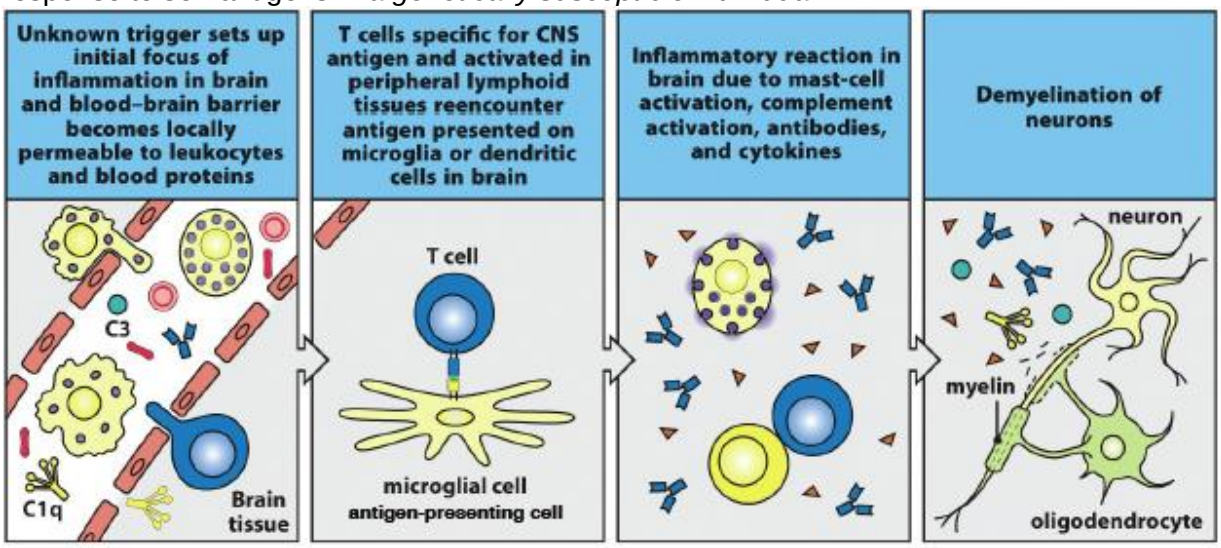
Type 1 Insulin Dependent Diabetes Mellitus

- **The insulin-secreting B cells** in the pancreas are targets of T cells
- Activated cytotoxic T cells find their way to pancreas
- B cell-specific CTLs kill the B ells
    - No insulin produced
    - The a and gamma cells are unaffected
    - highlights ***exquisite antigen specifically***



Multiple Sclerosis (MS)

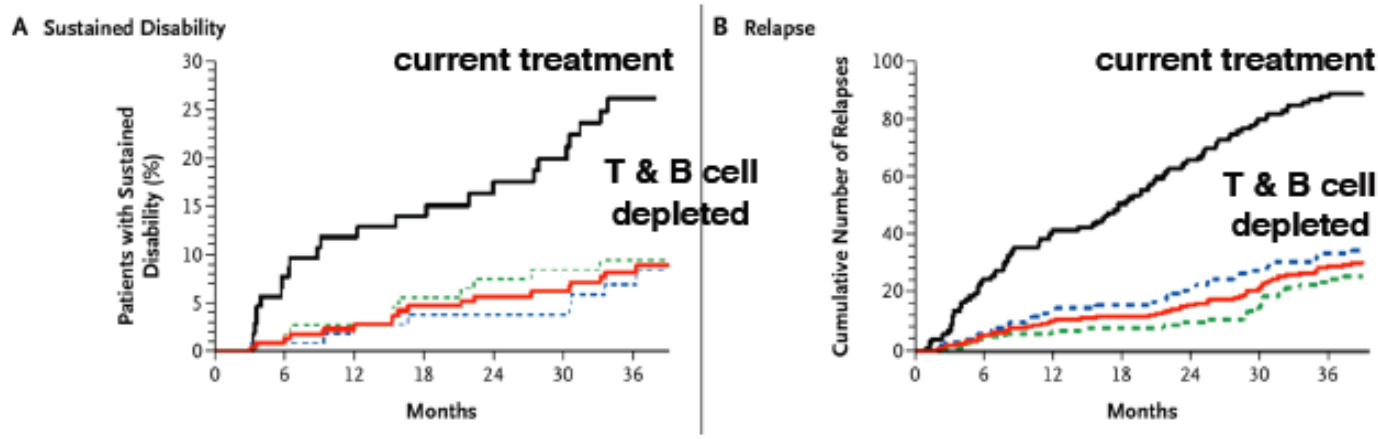
→ MS is a multifocal *demyelinating disease* with progressive *neurodegeneration* caused by an autoimmune response to self-antigens in *a genetically susceptible individual*.



Symptoms:

- motor control
  - coordination, balance, functioning of the extremities
- fatigue
  - including heat sensitivity
- other neurological symptoms
  - vertigo, pins and needles, neuralgia and visual disturbances
- continence problems
  - incontinence and constipation
- neuropsychological symptoms
  - memory loss, depression and cognitive difficulties

Treating MS by removing B cells and T cells:



Immune Surveillance:

Patients with tumours that have been infiltrated with lymphocytes have a *better* prognosis
→ Enlarged tumour-draining lymph nodes = better prognosis

When Transplanted tumours are rejected:
→immunodeficient patients have a higher *susceptibility to tumour growth*

- transplant patients on immunosuppressive therapy
- people with acquired immunodeficiencies:
  - older populations, those with certain infectious diseases, exposure to environmental carcinogens (ex. UV, radiations)

New cancer therapies that *target the immune system*

- bone marrow transplantation
    - certain types of leukemia
- re-engineered T cells
  → Chimeric Antigen Receptor (CAR) T cells
- antibodies
    - remove the immunological brakes
    - apply the immunological accelerator

W10L23 Where does the waste come from and how is it removed?

Learning objectives:

- Identify the different types of waste and where they are produced and excreted
- Discuss the function of the major excretory organs- the kidneys
- Describe the role of hormones in the regulation of urine volume and blood pressure

Examples of types of waste and the production location:

- Undigested food in the large intestines → faeces
- Excess ions and water
- Bilirubin
  → breakdown of haemoglobin
- Metabolism produces wastes
  - Breakdown of amino acids in the form of NH_3 (ammonia), toxic
    - liver combines ammonia with CO_2 to form urea, less toxic, adequate for storage and excretion in concentrated form
  - Breakdown of creatine phosphate (→ important for muscle contraction) in the form of creatinine
  - breakdown of nitrogenous bases from RNA in the form→ uric acid

Excretory organs:

1. Lungs- CO_2, water and heat
2. Skin
3. Liver (prepares waste)
   - Bilirubin
   - Converts ammonia into less toxic urea
4. Digestive system
5. Kidneys

Kidney functions:

- Kidneys filter about 180L fluid/filtrate from the blood per day
- 99% re-absorbed
- 1% (1-2L) forms urine to be excreted
- Main structures of Urinary system: kidney, ureter, urinary bladder, urethra
- **Micturition reflex**

Excretion

- Urine:
  - Urea
  - Poisons
  - Uric acid
  - Creatinine
  - Excess water and solutes such as sodium and potassium
  - Drugs ex. Penicillin

Homeostasis

- Water level determines blood volume
- Electrolytes balance in tissue fluid and blood
  - Sodium, potassium, etc.
- pH
- Blood pressure
- Regulating numbers of RBC entering the circulation by *secreting* ***erythropoietin***

Nephron- (1) tubules

- Bowman's capsule
- **proximal convoluted tubules (PCT)**
- Loop of Henle
- **Distal convoluted tubules (DCT)**
- Collecting ducts
- **Renal pelvis**

Nephron- (2) blood vessels

- From Renal artery
- Afferent arteriole
- Glomerulus
- Efferent arteriole (narrow)
  → increases pressure in glomerulus
- Peritubular capillaries
  → arterial end foes down *ascending* loop of Henle
  → venule end goes up *descending* loop of Henle

Bowman's capsule

- Movement of filtrate from *glomerulus (high pressure)* to Bowman's capsule (low pressure)
  - At this stage, glomerular filtrate contains nutrients, salts, wastes and water
- Blood cells and large proteins remain in glomerulus

Proximal convoluted tubules (PCT)

→ All glucose is removed in this process (except for diabetic patients)

- Sodium, amino acids, vitamins, glucose (active transport)
  - PCT (mitochondria release ATP) → peritubular capillaries
- Water (passive transport/ osmosis) along with solutes

Loop of Henle (1) Descending

- Walls permeable to water
- More water reabsorbed/ osmosis (Tubule with low concentration of solutions → Medulla → Venule)
- Walls moderately permeable to solutes
  - High relative concentration of solutes in surrounding tissue = some movement of solutes into tubule by diffusion (sodium, potassium and urea)
- Filtrate becomes more concentrated

Loop of Henle (2) Ascending

- Walls not permeable to water
- Permeable to solutes such as sodium, potassium, and chlorine
  → movement out of tubule into arteriole is by:
  - Diffusion (thin section)
  - Active transport (thick section)
- Filtrate becomes more dilute
- Counter current

Distal convoluted tubules (DCT)

→ Reabsorption

- of water (19%) by osmosis
- solutes by active transport
- *Controlled by Aldosterone and Anti-diuretic hormone (ADH)*

Hormones: **aldosterone**

- Secreted by adrenal gland when low plasma [Na^+], ex. after exercise or sweating
  - increases Na^+ absorption (from DCT and collecting ducts into the blood)
- If high plasma [Na^+], ex. consuming a salty meal
  - low aldosterone secretion
  - more Na+ excreted in urine (retained in DCT and collecting ducts)

Hormones: **anti-diuretic hormone (ADH)**

- Secreted by pituitary gland when *low plasma water concentration*
  - Increases permeability of DCT and collecting ducts
  - Increases resorption of water into blood
  - Increases blood volume and pressure
- Increases blood pressure
  - Negative feedback reduces ADH secretion
- *Alcohol and caffeine interfere with ADH*