TABLE OF CONTENTS:

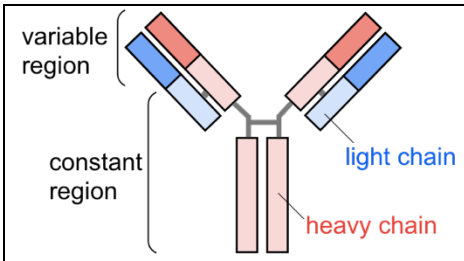
Note to reader: Drugs and drug classes are highlighted in green and bolded (e.g. **Paracetamol**). If a drug is withdrawn from the market (e.g. due to safety issues), then it will be grey and bolded (e.g. **Rofecoxib**).

LECTURE 5: Therapeutic Antibodies 06/03/18

Revision of Antibodies:

Structure of Antibodies:

- An antibody is a protein with **2 heavy chains** and **2 light chains**, each with variable and constant regions:



- **Variable region**: Contains antigen-binding site
  - The **complementarity determining regions** (**CDRs**) are the parts of the variable region that actually bind to the antigen and provide specificity for the antigen.
- **Constant region**: Contains effector functions
  - The antibody's isotype is determined by the *constant region* of the *heavy* chain.

Functions of Antibodies:

1. Recognition: The BCR is a membrane-bound antibody! The BCR's role is to recognise a specific antigen.
2. Neutralisation: Antibodies can bind to toxins in a way that prevents them from exerting its toxic effects.
3. Complement: Antibodies can activate the **complement cascade** (a series of enzyme reactions that lead to the formation of a **membrane-attack complex**, which causes lysis of cellular pathogens).
4. Opsonisation: IgM and IgG antibodies can opsonise (coat) pathogens. Opsonisation flags pathogens to phagocytes for phagocytosis.
5. Protection: Antibodies have long half-lives, thus providing long-lived protection. Thus, if we use antibodies as therapeutics, then we do not need to administer the drug frequently.
6. Medical uses: Antibodies can be used as therapeutics, for diagnostic imaging, as drug delivery systems

Polyclonal Versus Monoclonal Response:

- The immune response that occurs in our body is **polyclonal**, because it involves many different B cell clones, each recognising different epitopes.
  - The benefits of a polyclonal response is if there are multiple epitopes involved (e.g. snake venom contains many proteins), so you want broad coverage to protect you against all the possible toxins.
- In contrast, a **monoclonal** response involves binding to only *one* single epitope. Many antibody-based drugs work in this way, providing a more uniform response.

Monoclonal Antibodies (mAbs) as Therapeutics:

Production of Monoclonal Antibodies:

- **Monoclonal antibodies** (**mAb**) need to be produced on a large scale, if they are to be used as therapies. Biotechnology makes it possible for large-scale production to occur (e.g. hybridoma technology).

Hybridoma Technology:

1. The antigen is administered to the mouse. Since the antigen has many epitopes, a large number of B cells respond, proliferate and differentiate into plasma B cells.
2. The plasma B cell that produces protective antibodies must be identified.
3. The plasma B cell that produces protective antibodies is fused with a multiple myeloma cell. The plasma B cell provides Ig genes. The multiple myeloma cell provides immortality, but lacks Ig genes.
4. The fused cells are grown on a **HAT** (**hypoxanthine**, **aminopterin**, **thymidine**) medium, which selects for fusions between plasma B cells and multiple myeloma cells (i.e. **hybridomas**).

Problems with Antibodies as Therapeutics:

- ***Problem #1***: Mouse antibodies are recognised as foreign by the human immune system. With repeated exposure, humans are likely to develop **human anti-mouse antibodies** (**HAMA**).

LECTURE 8: Drugs to Treat Allergic Inflammation 13/03/18

- Some allergies are mediated by the **IgE pathway**. Affected persons make IgE against innocuous antigens (**allergens**). Elevated allergen-specific IgE levels (**atopy**) is characteristic of many allergic diseases (e.g. allergic rhinitis/ hay fever, allergic dermatitis, asthma, anaphylaxis
  - *Thunderstorm asthma*: Many people have IgE specific for rye grass. Normally, rye grass allergens do not penetrate into the airways, so it stays in the upper respiratory tract, causing nasal problems. But fragmentation of the pollen (by the storm process) allows it to penetrate into the airways, where it activates mast cells in the airways, producing severe bronchospasm.
  - *Anaphylactic food allergies*: Some people have IgE specific for food antigens (e.g. peanuts). Ingested peanuts spread and cause systemic degranulation of mast cells, resulting in anaphylaxis.

Mast Cells:

- Structure: The mast cell contains many granules in the cytoplasm, which are filled with bioactive mediators (e.g. histamine, proteases). If the mast cell is activated, degranulation occurs (where the granules fuse with the plasma membrane) and these mediators are released. These mediators can affect vascular tone (vasodilation), affect nerve sensations to pain and affect secretions by glands.
- Location: Mast cells occur in connective tissues. Mast cells are particularly prevalent at body sites that are in contact with the external environment (e.g. skin, gastrointestinal tract, lung).
- Physiological role: Do mast cells have a physiological role? Or is there only a pathophysiological role?
  - ***Research***: An experiment compared WT mice, with mast cell-KO mice. Venom was introduced in the mice. Mast cells were found to be protective against the venom, since the venom's responses were reduced in the WT mice. Mast cells released proteases that digested proteins in venom.

Mediators Released by Mast Cell:

Granular Mediators:	*De Novo* Mediators:	Transcriptional Regulation Mediators:	Membrane-Packet Mediators:
▪ **Histamine** ▪ **Tryptase**, **chymase** ▪ **Cytokines**	▪ **LTC_4** ▪ **PGD_2**	▪ **Cytokines** ▪ **Chemokines**	▪ **Exosomes**: Small vesicles that can be released

Activation of Mast Cells and Its Role in Allergies:

- Many stimuli can activate mast cells, including:
  - Antigen binding to IgE
  - Complement
  - Neuropeptides
  - Cytokines
  - Bacterial components
  - Physical trauma

IgE Pathway for Mast Cell Activation:

1. Sensitisation: IgE binds to the α-subunit of the FcεR1 receptor. The **FcεR1 receptor** has a high affinity for IgE. If IgE is present, then mast cells will be coated with IgE molecules. Once coated, the mast cell is said to be **sensitised** to the antigen that those IgE molecules bind to.
2. Degranulation: Allergen binds to IgE. The allergen is **polyvalent** (meaning it has many binding sites). Thus, it can bind multiple IgE, causing cross-linking of FcεR1 receptors, which causes degranulation.
3. Further release of mediators: Release of eicosanoids and cytokines (this is slower than degranulation).

FcεR1 Receptor:

- FcεR1 receptor has three subunits: α, β, γ
  - **α-subunit**: Binds to IgE. This subunit is heavily glycosylated – these sugar residues are thought to be important in preventing non-antigen induced cross-linking of FcεR1 receptors.
  - **β-subunit**, **γ-subunit** (γ-subunit exists as a dimer): No intrinsic kinase activity. These subunits have motifs in their cytoplasmic domains, which serves to recruit tyrosine kinases.
    - These motifs are called **ITAMs** (**immunoreceptor tyrosine-based activation motifs**).
    - ITAMs have a consensus sequence that has at least one tyrosine residue.

LECTURE 27: Drugs to Treat Hypertension 07/05/18

Anti-Hypertensive Drugs:

- Treatment for hypertension is long-term to decrease cardiovascular risk. The drug choice depends on the patient (e.g. age, side effects, concomitant disease).

Drug:	**Mechanism/ Therapeutic Effects**:	**Adverse Effects**:	**Indications/ Contraindications**:	**Notes**:
Class: β_1-blockers **Propranolol** **Atenolol**	• Inhibits β_1-receptors on nodal cells, which decreases HR. • Inhibits β_1-receptors on cardiac myocytes, which decreases the force of contraction and decreases SV. • Above effects result in reduced CO, and hence reduced blood pressure. • Inhibits β_1-mediated release of renin from juxtaglomerular cells in the kidney. Therapeutic Effects: • Lowers blood pressure *gradually*, which is good because it gives the body more time to reset the set-point.	• *Bradycardia*: Due to β_1 blockage on nodal cells • *Bronchoconstriction*: Due to β_2 blockage on bronchial smooth muscle. • *Dizziness, insomnia*: Especially the lipophilic drugs that can enter the CNS. • *Fatigability*: Due to β_2 blockage on blood vessels supplying skeletal muscle, which means that vasodilation to supply skeletal muscle does not occur easily when needed (e.g. during exercise). Also due to β_1 blockage on heart, which lessens the ability to raise blood pressure when needed. • *Cardiac depression*: Due to β_1 blockage on heart, which lessens the ability to raise blood pressure. • *Cold extremity*: Due to β_2 blockage on cutaneous blood vessels, reducing circulation to skin.	Contraindications: • *Poorly controlled diabetes*: β_1 blockers suppress warning symptoms of hypoglycaemia (e.g. tachycardia). • *Asthma*: β_1 blockers, particularly non-selective, can prevent β_2-mediated bronchodilation. • *Sinoatrial or atrioventricular nodal dysfunction*: β_1 blockers exacerbate these conditions • *Heart failure*: β_1 blockers should not be used as an initial therapy for patients with heart failure • *Peripheral vascular disease*	Drug Selection: • Selective β_1 blockers are preferred • Hydrophilic β_1 blockers are preferred over lipophilic (to minimise CNS side effects). Drug Withdrawal: • In response to β_1 blockers, the body upregulates β_1 receptor expression. Thus, if β_1 blockers are withdrawn, they need to be withdrawn slowly, because the patient will be sensitive to β_1 agonists (e.g. endogenous NA, endogenous ADR). • To avoid rebound hypertension, the dose of β_1 blocker is tapered.
Class: Thiazides **Chlorthalidone** **Hydrochlorothiazide**	• Inhibits C_1 (NaCl cotransporter) in DCT. Normally, Na^+ is reabsorbed in DCT via C_1 and Na^+/K^+ ATPase. However, blocking C_1 prevents Na^+ reabsorption from occurring, so Na^+ remains in the DCT. Via osmosis, more water remains in the DCT as well. • Thiazides activate K_{ATP} channels on blood vessels, causing vasodilation. Therapeutic Effects: • Inhibition of Na^+ reabsorption does not produce long-term antihypertensive effects because compensatory mechanisms. It is through K_{ATP} channels, that there is a maintained anti-hypertensive effect.	• *Impaired glucose tolerance*: Due to activation of K_{ATP} channels in islet cells of pancreas, inhibiting insulin secretion. • *Uric acid retention*: Since thiazides compete with uric acid for tubular secretion in the kidney. • *Allergic reactions*: Some people are allergic to thiazides. • *Hypokalaemia*: Due to more Na^+ arriving at the collecting duct, which promotes more Na^+ reabsorption occurs in the collecting duct, which promotes K^+ loss in the collecting duct.	Indications: • Recommended primary therapy for uncomplicated hypertension • Suitable for elderly (> 55 years) • Suitable for volume-based hypertension. Contraindications: • *Gout*: Due to thiazide's uric acid retention effects. • *Pregnancy*: Due to impaired placental perfusion.	Duration of Action: • Long duration of action.

LECTURE 29: Drugs to Treat Angina 10/05/18

What is Angina?

- **Angina pectoris**: Caused by ischaemic heart disease (e.g. obstruction of coronary arteries due to atherosclerosis), where there is reduced perfusion to the heart to fails to meet cardiac demand. This poor perfusion of the heart registers as chest pain.
  - Hypoxia results in the production of algesic substances (e.g. adenosine, lactic acid) that cause pain.

Oxygen Supply Versus Oxygen Demand:

- Angina can be conceptualised as a situation when O_2 demand is greater than the O_2 supply.

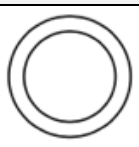
Oxygen supply:	**Oxygen demand**:
• **Oxygen supply** depends on coronary artery flow, where coronary arteries supply blood to heart. • To increase oxygen supply: **1.** Dilate coronary arteries **2.** Decrease the heart rate, so that the heart spends less time in systole. • During systole (when ventricles contract), parts of coronary arteries that are embedded in endocardium are compressed, which reduces perfusion.	• **Oxygen demand** depends on cardiac workload. If the heart is not working as hard, then it doesn't need as much oxygen. • To decrease oxygen demand: **1.** Reduce cardiac output: i.e. reduce HR, reduce SV or reduce both! **2.** Reduce preload (the stretch of the heart from venous return) **3.** Reduce afterload (the resistance that the heart pumps against)

Different Forms of Angina:

- **Stable angina** (*classic or effort angina*): Chest pain occurs during exertion or stress, where there is increased sympathetic tone (i.e. NA), increased heart rate and increased contractility (i.e. increased O_2 demand). This is associated with coronary artery disease (i.e. decreased O_2 supply).
- **Variant angina** (*vasospastic or Prinzmetal's angina*): Chest pain can occur whenever (*at rest* or during exertion) due to coronary vasospasm. The mediator is unknown and hard to predict.
- **Unstable angina** (*crescendo or rest angina*): Chest pain can occur whenever (*at rest* or during exertion). There is a crescendo of pain. There is complete occlusion of coronary arteries and myocardial infarction. There is potential for thrombus formation.

Stable Angina (Classic or Effort Angina):

- Healthy: During exertion (i.e. increased O_2 demand), there is increased sympathetic tone and increased ADR. The arteries and arteries dilate to increase supply to the myocardium (i.e. increase the O_2 supply).
- Angina: In angina, arterioles are already dilated to compensate for the partially occluded artery. Dilation of arterioles occurs from production of local mediators (not neural control). During exercise (i.e. increased O_2 demand), the arterioles cannot dilate much further, leading to hypoxia and pain (**acute angina attack**).

	Healthy:		**Angina:**	
	Rest	Exercise	Rest	Exercise
Artery				
Arteriole				
O_2 demand	+	+++	+	+++
Blood flow	+	+++	+	+

REVISION CHECKLIST FOR BLOCK 4:

Block 4: Respiratory Diseases

L #:	Content:	
Lecture 21	• Describe cells that are involved in the innate immune system and those involved in the adaptive immune system. What are the roles of each cell?	
	• Explain the different roles of MHC I and MHC II. What cells express MHC?	
	• Describe how cells in the innate immune system are activated (e.g. PAMPs).	
	- Understand that the activation of innate cells activates the inflammatory response.	
	- Understand that in disease states, if inflammation does not resolve, then this can lead to remodelling, which affects the function of the lung.	
	• Understand the pathophysiology and treatments of the different forms of asthma (e.g. allergic, non-allergic, neutrophilic).	
Lecture 19	• Understand the structure of the airways. Explain the function of the conduction zone and respiratory zone.	
	- Define airway resistance.	
	○ Explain why airway resistance is higher in larger airways than smaller airways.	
	• Describe the cross-section of the airways. Be able to draw a diagram.	
	• Understand the location, structure and function of airway smooth muscle.	
	- Define bronchial thermoplasty. What were the results of this therapy?	
	• Explain the physiology of airway smooth muscle contraction.	
	- What are examples of bronchoconstrictors? Explain the pathway for constriction.	
	- What are examples of bronchodilators? Explain the pathway for dilation.	
	• Compare and contrast asthma and COPD with respect to bronchodilator response.	
Lecture 20	• Define asthma. What are the risk factors for the development of asthma?	
	• Explain the pathophysiology of asthma.	
	- Describe the structural aspects of asthma.	
	- Describe the effects of bronchoconstrictor and bronchodilator in a person with asthma.	
	- Describe the cellular pathology in asthma (e.g. APCs, T cells, B cells).	
	• Describe the mechanism, therapeutic effects, adverse effects and indications of various drugs: SABAs, LABAs, CTRAs, glucocorticoids, omalizumab, dupilimumab, mepolizumab, azithromycin	
Lecture 22	• Define cystic fibrosis. What is the genetic defect? What are the signs and symptoms?	
	• Explain the pathophysiology of cystic fibrosis.	
	- Describe the protein that is affected in cystic fibrosis.	
	○ What are the different classes of CFTR mutations?	
	○ Explain the possible reason why there is a higher incidence in Caucasians.	
	- Describe the effect of absent or dysfunctional CFTR in the airways.	
	○ What happens in the lung?	
	○ What happens in the gastrointestinal tract?	
	- Describe the vicious cycle of infection and inflammation.	
	• Describe the mechanism, therapeutic effects, adverse effects and indications of various drugs: physiotherapy, mucolytics, pancreatic enzymes, azithromycin, aztreonam, ibuprofen, prednisolone, ivacaftor, ivacaftor/lumacaftor	
Lecture 23	• Define COPD. What are the 3 disease sub-groups in COPD?	
	• Explain the pathophysiology of COPD:	
	- Describe what happens in emphysema, and the various consequences on lung.	
	- Describe various factors that can lead to emphysema.	
	- Why are COPD patients susceptible to chest infections and cancer?	
	• What is meant by the COPD clock?	