Fundamentals of Immunology Notes

1.3 Pathogen Varieties

- Different types of pathogens:
  - Virus: Unstructured DNA, Protein Coating.
  - Bacteria.
  - Fungus.
  - Unicellular Eukaryotes.
  - Multicellular Worm.

1.4 Pathogen Recognition

- Neutrophils attach to bacteria prior to phagocytizing them.
- Lysozyme, an enzyme that cuts up bacterial cell wall peptidoglycan.
- Pore in pathogenic membrane constructed by complement MAC.
- Leucine-rich hook domain found in many pattern recognition receptors.
- Properties of Innate vs Adaptive Defenses:

Innate	Fast: Minutes after exposure	Always there	Recognizes patterns: types of molecules that a pathogen might have and you would not have	Neutrophils, macrophages, NK cells, proteins, barriers
Adaptive	Slower: approximately two weeks after first exposure and three days after subsequent exposure	requires gene rearrangement	recognizes specific parts of proteins characteristic of a pathogen	B cells, antibodies, T_C cells, T_H cells.

1.7 Innate vs Defensive Responses

- Responding to foreign antigen:

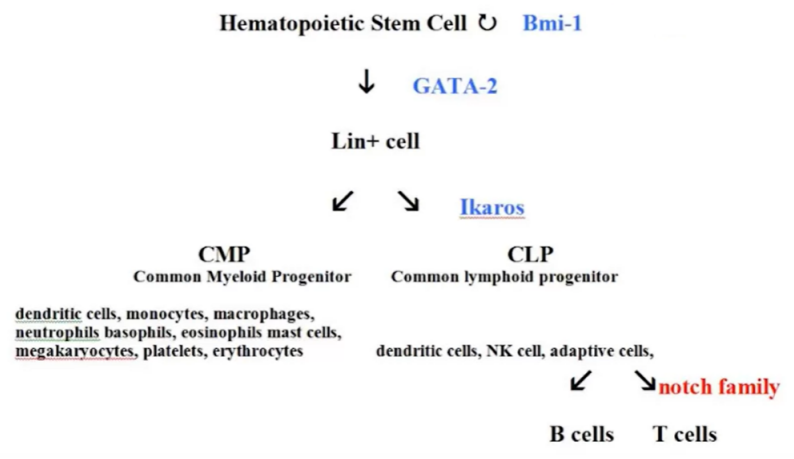
Responding Cell	T_H (Helper)	T_C (Cytotoxic)
Response	Coordinates immune response	Attacks and kills cell
Binds antigen with	$\alpha\beta$ T-cell receptor	$\alpha\beta$ T-cell receptor
Co-receptor	CD4	CD8
Antigen presented/displayed on	Class II MHC	Class I MHC
Cells presenting/displaying	Sentinel dendritic, macrophages, B cells	All nucleated cells except sperm
Source of antige	phagocytosis	synthesized in cell
Antigen hydrolyzed in	phagolysosome	proteosome

2.1 Terminology

- The primary organs are where the genes rearrange to make various recognition molecules. They include:
  - Bone marrow for B cells.
  - Myeloid cell.
  - Thymus for T cells.
- The secondary organs are where the immune cells are activated and counter-antigen. They include:
  - Lymph nodes.
  - Spleen.
  - Regions of the gut.
- Myeloid and lymphoid cells:
  - Myeloid cells are all innate and are found everywhere.
  - Lymphoid cells are only present in lymphs, including B cells, T cells, NK cells, and sentinel dendritic cells.
- Cluster of Differentiation (CD) refers to how cells come out of various separation procedures that involve cytometry. Hence, CDx cells are only informative in the chronological order they were discovered.

2.2 Hematopoiesis

- Pluripotent stem cell has many possible developmental phase.
- Different blood cells:
  - Erythrocytes or red blood cells:
    - Makes up the majority of the cells.
    - Minor cooperation with the immune system.
    - Maintain oxygen supply and pH in blood.
  - Thrombocytes or platelets:
    - Little fragments cells.
    - Pinched off from megakaryocytes.
    - Help stimulate the immune system but not considered part of the white blood cells.
  - Megakaryocytes:
    - Produce platelets to repair blood vessels.
    - Has thrombopoietin receptors, whose activation urges the up-regulation of platelets.
- Workflow of a hematopoietic stem cell:



2.3 Myeloid Granulocytes

- Granulocytes have granules and funky-looking nuclei.
  - Neutrophil:
    - Initial response to a threat.
    - Strongly phagocytic, typically only lives for a day.
    - Multi-lobe nucleus.
    - Granules are both acidic and basic.
    - Has FC receptors that binds the antibody stems and targets threats.
  - Basophil:
    - Complex C-shaped nucleus.
    - Not phagocytic.
    - Part of the response to worms and environmental threats.
    - Granules have C-shaped nucleus.
    - Has FC receptors that hold E-class antibodies and go looking for antigen.
  - Mass cell:
    - Tends to stay at one location.
    - Has lots of granules with histamine.
    - Signals running nose, mucus production, and itching if has an allergic response.
    - Nucleus has no lobes.
    - Works with basophils to respond to worms and environmental pollutants.
    - Has FC receptors that hold E-class antibodies and go looking for antigen.
  - Eosinophil:
    - Has a bilobed nucleus.
    - Granules are red as they are staining with eosin red and acidic stain.
    - Also attacks worms.
    - Weakly phagocytic, most of the time secretes substances from those red staining granules at the warms.

- Hygiene hypothesis states that early childhood exposure to particular microorganisms protects against allergic diseases by contributing to the development of the immune system.

2.4 Myeloid Antigen Presenting Cells

- Myeloid antigen presenting cells:
  - Monocyte:
    * Makes lot of inclusions and other molecules important in hydrolyzing pathogens, breaking them up, and using them to alert T_H cells.
    * Not only phagocitize but also reach out and attempt to transmit information or grab pathogens.
    * Mobile and has active surface, strongly phagocytic.
  - Sentinel Dendritic Cell:
    * First to present an antigen to naive T_H cells.
    * Gatekeepers that decide whether or not a T_H cell will respond to a new antigen.
    * Not migrating but lying in wait in a tissue to phagocytize antigen, digest it, and then present it to a T_H cell.
    * Some belong to the lymphoid lineage.
  - Follicular dendritic cell:
    * Incredible number of extensions that can trap exosomes, including particles and antigen-antibody complexes.
    * Instructs B cells by giving them a chance to bind to a particular antigen-antibody complex and thus get some encouragement in their development.

2.5 Lymphocytes

- B cells:
  - Developped in the bursa of Fabricius, which is the dorsal wall of the cloaca (common exit) of both the digestive and urogenital system of the bird.
  - Has membrane-bound antibody.
  - Has class II MHC molecules, part of its their way of communicating with T_H cells.
  - When stimulated to divide and develop and secrete antibodies, it becomes much larger and its protein synthesis apparatus will be up-regulated.
- T cells:
  - Either becomes cytotoxic (T_C) cell or helper (T_H) cell. T_C has CD8 and T_H has CD4.
  - T_H cells are quite diverse, including T_H1, T_H2, T_{reg}, T_H17, etc.
- NK cells:
  - Many granules and a round nucleus.
  - Has a trident of Fas ligand (FASL) that up-regulate apoptosis of the cell it sticks into.

- If it synapses with a rogue cell or viral-infected or cancerous cell, it has a lot of ammunition in the form of granzymes (serine proteases promoting cytotoxicity of rogue cells) and perforin (glycoprotein responsible for pore formation in cell membranes) that help to break up the cell and send it into asbestosis.
- Has MHC1[1] regulator that down-regulates to stop from attacking.
- Has FC receptor so that if it finds antibodies stuck in the surface of a cell, it will attack the cell.
- In a T_C cell, the MHC receptor will recognize foreign antigen on MHC1 and attack the cell. If a virus down-regulates the production of MHC so that a T_C cell cannot be alerted to the presence of foreign antigen, NK cells attack it as well. This process is an innate response.

2.6 Primary Organs

- Three primary lymphoid organs: bursa, bone marrow and thymus.
  - Bone marrow:
    * Produces most of the immune cells.
    * Bone marrow has lots of fat that sends out signals that regulate hematopoiesis.
    * Produces lymphoid cells.
    * NK cells arise here and B cells rearrange their genes as part of their development.
    * T cells start out here and migrate into the thymus to rearrange their genes.
    * Primarily the femur[2], humerus[3], arm bones, hip bones, and sternum[4] that do the production of most of blood cells.
  - Thymus:
    * Rearrangement site for the T cells.
    * Has a cortex (outer layer) and medulla (inner layer).
    * T cells start from the cortex. T cell progenitors come in in a relatively undifferentiated state. Then,
      · Rearranging genes occur. If no productive rearrangement is derived, the cell dies off.
      · They undergo a positive selection in the cortex to make sure they can identify antigen on an MHC molecule. If so, they migrate towards the medulla.
      · They undergo a negative selection in the medulla to get rid of T cells that recognize our own self antigens to prevent auto-immune diseases.

      Cells pass the three tests leave the circulation and head out to the secondary lymphoid organs to coordinate your immune response, and cells do not pass any of the test will die. Over 95% of the cells undergo apoptosis as part of this process.

2.7 Secondary Organs

[1] Major Histocompatibility Complex (MHC) is a large locus on vertebrate DNA containing a set of closely linked polymorphic genes that code for cell surface proteins essential for the adaptive immune system.

[2] Thigh bone.

[3] Bone of the upper extremity.

[4] T-shaped vertical bone that forms the anterior portion of the chest wall centrally.

- The secondary organs of the immune system form an interconnected surveillance system where the immune cells gather and exchange information, including the fluid, vessels and nodes.
- Circulation involves both the blood vessel (round trip) and the lymph (one-way from the organs out). The plasma from the blood filters into the tissues carrying the proteins, only the lymphoid cells crossover but not the blood cells or myeloid cells.
- The lymphatic system picks up fluid throught the body. This drained interstitial fluid from tissues picking up lots of information is gathered up and filtered through the lymph nodes where the antigen is trapped and acted on. Eventually, these vessels grow into larger ones that in turn empty into[5] the thoracic duct, left subclavian vein, and then the heart to rejoin the blood circulation.
- The lymph system also extends into the head, named as the meningeal system. It connects with the deep cervical or neck lymph nodes. It lies between the dura mater and the meninges outside the brain and between the meningial arteries and the dural sinuses, which are the veins. The sagittal sinus over the top of the head, transverse sinuses runnning along the side, a lymph vessel is buried in there. It will divide into two transverse branches that contains valves only in the skull, leading out to the cervical lymph nodes.
- Fluids come into a lymph node and come out from the efferent vessel. The cortex of a lymph node holds the follicles and the B cells, the power cortex refers to the structure below the cortex, and the medulla sits inside.
- B cells activated by antigen are to wind up in follicles. The germinal center is where the B cells develop in response to signals from the follicular dendritic cells, T_H cells, and macrophages will clean up the debris. B cells learn how to bind more tightly to the particular pathgenic antigen here.

2.8 Other Secondary and Tertiary Organs

- The lymph nodes, spleen, and gut mucosa are all secondary lymphoid organs.
  - Spleen:
    - Lies above the abdomen.
    - Filters and monitors blood but not lymph.
    - Has red pulp tissue where the macra fascias recycle old red blood cells.
    - Has white pulp tissue where T cells participate in immune surveillance.
    - Has a marginal zone that has B cells in follicles.
  - Mucosal Associated Lymphoid Tissue (MALT) is a collection of tissues including Bronchial Associated Lymphoid Tissue (BALT), Nasal Associated Lymphoid Tissue (NALT), and Gut Associated Lymphoid Tissue (GALT).
  - The tonsils, appendix, and Peyer's patches (bunch of follicles) in the intestine of some animals also participate in the activation of B cells.
  - GALT:
    - Picks up antigens from the lumen and transports into a developping follicle. Similar in aforementioned other issues.

[5] Flows into.

    * M cell is sequestering a bunch of immune function cells to facilitate information flow.

- The skin forms a tertiary organ:
  - Made up of the epidermis, epithelial tissue that sits on a basement membrane, and dermis.
  - The epidermis derives from embryonic ectoderm, and the dermis derives from embryonic mesoderm. The skin is produced by combining cells from two different tissue layers. It secrets lots of commounds to protect us from pathogenic attack.
  - Keratinocytes are epithelial cells that upon death retains the keratin and helps to make the skin waterproof. They also express MHC2 and present antigen to T cells. Setenal dendritic cells phagocytizes antigens here as well that can leave the epidermis and go off and look for T cells someplace else.
  - There is a speical kind of T cells reside in the epidermis and other mucosal tissues that have special receptors. They have built-in quasi-innate recognition for the possible dangerous organisms.

2.9 Final Issues

- Apoptosis is a controlled way of killing off a cell and disposing of the contents.
- Necrosis means a cell swelling, breaking open, spilling its guts, and causing inflammaton.

3.3 Innate Targeting of Pathogens

- People tend to only recognize few protein molecules innately, including
  - Flagellin that makes up bacterial flagella.
  - Profilin that turns out to be the one that people use to help organize microfilaments.
  - β 1-3 glucan that is part of fungi cell-coating.
  - Peptidoglycan that is often found in the cell wall of gram-positive bacteria.
  - Lipopolysacchride (LPS) that is a strong trigger of pattern recognition reception that leads to an inflammatory response.
- Human can also recognize wrong kinds of nucleic acids: ssDNA, dsRNA, or DNA with long methylation patterns.
- Protective molecules:
  - Lysozyme can break up peptidoglycan.
  - Psoriasin found on skin is against E.coli. Too much of it exposed on sunlight can trigger eczema.
  - Mannose-binding lectins in the plasma.
  - C-reactive protein recognizes microbes and damaged self cells. It is a sign of general long-term inflammation and is associated with heart attack.
  - Nucleotide-binding oligomerization domain (NOD) proteins in cytoplasm sometimes bind nucleic acid and sometimes bind cell wall compounds.

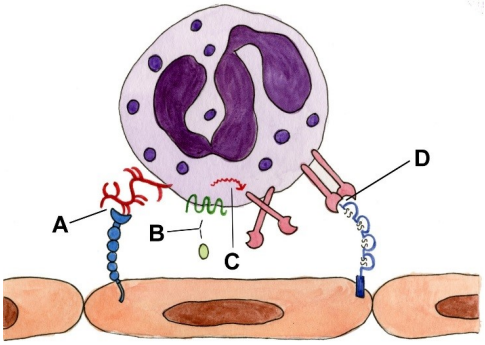
- Toll-like receptors (TLR) represents a huge family of molecules. It recognizes parasitic surface proteins using leucine-rich hooks extending into the exterior of the cell and can trigger inflammatory response that will activate NF-kB, a transcription factor that will turn on many genes to fight various infections.
  - Plasma membrane TLR recognizes surface characteristics of pathogens, such as cell wall, capsule compounds and flagella.
  - Endosome TLR recognizes pathogenic nucleic acids after their release during digestion.
  - Some is found in the plasma membrane, and the other in endomembranes.
  - They trigger the recognition of a threat in phagocytic organisms and that is partly what send them off to present antigen to a T_H cell.

3.4 Myeloid Cell Function

- After phagocytosis, pathogens in the phagolysosome die when their cell membranes are punctured by defensin and their organic molecules are oxidized by HOCl.
- A compound in the membrane of the phagolysosome takes NADPH and takes the hydrogen off of it to generate H_2O_2. This is an oxidative process, considered to be a form of respiration. It is not generating ATP but some toxic compounds to kill off pathogens. When a neutrophil or a micropahge pahgocytizes a bacterium, a respiratory burst takes place, a rapid oxygen uptake as the enzymes are activated. Simultaneously, intake of K^+ takes place to make the vacuole hypertonic. Outside the membrane, we also polymerize actin that acts like a mini-cell wall to keep it from swelling.
- Several toxic compounds:
  - Reactive oxygen species:
    - Superoxide radicals: $\dot{O}_2^-$.
    - Hydrogen peroxide: H_2O_2.
    - Hypochlorous acid: HOCl.
  - Reactive nitrogen species:
    - Nitric oxide: NO·.
    - RNS thiols: RNSO.
    - Peroxynitrous oxide: ONOOH.
- Though the previous mechanism can kill off most pathogens, some may be able to survive and use macrophages to transport, like tuberculosis and leprosy.

3.5 Quizzes

- Neutrophils leave the blood vessels and enter into infected tissue via a multistep process.



A: It indicates the non-covalent linkages between the blue lectin and the red mucin. As these are made and broken the neutrophil sticks and releases from the endothelium, causing it to roll.

B: It indicates the chemokine signal from the endothelium to the neutrophil, which will lead to internal changes in that cell.

C: It indicates the internal cascade pathway kicked off by the chemokine receptor leading to conformational and organizational changes in the neutrophil.

D: It indicates the specific conformation of the integrin that allows it to form a tight non-covalent link to the immunoglobulin CAM, leading to arrest, a full stop, not a roll.

- Sleeping sickness and Chagas, malaria, and giardia and leishmaniosis are all caused by eukaryotic trypanosomes whose flagellae are different from that of the prokaryotes.

4.2 The Immunoglobulin Superfamily

- The immunoglobulin domain is referred to a region of the protein where the peptide has folded back up so that hydrophobic parts are inside, hydrophilic parts are outside, and they are stabilized with a disulfide bond. A typical immunoglobulin protein in the subfamily has one or more of these domains, and is typically embedded in the plasma membrane.

4.3 Ig Receptors and Antibodies

- If you take blood plasma and put it in electrophoresis apparatus, you get again a series of peaks:
  - The first peak is albumins: about 60000 molecular weight small blood proteins that help carry things around and also keep blood osmotic pressure under control.
  - The other peaks are called α_1, α_2, β and γ, respectively.
  - These proteins are called globulins because they are soluble, in contrast to fibrous proteins which are not soluble.

4.4 Form and Function

- A typical antibody has two Fab fragments, a Fab_2 fragment, and a Fc fragment:
  - A Fab fragment is also known as light chain[6] with molecular weight about 50k.
  - Fab_2 fragment and Fc fragment forms a heavy chain with molecular weight about 100k.

[6] A chain in immunology is a synonym for peptide.

  - Fc parts of antigens are very similar, but loop regions at the end of the antibody tends to be variable.
  - A carbohydrate (oligosaccharides) as part of the Fc stage is attached the second chain from the C-terminal and pulls apart the heavy domains in the Fc, whose main significance is in signalling to other parts of the immune system just how to treat whatever this antibody is hooked up against.

- A light chain is a joint of a constant region and a variable region. The whole light chain can either be κ or λ, dictated by two different genes. Human has 4 different choices to put into the λ. Any cell can either make the κ or the λ, that is, the two light chains in an antibody have to be the same type.
- There are 5 different types of antibodies, based on the specificity of heavy chains. However, any heavy chain (also pick by that specific cell) can be associated with any light chain, which renders the antibody the capability of binding different antigens.

4.5 **Immunoglobulin Classes**

- There are two major classes of immunoglobulins. Both of them have the variable chain, one constant domain called C1, either a hinged region or another constant domain (C2) that tells the classes apart, and the Fc stem region that has two constant domains, C2 (C3) the bendy ones and C3 (C4) the rigid ones.
- The hinged region turns out to be a string of prolenes that are stabilized by typically two disulfide bonds.
- The bottom is the C-terminus of this antibody where attached to the membrane.

4.6 **Specific Ig Types**

- Antibody classes:

Ab type	hinge or rigid	forms com-plexes	J chain	sub-classes	timing	membrane-spanning Ig receptor	role
M	rigid	yes	yes	no	first class produced in maturing B cells	yes, naive and memory	general; activates complement, summons phagocytes, crosses epithelia
D	hinge	no	no	no	produced as Ig receptor on mature but naive B cells	on naive cells, rarely soluble or memory	aids naive B cell activation
G	hinge	no	no	4	after class switching in activated B cells	memory cells only	specific responses to acute infections
A	hinge	yes	yes	2	after class switching in activated B cells	memory cells only	crosses epithelia, protects boundaries
E	rigid	no	no	no	after class switching in activated B cells	memory cells only	T_H2 response: defense against worms pollutants & chronic infections; allergies

- G-class antibodies:

Class	hinge length (# disulfides)	complement activation	phagocyte activator	function
1	2	strong	very strong	inflammatory: T_H1 response to serious threats
2	4	weak	no	only mildly inflammatory; may cooperate with A and E antibodies during T_H2 responses
3	11	very strong	very strong	highly inflammatory: T_H1 response to intracellular pathogens
4	2	no	strong	intermediate response (possibly mop-up)

- M class antibody is secreted with the J chain (which allows molecules to cross the epithelia) and in an association of typically five of the units put together with disulfide bonds.

- A class antibody comes with 2 sub-classes. They can be found on the membrane but also secreted in dimers/trimers. They can go to mucus, tears, saliva, breast milk, and one can make up to 15 grams of this a day. It is as a barrier to an assortment of pathogens that prevents one from getting sick. Sometimes bacteria can make protease to attach the hinge region of this antibody to fight it off.
- E class antibody only occurs in the form of monomer. Its Fc region attaches and even pre-attaches to the FC receptors of basophils, mast cells and eosinophils.

4.7 Immunoglobulins in Action

- Haptens are something that is too small to cross-link two receptors in a B cell.
- Haptens are too small to be immunogenic, so we can manipulate by sticking lots of haptens to enlarged proteins such as bovine serum albumin or BSA.

4.8 Fc Biological Activity

- An antibody, instead of summoning basophils, phagocytes, etc., can also activate the complement system, part of the immune system that enhances the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attach the pathogen's cell membrane.
- Antibody-dependent cellular cytotoxicity (ADCC) is a mechanism of a cell-mediated immune defense whereby an effector cell of the immune system kills a target cell, whose membrane-surface antigens have been bound by specific antibodies.
- J chains of the A and M classes antibodies can trigger transcytosis, a type of transcellular transport in which various macromolecules are transported across the interior of a cell, by providing a hook for a membrane embedded transport protein to pick up an antibody complex and transport it from the plasma, across the epithelia, and into gut or breast milk.
- Idiotype is a shared characteristic between a group of immunoglobulin or T-cell receptor (TCR) molecules based upon the antigen binding specificity and therefore structure of their variable region. For example, it can refer to an M class antibody and an A class antibody that have the same N-terminus of the variable chain.
- Isotype can refer to the same class of antibodies that are bound to different antigens (different N-terminus of the variable chain).

4.9 The B Cell Receptor

- Immunoglobulin receptors are just Ig antibodies stuck on cell membrane. They can slide aside the cell membrane but not getting out or in. When B cells recognize foreign antigens, they recognize it when two neighboring receptors will bind to that antigen and cross-link. A single receptor cannot begin the signalling process because their cytoplasmic regions are short and don't contain any signalling domains.
- CD79A and CD79B (previously known as Igα and Igβ) form a hetero-dimer on the plasma membrane that appears next to an Ig molecule, joined by a disulfide bond. Their cytoplasmic region, specifically a region called immuno-tyrosine activation motifs (ITAMs), can pick up a covalent phosphate (put on a tyrosine) and trigger a response.

- One can actually make memory cells for all the different classes of antibodies. These memory cells will lie in wait in case that antigen shows up again. Once a class switches away from the naive cells, from then on, in whatever class of antibody one is producing, that is the only class of receptor present in the surface of the cell.

4.10 Monoclonal Antibodies

- Typically, a B cell can only live for several weeks. What we can do to make it immortal is via hybridoma, that is, to fuse a desired B lineage cell with a B cancer cell:
  - Put B cells extract from plasma together with myeloma cell (B cancer cell) that makes no antibody in polyethylene glycol (to disrupt membranes), and they tend to fuse together.
  - Put them into an hypoxanthine aminopterin thymidine (HDT) medium. As rapidly dividing cells, these cells need to make DNA. There are several pathways for these cells to do this: a de novo pathway to make everything from scratch, or a salvage pathway to pick up materials to salvage them (here supplied by hypoxanthine and thymidine). Here, aminopterin can block the de novo pathway so that only the hybridoma can be retained, as myeloma cells cannot reproduce via the salvage pathway.
  - Engineer the antibody-producing cells to make a human, not a mouse antibody.

4.11 Quizzes

- Adjuvant is a substance that increases or modulates the immune response to a vaccine. The most common category of adjuvants in US vaccines is aluminum salts, which activate cytoplasmic NOD receptors to improve the effectiveness of antibody production. Bacterial cell wall peptides have been used in the past, but these can produce an excess inflammatory response.

- Mercaptoethanol can reduce the disulfide bonds; pepsin is an endopeptidase that breaks down proteins into smaller peptides.