TABLE OF CONTENTS:

Notes to reader:

- Drugs and drug classes are highlighted in green and bolded (e.g. **Infliximab**).
- Drugs that are not approved for clinical use, are highlighted in grey and bolded (e.g. **Theralizumab**).

LECTURE 1-3 B-Cell Biology 28/02/18, 01/03/18, 02/03/18

Primary Immunodeficiency (PID):

- **Primary immunodeficiencies** (**PIDs**): A group of rare disorders characterised by an impaired ability to produce immune responses. Hence, people with PIDs can have recurrent/ severe infections. Most PIDs have genetic causes. PIDs are *not* caused by other diseases (e.g. AIDS), treatments or toxins.
- **Secondary immunodeficiency**: An impairment in the immune system due to environmental factors.

Two Classes of Primary Immunodeficiencies:

1. **Antibody deficiency** (e.g. XLA, HIGM, CVID): A PID where there is loss of humoral immunity (but cellular immunity is intact). In antibody deficiencies, B cells are affected (i.e. B cells may be absent, reduced in number, have developmental defects, or have differentiation defects).
2. **Combined immunodeficiency** (e.g. SCID): A PID where there is a combined loss of humoral and cellular immunity. In combined immunodeficiencies, either: **1)** Only T cells are affected or **2)** Both T cells *and* B cells can be affected. *Why would defects in T cells alone cause defects in both humoral and cellular immunity?* Because **$CD4^+$** (**helper**) **T cells** *regulate* B cells.

Possible Reasons for Failing to Respond to an Infection:

- Lack of B cells (e.g. in XLA)
- Lack of T cells (e.g. in SCID)
- Lack of T cells *and* B cells (e.g. in SCID)
- Failure of $CD4^+$ T cells to give signals to B cells
- Failure of B cells to respond to signals from $CD4^+$ T cells
- Failure of B cells to respond to antigen binding

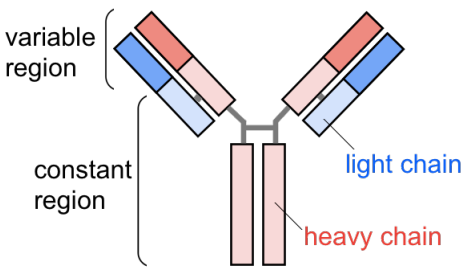
Treatment for Primary Immunodeficiencies:

- Severe PID: In severe forms of PID (e.g. SCID), where the immune system is largely missing, we can replace the whole immune system with **haematopoietic stem cell transplantation** (**HSCT**).
  - In a HSCT, a person receives **haematopoietic stem cells** (**HSCs**) from a healthy donor to replace their immune system. HSCs are the cells that give rise to blood cells (e.g. B cells, T cells).
  - Other treatments could include **gene therapy**.
- Less severe PID: In less severe forms of PID (e.g. XLA, HIGM, CVID) where people have *some* of their immune system, it is not appropriate to do HSCT because we would need to use immunosuppressive treatments to manage the risk of graft-versus-host disease.
  - Other treatments include **intravenous Ig** (IVIG) or **subcutaneous Ig** (SCIG) to replace antibodies.
  - Other treatments could include gene therapy.

Antibodies:

- **Antibodies** (or **immunoglobulins** (**Ig**)) are produced by **plasma B cells**. The function of antibodies is to bind *specific* epitopes to neutralise foreign pathogens.
- The structure of an antibody has two **heavy chains** and two **light chains**:

Note: The light chain can be κ or λ.



- **Variable regions** have antigen-binding functions. The tips of an antibody are the **hypervariable regions**, which are extremely variable (allowing for millions of different antigen-binding sites to exist).
  - Each antibody binds to a single kind of **epitope** on an **antigen** (**Ag**). Different antibodies have *different* variable regions that recognise *different* antigens.
- **Constant regions** have effector functions. An antibody's **isotype** (**class**) is defined by the constant region of the antibody's *heavy chain*.
  - Each isotype has different effector functions.

LECTURE 6 Autoimmunity 08/03/18

Tolerance:

- ***Recall***: Antigen receptor gene rearrangement is random. So it is not surprising that some of the produced antigen receptors can recognise self. These antigen receptors are termed **self-reactive** (autoreactive).
- **Tolerance** describes the suppression of self-reactive lymphocytes via deletion (apoptosis) or silencing.

B-Cell Central Tolerance (In Bone Marrow):

- Following gene rearrangement and success signalling, BCRs are checked for self-reactivity. This may be paradoxical, but all BCRs must show some degree of self-reactivity, otherwise they will die by neglect. But BCRs cannot be *too* self-reactive, otherwise that B cell will die by **deletional tolerance**. B cells with highly self-reactive BCRs have another chance to escape deletional tolerance by **receptor editing**.
- Some B cells with self-reactive BCRs, but do not become deleted and are able to leave the periphery.
  - ***Note***: Self-reactive B cells are normally not a problem for most of us, unless genes/environment cause the activation of these cells to cause autoimmune disease.
- Some B cells have BCRs that are **poly-reactive**, meaning that the BCRs can recognise multiple antigens. Poly-reactivity is associated with autoantibodies, so these B cells tend to also be removed.

T-Cell Central Tolerance (In Thymus):

- Following gene rearrangement and production, TCRs are checked for self-reactivity. Again, all TCRs must show some degree of self-reactivity to avoid death by neglect. But TCRs cannot be too self-reactive, otherwise they will be removed by **deletional tolerance**.
  - Strongly self-reactive CD4+ T cells become **regulatory T ($\mathbf{T_{reg}}$) cells**. These T_{reg} cells dampen down immune responses, protecting against autoimmunity.
  - Since B cells depend on T cells, T cell tolerance is particularly important in preventing autoimmunity.
- How are T cells screened for self-reactivity? To check for self-reactivity, T cells are exposed to a vast array of self-antigens. The transcription factor, **AIRE** (**autoimmune regulator**), activates the expression of many genes in the thymus (e.g. genes from the pancreas), which provides the opportunity for self-reactive T cells to be exposed to many self-antigens.

Mechanisms of Tolerance:

- Tolerance can occur through several mechanisms:
  1. **Deletion**: Apoptosis, which tends to be the fate for high-affinity self-reactive B and T cells.
  2. **Anergy**: Functional silencing, which tends to be the fate for lower-affinity self-reactive B and T cells.
  3. **Exhaustion**: Rendered silencing after extensive proliferation. This extensive proliferation occurs due to an undiminishing antigen load (e.g. organ, over-proliferating tumour).

Tolerance is Imperfect…Intentionally:

- B and T cells depend on weak signals through their BCRs / TCRs for survival (**positive selection**). If B and T cells do not have enough self-reactivity, then B and T cells cannot generate a survival signal, and therefore die by neglect.
- B and T cells with high-affinity self-reactive BCRs / TCRs are deleted (**negative selection**).
- B and T cells with lower-affinity self-reactive BCRs / TCRs become anergic. While it can be dangerous to have self-reactive B and T cells, it may be advantageous because it limits the extent to which pathogens escape the immune system by displaying antigens that resemble our antigens.

Autoimmune Disease:

- Self-reactive T and B cells exist because tolerance is imperfect. This can lead to autoimmune diseases (e.g. rheumatoid arthritis, psoriasis, Crohn's disease).

LECTURE 7 Disease Manifestations of Rheumatoid Arthritis 14/03/18

Introduction to Rheumatoid Arthritis:

- **Arthritis**: An umbrella term encompassing *over 100* subtypes, each characterised by the inflammation of the joints. Hallmarks of arthritis include pain, stiffness and swelling. 95% of arthritis cases are due to osteoarthritis, rheumatoid arthritis and gout.
  - **Osteoarthritis**: The subtype of arthritis that is associated with ageing.
  - **Rheumatoid arthritis** (**RA**): A chronic inflammatory autoimmune disease (of unknown aetiology).
    - Without treatment, RA causes progressive disability, reduced life expectancy and socioeconomic costs (e.g. direct costs of medical care, indirect costs of missing work due to illness).

Onset of Rheumatoid Arthritis:

- ***Note***: Morning stiffness is one of first hallmark features of rheumatoid arthritis.

Onset:	**Description:**
Insidious	• The onset of articular manifestations occurs over weeks to months. **Morning stiffness** that lasts for over an hour. This is due to inflammatory exudate that builds up over night (due to lack of movement), which gives a sense of stiffness. As time goes on, patients notice pain and swelling developing.
Intermediate	• The onset of articular manifestations occurs over days to weeks.
Acute	• The onset of articular manifestations occurs within a few days. - Patients describe when it suddenly manifested and rapidly progressed.

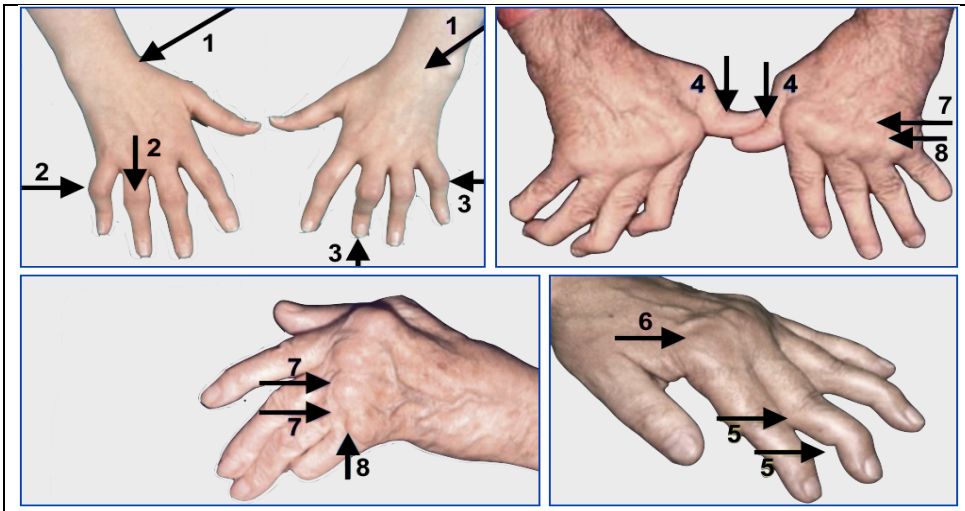
Clinical Features (Manifestations) of Rheumatoid Arthritis:

Articular Manifestations of Rheumatoid Arthritis:

- In RA, the distribution of the involved joints is:
  1. Symmetrical: If one hand is affected, the other hand tends to be affected in a symmetrical fashion.
  2. Commonly affected joints: In RA, small joints are particularly affected.
  3. Characteristic deformities: In RA, deformities can develop when inflammation is untreated.
     - Radial deviation of wrist
     - Ulnar deviation of MCP
     - MCP subluxation
     - Boutonniere's deformity
     - Swan neck deformity
     - Z-deformity

Characteristic Deformities in Rheumatoid Arthritis:

- ***Review BIOM20002***: The small joints in the hand include metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints and distal interphalangeal (DIP) joints. The small joints in the foot include the metatarsophalangeal (MTP) joints. These are all the joints you need to know for RA.
- Hand deformities in RA:



1. Radial deviation (wrist)
2. PIP fusiform swelling
3. Boutonniere deformity
4. Z deformity
5. Swan neck deformity
6. MCP swelling
7. Ulnar deviation (MCP)
8. MCP subluxation

LECTURE 8 Synovium in Health Versus Disease 15/03/18

Introduction to Synovitis in Rheumatoid Arthritis:

- ***Note***: The primary manifestation of RA is **synovitis**. In synovitis, the synovium becomes hypertrophied, becomes infiltrated with inflammatory cells (e.g. fibroblasts, macrophages) and becomes inflamed. The inflamed synovium is called **pannus**. Pannus erodes material in its way, leading to erosion of articular cartilage and bone (*which was observed in X-rays from Lecture 7*).

Structure and Function of the Synovium:

- The **synovium** is the thin membrane that lines the joint capsule of diarthrodial joints. This membrane extends from the interface between cartilage and bone). This membrane consists of two layers:
  1. **Intima** (innermost layer), which contains **synoviocytes** and is 1-3 cells thick.
  2. **Subintima**, which contains connective tissue/ **extracellular matrix** (**ECM**), and has blood vessels, lymphatic vessels and nerves. It is normally acellular.
- There are two distinct populations of synoviocytes:

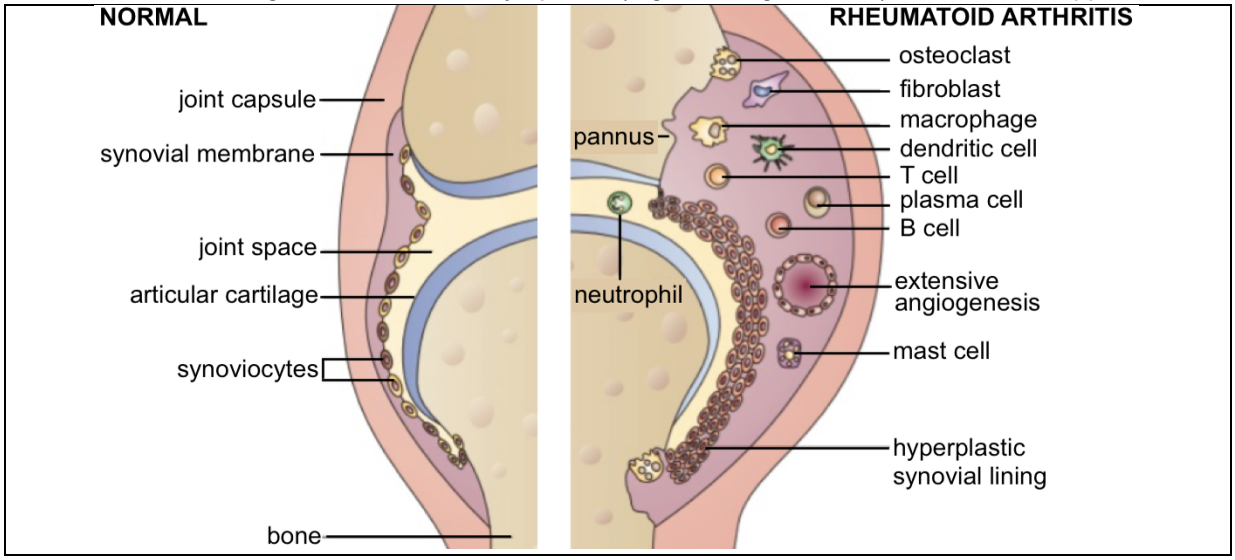
Fibroblast-like synoviocytes (**Type B**): 80%	**Macrophage-like synoviocytes** (**Type A**): 20%
• Type B (synovial fibroblast): - Mesenchymal in origin. • Produces hyaluronan, lubricin: - Joint lubrication • Produces collagen and fibronectin: - ECM is important for cell adherence and the formation of subintima.	• Type A (synovial macrophages): - Basically tissue-resident macrophages. - Are derived from blood mononuclear cells • Phagocytic: Clearance of debris in joint • Express receptors for Fc domains of IgG, so they can recognise immune complexes

  - In healthy joints, Type A synoviocytes are not pro-inflammatory and constantly produce cytokines.
- The joint capsule contains **synovial fluid**, which allows chondrocytes to receive nourishment.
- The function of a synovium is to facilitate the movement of a joint. The synovium is deformable with non-adherent properties (so it does not stick to bone or cartilage). Synovial fluid contains two main lubricants:

Hyaluronan (part of synovial fluid):	**Lubricin** (part of synovial fluid):
• High molecular weight polysaccharide - Viscous - Provides shock absorption - Prevents fluid loss from the joint space	• Mucin-like proteoglycan - Protects cartilage surfaces from protein deposition and cell adhesion, - Inhibits synovial cell overgrowth.

State of Synovium in Rheumatoid Arthritis:

- ***Note***: Inflammation begins before clinical symptoms (e.g. morning stiffness) occur. What happens in RA?



- In RA, there is hyperplasia of Type B and Type A synoviocytes within the intima. Synoviocytes become activated and release factors into the fluid and subintima.

LECTURE 15 Clinical Aspects of Muscular Dystrophy 29/03/18

Dystrophinopathies:

- **Duchenne muscular dystrophy** (**DMD**) is the most common form of muscular dystrophy. It is caused by frameshift mutations, resulting in lack of dystrophin in the muscle. **Becker muscular dystrophy** (**BMD**) is less common and caused by in-frame mutations, resulting in less functional dystrophin.
- There are other forms of dystrophinopathies (e.g. X-linked cardiomyopathy, X-linked cramps-myalgia syndrome, isolated quadriceps myopathy). Females typically do not manifest signs and symptoms, but, there are instances of **manifesting carrier females**, who experience some muscle weakness.
  - ***Clinic***: A cardiology assessment is done for female carriers since they are at risk of cardiomyopathy.

Onset:

- ***Note***: DMD used to be a little more frequent, however it is less frequent with genetic counselling.

	DMD:	**BMD**:
Onset	Less than 5 years	Over 5 years (very variable). Some people remain asymptomatic for many years.
Diagnosis	Typically 2 to 4 years	
Incidence	1 in 5,000 (most common MD)	1 in 35,000 births
Wheelchair	Less than 13 years	More than 16 years (perhaps 30s, 40s, 50s)

Clinical Presentations (Symptoms) of Duchenne Muscular Dystrophy:

- Parents and guardians may notice the majority of the following symptoms:
  1. Delayed motor milestones (***Note***: The normal age of walking is ≤18 months)
  2. Gait difficulties: broad-based, waddling gait, proximal weakness, truncal weakness
     - Trouble climbing steps, persistent toe walking and flat feet
  3. Trouble getting up off the ground: May use Gower's manoeuvre to get up off the ground
  4. Fatigability, frequent falls, inability to run or keep up with peers
  5. Calf cramps, thigh cramps, muscle enlargement (pseudo-hypertrophy)
  6. Speech delay, learning problems
     - There is increased incidence of ADHD and autism in children with DMD.

Physical Examination by Clinician:

- Waddling gait: Clinician asks the child to walk up and down the corridor.
- Proximal weakness: Weakness occurs particularly around the lower limbs, hip girdle, shoulder girdle
- Positive Gower's sign: Clinician asks the child to lie flat on the ground and get up without using the hands. Normally, you should be able to sit up and get up, but DMD boys struggle.
- Enlarged, rubbery muscles: There is muscle 'hypertrophy' of calves (classic). There may or may not be muscle 'hypertrophy' of quadriceps, gluteals, tongue, brachioradialis, deltoid.
  - There is increased muscle, but it is not more of normal muscle, it is more of abnormal muscle.
- Lumbar lordosis (sway back): This occurs because of lack of strength around the hip girdle and trunk.
- Weak neck flexors: Clinician asks the child to lie flat on a bed with their neck over the edge and to bring their head up. DMD boys cannot bring their head up because of weakness in neck flexors.
- Facial muscles *spared*: Some muscular myopathies have severe muscle weakness of the face, however the facial muscles in DMD are normal. DMD boys can smile normally.
- Extra-ocular muscles *always spared*: Some muscular dystrophies affect eye movement (e.g. FSH), but DMD boys can close their eyes normally.

Gower's Sign:

- The **Gower's manoeuvre** is used because of truncal and proximal weakness. It is an indication of truncal and proximal weakness, which is not unique to DMD and BMD. It can be a sign of other muscle disorders.

LECTURE 16 Other Muscular Dystrophies 09/04/18

Importance of Making a Diagnosis:

- Prognosis: To know the likely disease course (e.g. life expectancy, level of independence).
- Monitoring: To enable monitoring of likely complications (e.g. respiratory, endocrine). A correct diagnosis also minimises doctor's visits by not wasting time monitoring for things that the patient is not at risk for.
- Treatment: To ensure that treatment is appropriate (and to avoid inappropriate tretments).
- Genetic: To enable genetic counselling about the risk of having another child with muscular dystrophy. It could involve counselling other family members (e.g. siblings of the parents) about their risk.
  - Genetic counselling allows parents to have options (e.g. pre-implantation testing, prenatal testing).
  - Genetic counselling can lead to **cascade diagnosis**, where the diagnosis of one person leads to the recognition and diagnosis of other family members.

How Do We Classify Muscular Dystrophies:

- The classification of muscular dystrophies is made based on:
  - Age of onset (e.g. infantile, adult onset)
  - Pattern of inheritance (dominant, recessive)
  - Pattern of weakness (e.g. generalised, focal)
  - Involvement of other systems
  - Specific abnormalities on muscle biopsy
  - Genetic testing for the causative gene

Normal Versus Dystrophic Muscle:

- Generally, muscle biopsies are done only if the genetic test is inconclusive. In 50% of cases, we do not find the cause for congenital muscular dystrophies, because those genes are yet to be identified.

Normal muscle:	**Dystrophic muscle**:

• Regular fibre size (low variation in fibre size)
• Peripheral nuclei
  - <4% of fibres may show central nuclei
• No increase in connective tissue or fat
	• Increased fibre size variation
	  - Fibre regeneration (darker stained areas)
	• Increase in central nuclei
	• Increase scar tissue (fibrosis)
	• Increased fat (white areas: no staining)

Immunohistochemical Staining with Fluorescent Antibodies:

- Use an antibody that binds to a sarcolemmal protein (e.g. dystrophin, lamin). The clinician will give the pathologist an idea of what condition is suspected (e.g. EDMD, DMD), so that the pathologist has some idea of what stain to test for first (e.g. emerin, dystrophin).
  - If the pathologist observes no staining, then that protein must be absent.
  - If the pathologist observes patchy or decreased staining, then that protein must be decreased. It could be a primary abnormality (because that protein is defective in the patient), or a secondary abnormality (because the structure that is anchoring that protein in place is absent).

Involvement of Other Systems:

- Brain:
  - Abnormalities of brain development
  - Cognitive abnormalities
- Eye:
  - Structural or retinal abnormalities
  - Cataracts
- Musculoskeletal: Due to muscle weakness itself
  - Spinal rigidity (loss of flexion, or extension)
  - Scoliosis
  - Joint contractures (e.g. Achilles, iliotibial band, elbow, wrist)
- Endocrine

FKRP Gene Mutations Cause Congenital Muscular Dystrophy, Mental Retardation and Cerebellar Cysts:

- Abnormal white matter development
- Calcification
- Cerebellar cysts
- Eye movement abnormalities
- Facial muscle weakness
- Cognitive problems
- Marked loss of muscle bulk
- Contractures

REVISION CHECKLIST FOR MODULE 1, MODULE 2 & MODULE 3:

About MST 1:

- In 2018, MST 1 ran for 50 minutes (5 minutes reading time, 45 minutes writing time). It was worth 20% of our final mark. Just as you may have experienced in BIOM20001 and BIOM20002, you do need to devote some time to *memorisation* as well as devote time to *understanding* the content. This is not a subject that you can bludge and do well in.
- ***Note***: The order of modules can differ each year. In 2018, the first two modules were B-cell diseases and rheumatoid arthritis, but in the past other modules were done first. So don't assume that your MST 1 will involve the same modules as the 2018 MST 1.

Class Results from MST #1:

- In 2018, one of the questions on MST 1 was removed after an analysis revealed that most students were guessing on that question. Consequently, our final marks were out of 39 instead of 40.
- In 2018, the average mark was 29.18 / 39 and the median mark was 30 / 39.

Preparing for MST 1 and Exam:

Revision Checklist for Module 1 (B-Cell Diseases):

- Here's a checklist:

From:	Content:	
Lecture 1-3	• What are primary immunodeficiencies? How do primary immunodeficiencies differ from secondary immunodeficiencies?	
	• What are the two classes of primary immunodeficiencies and how do they differ?	
	• What are the treatment options for patients with immunodeficiencies?	
	• Explain four reasons why immunoglobulin diversity exists.	
	• Explain the key processes involved in B cell development in the bone marrow.	
	- Explain somatic recombination and the steps involved.	
	○ Explain mutations involved in somatic recombination that can lead to PIDs.	
	○ Explain the incidence, symptoms, diagnosis, and treatment of SCID.	
	- Explain 'success signalling' and the steps involved.	
	○ Explain mutations involved in 'success signalling' that can lead to PIDs.	
	○ Explain the symptoms, diagnosis, and treatment of XLA.	
	- Explain central tolerance.	
	• Explain the key processes involved in B cell development in the periphery.	
	- Explain what occurs during the primary and secondary immune response.	
	- Explain somatic hypermutation and the steps involved.	
	○ Explain affinity maturation.	
	- Explain class-switch recombination (isotype switching) and the steps involved.	
	- Explain mutations in B cell differentiation (i.e. mutations in T cell help, signalling pathways) that can result in PIDs.	
	○ Explain the symptoms, diagnosis and treatment of HIGM.	
	○ Explain the incidence, diagnosis and treatment of CVID.	
Lecture 4	• What is a monoclonal response? What is a polyclonal response?	
	• How are polyclonal antibodies used as therapeutics? What are their advantages and what are their disadvantages?	
	• How are monoclonal antibodies made?	
	• Explain how hybridoma technology works.	
	• What are mouse monoclonal antibodies? What is their representative ending (suffix) in drug nomenclature? What are their limitations?	