Drugs treatment of disease notes

Lecture 1: Introduction

- A chemical becomes a therapeutic drug through **scientific development** (target selection-in vitro, lead finding and optimisation and pharmacological profiling-determining the mechanism of action, animal testing, understanding of pharmacokinetics, formulation and synthesis and understanding the short-term safety-we identify a drug candidate that we need to optimise), **clinical development** (human testing of the drug through phases 0, 1, 2, 3 and may have accelerated development if required- we also develop an understanding of the long term safety of the compound and we start clinical development with the development compound) and then we have **clinical use** (we have an approved drug which has post-marketing surveillance- phase 4 where the drug may be unsafe and taken off the market).
- In drug development we need to translate scientific principles into clinical practice where we may start with molecules and through molecular pharmacology, cell and tissue pharmacology, organ pharmacology, whole body pharmacology and then in the population we get an understanding of the benefits and harm of the agent such that we can produce a medicine. We need to bring the compound from the bench to the bedside from pharmacology to applied pharmacology.
- Individuals with the condition **dropsy** suffered from fatigue, oedema in legs, shortness in breath and heart palpitations but the use of the digitalis foxglove flower led to symptomatic relief of this condition (used safely without understanding the mechanism of action). It's now known that this flower contained **cardiac glycosides** which inhibit the Na+/K+ ATPase where the intracellular changes in [Ca2+] and [Na+] lead to changes in electrical properties of cardiac cells. Thus cardiac glycosides will act to increase contraction and slow the ventricular rate of the heart (heart works more efficiently) and is thus useful in heart failure and provides relief from atrial arrhythmia (but is **arrythmiogenic**- can cause ventricle arrhythmia-side effect). An example of a cardiac glycoside is **oubain** with the benefits and harms being based upon the therapeutic index and margin of safety.
- It was found that acetylcholine only relaxes blood vessels with an intact endothelium as acetylcholine releases an **endothelium dependent relaxing factor** (**EDRF**). If the endothelium was rubbed off, then acetylcholine has no effect. Over time we are understanding new ways that the endothelium will act.
- The endothelium has multiple roles such as in **matrix remodelling** (collagen, fibronectin, thrombospondin and vWF), **transcellular** (megakaryocytes, leukocytes, platelets, tumour cells and eicosanoids), **angiocrines** (metastasis, haematopoiesis, embryonic organogenesis, lung regeneration and liver regeneration) **non-constitutive activated thromboregulatory** (cell adhesion molecules, tissue factor, plasminogen activator inhibitor and vWF) and **constitutive anticoagulant anti-inflammatory** (thrombomodulin, tissue factor inhibitor, anti-thrombin and plasmin assembly).
- The endothelium can initiate the actions of macrophages and inflammatory pathways with potential roles in atherosclerosis. We thus need to evaluate low MW and immunological approaches as therapeutics. Macrophages have many ways to be activated and we need to integrate pharmacology and immunology to exploit this system of macrophage activation.

- The drug targets in the post-genomics era have mainly been GPCRs, enzymes, carriers/transporters while there has been less targeting of cytotoxics, protein kinases and nucleic acids (has been rising).
- The EDRF mediator is a diffusible factor with a short-half-life (issue as it's a signalling molecule) and matches the effects of NO. This mediator was hypothesised to be NO. NO is synthesised from arginine by **nitric oxide synthase** (**NOS**) with the by-product being **citrulline** (part of urea cycle). NO thus has a role in vasodilation. Thus when endogenous molecules (acetylcholine, bradykinin and substance P) or mechanical stress cause activation of NOS it can initiate NO synthesis through [Ca2+] increases such that when NO is produced, it can act on smooth muscle cells to cause relaxation of the smooth muscle cell.
- We thus have therapeutic applications that exploit the action of NO, such as for **pulmonary hypertension** we inhale NO gas, for **angina** we use NO donors, for **erectile dysfunction** we use **sildenafil** (inhibits cGMP specific type V phosphodiesterase) and for **atherosclerosis** we use **L-arginine**.
- There are three isoforms of **NOS** such as **eNOS** (endothelial cells), **nNOS** (nerves and epithelial cells) and **iNOS** (macrophages and smooth muscle) - cell specific isoforms.
- In oxidative stress (too much O2), the NO produced will bind to the O2 (becomes an inactivating molecule) causing production of **peroxynitrite** (ONOO-) which will cause cell damage (bad effect of NO). We thus need to find a balance to control the effects of oxidative stress on NO (not just through taking antioxidants).

Lecture 2: Innate Immunity

- We try to fine tune the **innate immune system** so it sends the right signals to the **adaptive** (specific) **immune system** so it gives an appropriate response.
- In the innate immune response we can see **microinjury** result from mechanical scraping of the thumbnail on the arm where we will see brief **vasoconstriction** (next to site of injury to limit blood flow to areas nearby and direct flow to the injured site) and **redness** followed by **swelling**. **Histamine** is released by local **mast cells** to cause this response and it's then followed by an infiltration of **inflammatory** cells, mediated by **cytokines** and **interleukins** and causing white blood cells to stick to the blood vessels and be prepared to attack potential bacterial infection. If there was no innate immune response, then the response wouldn't occur as rapidly.
- The term **immunity** is the ability of an organism to resist a particular infection or toxin by the action of specific antibodies or sensitised white blood cells. Immunity is an old idea which was used in Roman law to denote exemptions from punishment and was used in 20AD to denote resistance to snake bites and later on to immunity to the plague. **Acquired resistance** to infection was determined to exist for certain infections in BC times where upon exposure, individuals were less likely to suffer from the plague.
- The immune system is required to defend against infection and to discriminate infectious cells from self and determine if self-tissue is normal or not (infected). We make new drugs to exploit the molecular pathways of the immune system.
- The innate immune system is quick, stereotyped responses (responses don't change in nature) and has no memory (of types of pathogens) while the adaptive immune response is slow, flexible (can act with immense specificity-highly advantageous) and has memory (amplified upon second exposure) and recall abilities. The innate immune response is essential and normally quite strong. The main way that the innate immune response breaks down pathogens is through **proteases** or through **molecular oxidants** which are the main killing mechanisms of the innate immune response. The adaptive immune response (not all species possess adaptive immunity-particular to mammals and fish) would take 2 weeks after an injury for its defensive mechanisms to peak.
- The classes of drug molecules that target the innate immune response include **adjuvants** (components of vaccines that kick-start and fine tune the adaptive response- **vaccine class** sits between innate and adaptive immune response in terms of which it targets)**, anti-inflammatories (**glucocorticoids) **NSAIDs** (non-steroidal anti-inflammatory drugs) and **anticancer agents.**
- The classes of drug molecules that target the adaptive immune response include **immunosuppressives** (for organ transplantation)**, immunomodulators** (restore immunity-strengthen immune system)**, immunostimulators, monoclonals** (artificially develop an immune system) and **engineered T cells** (engineer lymphocytes to target cancer antigen and reintroduce to patient to eliminate cancer-self therapy)**.** This is because many of the slow, chronic, debilitating diseases are often mediated by excessive immunity or a defect in immunity (too strong/too weak) which lead to long term pathologies.
- At the cell surface we have a series of receptors which all have **PRR** (**pattern recognition receptors**-unique to innate immune system) which responds to **PAMPs** (Pathogen associated molecular patterns- key molecular signatures/proteins on surface

of infectious agents required for the pathogen which cannot be removed without losing viability). PRRs have been developed due to selection pressure to respond to PAMPs such as the **lipopolysaccharide complex** (dimer of CD14 and **Toll-like receptor 4**). The **TLR family** (Toll-like receptor) responds bacteria and surface elements to certain pathogens to produce a signal. There are other molecules that responds to other PAMPs such as **dectins, mannose-binding lectins, trem** and components of the **immunoglobulin family**. All these PRRs converge down to 3 primary effector systems such as inducing **type-1 interferons** (recognition, transduction and induction of type-1 interferons give us high temperature and flu like symptoms- type-1 interferons are overstimulated in **lupus disease**), **pro-inflammatory genes** (attracts white blood cells to a site of injury and put them into an activated state- there is convergence of pathways- producing chemokines) and there is also the **healing program** (in a pre-activated state- stay dormant until pathogen is cleared away by immune system will activate a repair and healing process-**catabasis**-active resolution-we don't passively get back to the ground state- we need to heal and repair tissue).

- Adaptive immunity is the action of lymphocytes. There are two types: **B cells** (make antibodies which bind to targets to neutralise them, make it easier for white blood cells to recognise and destroy targets and they neutralise toxins from the targets) and **T cells** (**cytotoxic** recognise infected cells and destroy it by punching holes in the surface and introduce enzymes that proteolytically destroy key structures in the cells and the **helper** T cells assist in the B and cytotoxic response). The **APC** (**antigen-presenting cell** which constantly does **micropinocytosis-**constantly takes in small amounts of ECF and has a surface covered in PRRs which if they are activated by PAMPs and they take in an antigen, the PRR activation will trigger the APC to place fragments of the antigen on the APC's surface and present it to the helper cell- type of PRR shapes whether the response heavily favours cytotoxic or B cell responses). After first exposure, we have the ability to form long-term memory helper T cells and B cells so upon second exposure we can have a strong, quick response.
- The innate/adaptive immune processes are relevant to any disease where tissue is damaged or exposed to foreign materials. Thus we target these processes as they are the richest sources of new medicines, they are the basis of all inflammatory disease, graft rejection and immunosuppressives, vaccines (including therapeutic vaccines against cancer), gene therapy (limited as introducing foreign genes initiates innate immune system as it thinks it's a virus and initiates nucleotide specific PRRs), stem cell therapy and regenerative medicine and are probably the most developed area of biological science to date.
- The interface between innate and adaptive immunity is where real immunity lies. The immune system has evolved to discriminate infectious non-self (gut has commensal bacteria which are not dangerous and not targeted against by immune system) from non-infectious self.
- The types of pathogen that the immune system has evolved to defend against include polio, cholera, intestinal worms, anthrax, aspergilus, malaria, amoeba, pollen and cancer. We distinguish between **unicellular organisms** (bacteria, mycobacteria and fungi), **viruses** (influenza, rhinovirus, corona virus, adenovirus and respiratory syncytial virus) and **multicellular pathogens** (helminths).

- The main processes of the immune response are to **recognise** (dangerous non-self from self), **kill or contain** (inflammatory response) and **resolve** (organ protective catabasis).
- In Tuberculosis, the tuberculosis pathogen is contained rather than killed by putting a wall of fibrous tissue around the pathogen.
- Upon adherence of pathogen to the epithelium there is protection against infection by normal flora, local chemical factors and phagocytes. Upon local infection/penetration of epithelium by pathogens there is wound healing induced, anti-microbial proteins and peptides, phagocytes and complement destroy the invading microorganisms and there is action of **γδT** cells. Upon local infection of tissues there is complement activation, phagocytes, cytokines and NK cell activation, activation of macrophages, dendritic cells migrate to lymph nodes to initiate adaptive immunity. Adaptive immunity sees infection cleared by a specific antibody, T-cell dependent macrophage activation and cytotoxic T cells.
- Upon a cut, previously useful bacteria on the surface of our skin may be introduced into the bloodstream, within microseconds, the physical trauma and the PRR activation is activating **macrophages** (eat and destroy cells) and **dendritic cells** (trigger immune response- APC cells) in the tissue. If there is a dangerous signal, the dendritic cells will consume the antigen, there will be chemicals released by the transduction process of the PRRs which prime them/instruct cells on certain type of infection (type of pathogen), it will make certain types of cytokine (matched to the pathogen) and it will break large proteins of pathogen down into peptides (8-12 amino acids in length) and puts these peptides on their cell surface. The early inflammatory response informs the dendritic cells to leave the tissue once it has captured a pathogen and go into the lymphatic system. While this is occurring the inflammatory genes will instruct white blood cells in the blood to adhere to venules in the microcirculation, to move out from the blood and into the tissue, thus we see the release of **white blood cell growth factors** (keep them alive for longer) and **activating factors** (push the white blood cells into a destructive state-when they leave the tissue they remain destructive- with a pimple we see local tissue breakdown through release of proteases). The APCs then trigger the adaptive immune response, with the same signal that attracts the white blood cells to the site also attract lymphocytes to the site of infection (lymphocytes exit through the same lymph nodes- do this as lymph nodes take large areas of body surface and funnel it down to a very anatomically small location).
- The intrinsic epithelial barrier to infection include **mechanical** defence (epithelial cells joined by tight junctions, longitudinal flow of air or fluid across epithelium and movement of mucus by cilia), **chemical** (fatty acids-skin, enzymes, lysozymes- saliva, sweat and tears, and pepsin-gut, low pH-stomach, **antibacterial peptides**-defensins-skin and gut or cryptidins-intestine) and there is **microbiological defence** (normal flora compete for nutrients and attachment to epithelium and can produce antibacterial substances). Mucus (captures bacterial and has many anti-bacterial factors in it) and surfactant proteins A and D are important in the lung. The **metagenome** is all the normal flora and are quite diverse (vary slightly by body site) but as people lose immunity and move towards illness, the metagenome becomes less diverse and the wrong types of commensal bacteria are present, allowing other bacteria to take over a

niche position in the ecology of bacteria on our surfaces. Chronic diseases usually arise from a shift in the metagenome where pathogens are able to grab hold of a region and have a long term colonisation (occurs in obesity and inflammatory bowel disease-subclinical and clinical conditions).

- There are 4 families of PRRs that have been discovered. There are **TLRs** (1-11) which recognise bacteria, viruses, protozoa and fungi, there are **NOD/NLRs** (nucleotide binding and oligomerization domain- like receptors) which recognise bacteria that get into the cell, there are **RLR** (RIG-I – retinoic acid inducible gene 1 – like receptors) which recognise viruses (innate immune receptors inside the cytosol of the cell) and there are **CLRs** (C-type lectin receptors) which recognise fungi and are found on the cell surface. All these release chemokines, type-1 interferons and are linked to catabasis.
- There are common motifs between internal and external parts of PRRs. The molecular structures of PRRs reuse 7 conserved domain elements being LRR (leucine-rich repeat), TIR (Toll/IL-1 receptor), CARD (caspase recruitment domain), helicase domain, CTLD (C-type lectin domain), immunoglobulin domain (shared with adaptive immunity) and ITAM (immunoreceptor tyrosine-based activation motif- shared with adaptive immunity). There is a structural basis of recognition and responses in the innate immune system- not every domain is linked to every transduction system.
- The signal transduction from PRRs entrain similar downstream intermediates and effectors. The **TLRs** signal through **NF-κB**, **IRFs**, and **MAPKs** which lead to production of cytokines, chemokines, anti-viral proteins and proIL-1 and pro-IL-18 which then become mature, the **NLRs** produce the same things but their signal is through **caspase-1.** The RLRs signal through IRFs and produce anti-viral proteins while the CLRs signal via NF-κB and MAPKs to produce cytokines and chemokines.
- There are multiple types of toll-like receptors as a result of selective pressures such that there is a diversity of TLRs. TLR4 recognises **liposaccharide** (from gram-negative bacteria), TLR2 and TLR6 complexes recognise diacylated lipopeptides, TLR2 and TLR1 complexes recognise triacylated lipopeptide, TLR3 recognises double stranded RNA, TLR 5 recognises flagellin, TLR7 recognises single stranded RNA and TLR9 recognises bacterial or viral DNA. All these TLRs lead to the activation of NF-κB leading to a strong inflammatory reaction (NF-κB is the master regulator).
- The NLRs also have variation for specificity and discrimination where there is the CARD subfamily, the pyrin subfamily and the BIR subfamily.
- Many of the PRRs converge on NF-κB such as the TLRs, CLRs and the NLRs leading to induction of type-1 interferons. Intercellular PRRs also converge on NF-κB. There is a family of receptors that are proteolytically activated and form a complex to activate a **caspase** which convert some inflammatory modulators (IL-1 and IL-18) from an inactive state into an active one (this pathway is often overstimulated leading to pathologies-thus has been targeted for certain diseases).
- Inflammation leads to heat, redness, swelling, pain and loss of function. These are the main components of inflammation. Most conditions in affluent countries have an important inflammatory component (atherosclerosis, heart disease, Alzheimer's etc.)- long term pathologies.

- Failure to mount inflammatory defence is fatal. **IL-2** is the growth factor for lymphocytes so if you lack IL-2 you can't expand the lymphocyte population (leading to a very weak adaptive immune response). A genetic defect in IL-2 production is associated with **SCID disease** (severe combined immune deficiency) which has now become curable through gene therapy where lymphocytes are taken out, genetically corrected and reintroduced.
- Interleukins are a communication mechanism between white blood cells which help to selectively stimulate certain white blood cells as well as going to the bone marrow to cause creation of more white blood cells.
- The field of **predictive medicine** is where we can do genetic tests to inform us on the likelihood of getting certain types of conditions. Therapy has mainly focused on blocking various molecules in the inflammatory response (monoclonal antibodies are a common therapeutic) to neutralise them as the inflammatory response can cause pathology, but blocking the inflammatory response can increase the likelihood to get an infection, thus we need to balance the blocking and activation of the inflammatory response (hard to treat conditions like MS).
- **Endotoxin** activates the toll-4 pathway and causes pneumonia (serious chest infection). During pneumonia there is an increase in the white blood cell count and thus may result from growth factors to lead to this increase in white blood cell population (haematopoiesis). For haematopoiesis, various stimulating factors are required such as **M-CSF** (important anti-inflammatory drug target as it activates monocytes and dendritic cells)**, GM-CSF (**profoundly pro-inflammatory molecule) and **G-CSF** (used in recombinant form after being given aggressive chemotherapy to rapidly regenerate the neutrophil population).
- Important cytokines and induced inflammatory genes include **IL-1β** (highly pro-inflammatory-can be blocked in treatment of atherosclerosis), **IL-8** (neutrophil chemokine), **TNFα** (highly pro-inflammatory and induces COX- are used very frequently to aggressively destroy certain pathologies- introduce **remission** in the disease such that it doesn't progress), **IL-6** (upstream regulator-can be blocked to treat rheumatoid arthritis and stopping the side effects such as cytokine storms from immunotherapy for cancer), **complement** and **kinins** (amplification cascades), **GM-CSF, G-CSF and M-CSF** (leukocyte survival and priming), **COX1/2** (induces eicosanoids and prostaglandins, with PGE2 being an important product in pain-sensitises pain fibres-more likely to fire once sensitised, PGE2 has 4 receptors and it cooperates with the inflammatory pathway to increase leukocyte traffic and swelling and it also works in the brain to regulate **pyrogenesis**-increased body temperature during infection, thus an aspirin like drug-NSAID, will reduce pain, inflammation and fever but they don't fundamentally alter destructive pathology-need combination therapy for chronic condition- used as palliatives to relieve pain) and **proteases** (such as MMPs for tissue destruction). These mediators have been targeted by many therapeutics.
- Two of the most important cells in the inflammatory response are **neutrophils** and **macrophages** (phagocytosis-engulf pathogens and put them into an intracellular capsule and expose to proteases and oxidants). If one has genetic defects affecting either neutrophils or macrophages, it's unlikely for a child to live until 2 without dramatic medical intervention (will die from overwhelming infection). During cancer

therapy, the cytotoxic agents kill stem cells that make neutrophils and thus the host is vulnerable to infection. Neutrophils release destructive proteases which destroy targets by cutting them proteolytically or they can release oxidants which are able to destroy membranes by making holes in the target to destroy them chemically (2 forms of host cell defence). When either of these cells are activated (excessively or inappropriately) they can cause tissue pathology.

- In cystic fibrosis, the cilia don't work properly as the mucus layer is perturbed by an alteration in fluid and infections can occur and then the host immune system causes the disease as once the infection occur in the thick mucus, the host tries to eliminate the pathogens by sending waves of neutrophils and macrophages, but these pathogens can't be eliminated as they hide in the thick mucus such that the immune system will end up destroying the lung.
- The steps in how white blood cells (leukocytes) move from the blood into the affected tissue include **capture**, **rolling**, **slow rolling, firm adhesion** and **transmigration**. When there is inflammation, it activates the endothelium to produce molecules that initially lightly tether (chemoattractant) these leukocytes and as the inflammatory signals get stronger, they cause the cells to stick, flatten and to undergo **diapedesis** (move through the junctions between the cells of the endothelium) all controlled by adhesion molecules called **selectins** and **integrins** (can block isoforms of certain selectins and integrins so only certain isotypes of the leukocytes can get access into the tissue).
- A large family of GPCRs are **chemokine receptors** that respond to inflammatory signals very specifically. There are many varieties of chemokines and their receptors but these receptors haven't been able to produce therapeutic benefit as there is ligand redundancy (hard to make selective blockers as multiple receptors do the same thing), was selective pressure in the past for variation of these receptors as it would make it hard for a pathogen to block all the different types of receptors that converge on a similar function. HIV uses one of these chemokine receptors as a co-receptor to enter cells, so blockers of the co-receptors are useful in reducing the chance of HIV infection but not as effective as anti-retrovirals which are now used instead.
- The **arachidonic acid** cascade is important where during tissue injury phospholipids from the membrane release arachidonic acid (20 carbons) and various enzymes take arachidonic acid and push it through different pathways (3 main pathways) to produce **prostaglandins** (by COX- most of the prostaglandins lead to inflammation but PGI2 is a protective molecule- relaxes vascular smooth muscle for pulmonary hypertension, anticoagulants work on TXA2 while anti-inflammatory analgesics work on prostaglandins), **leukotrienes** which cause constriction in asthma (we have therapeutics that target here), **lipoxins** (play role in catabasis-we have synthetic lipoxins to assist healing) and **EET dihydroxyacids** (promising roles in cardiovascular health). Arachidonic acid is released from the membrane by activation of **PLA2** (phospholipase A2).
- Cells have limited lifetimes which can be extended by growth factors or shortened by factors that induce **apoptosis** (active cell death). Cells can either die via apoptosis (physiological programmed cell death- apoptotic cells are removed by macrophages without causing any problems- macrophages know to remove this tissue due to chemical signalling) or **necrosis** (cell breaks down and intracellular contents spill into

surrounding tissue- intensely pro-inflammatory- can result in resolving inflammation or chronic inflammation). When apoptotic phagocytosis occurs, it prevents inflammation from occurring assisting in healing. Apoptosis is anti-inflammatory and pro-resolution with a key chemical mediator being **IL-10** while necrosis is pro-inflammatory with key chemical mediators including **HMGBP1** (high mobility group box protein 1- is a ligand) and **RAGE ligands** (receptor for advanced glycosylation endpoints-is a receptor)**.**

- The major pro-resolution processes is that there is clearance of the pathogen which then lead to downregulation of inflammation and survival factors (less survival factors produced as PRRs aren't reacting as much) leading to apoptosis (natural apoptosis of neutrophils) and there will thus be **efferocytosis** (engulfment by phagocytosis) of the apoptotic cell (neutrophils) by macrophages lead to the production of IL-10 and TGFβ and thus lead to tissue healing.
- Arachidonic acid metabolites are both pro and anti-inflammatory. The metabolites involved in **initiation** include prostaglandins and leukotrienes through initiating production of cytokines while those involved in **resolution** are **lipoxins** which initiate apoptosis and phagocytosis. Arachidonic acid therefore, firstly goes through the inflammatory pathway through the production of the COX metabolites (prostaglandins) and then transitions later to a pro-resolving pathway with the tipping point between the two pathways being the availability of **15-lipoxygenase** (not normally expressed in tissue but its level rises during catabasis- resolving pathway)**.**
- Some important drugs involved in the arachidonic acid pathway include **aspirin** and **indomethacin** (block COX), **antibodies** which may target TNFα (etanercept), IL-6 (tocilizumab), IL-1, complement, chemokines and cytokines, **GPCR blockers** (chemokine receptors, kinin antagonists and anti-histamines), **integrins** (antibodies), **catabasis** (LTA4 derivatives) and **glucocorticoid steroids** (largely don't effect innate immunity).
- The classical drugs used to treat inflammation include anti-inflammatory agents such as **NSAIDs**, (nonsteroidal anti-inflammatory agents), **salicylates** and **selective COX-2 inhibitors**. Other drugs include **DMARDs** (disease-modifying anti-rheumatic drugs) such as **methotrexate, hydroxychloroquine, sulfasalazine** and **leflunomide**. Agents that are less commonly used include **azathioprine, D-penicillamine, gold, minocycline** and **cyclosporine** while **colchicine** is used to treat acute gout. These drugs are being pushed out by the newer biological agents (more expensive than these drugs though).

  Innate immunity is essential for host defence, multiple recognition systems have evolved, use of modular architecture (intracellular and extracellular) means responses are similar, macrophages and neutrophils are critical effector cells, chemokines and adhesion molecules govern leukocyte recruitment, chemical mediators are important drug targets and resolution is an active and coordinated process (not passive).