

Fundamentals of Immunology



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BIOC 372x Study Guide

This study guide covers Part One: Antibodies and Innate Immunity.

Lecture L01- Introducing the Metaphors

Lecture L02a- Surveying the Cells & Organs of the Immune System

Lecture L02b- Surveying the Cells & Organs of the Immune System

Lecture L03-Innate Immunity

Lecture L04- Antigens & Antibodies

Lecture L05-Organization & Expression of Immunoglobulin Genes

Lecture L06- Development of B Cells

Lecture L07- Complement

Glossary

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Lecture L01

Introducing the Metaphor

Infectious disease is one of the few genuine adventures left in the world. The dragons are all dead and the lance grows rusty in the chimney corner....About the only sporting proposition that remains unimpaired by the relentless domestication of a once free living human species is the war against those ferocious little fellow creatures, which lurk in the dark corners and stalk us in the bodies of rats, mice, and all kinds of domestic animals, which fly and crawl with the insects and waylay us in our food and drink and even in our love. Hans Zinsser, 1935



Metaphor ... is the lifeblood (ha!) of good scientific prose.”– Matt Ridley, 2003

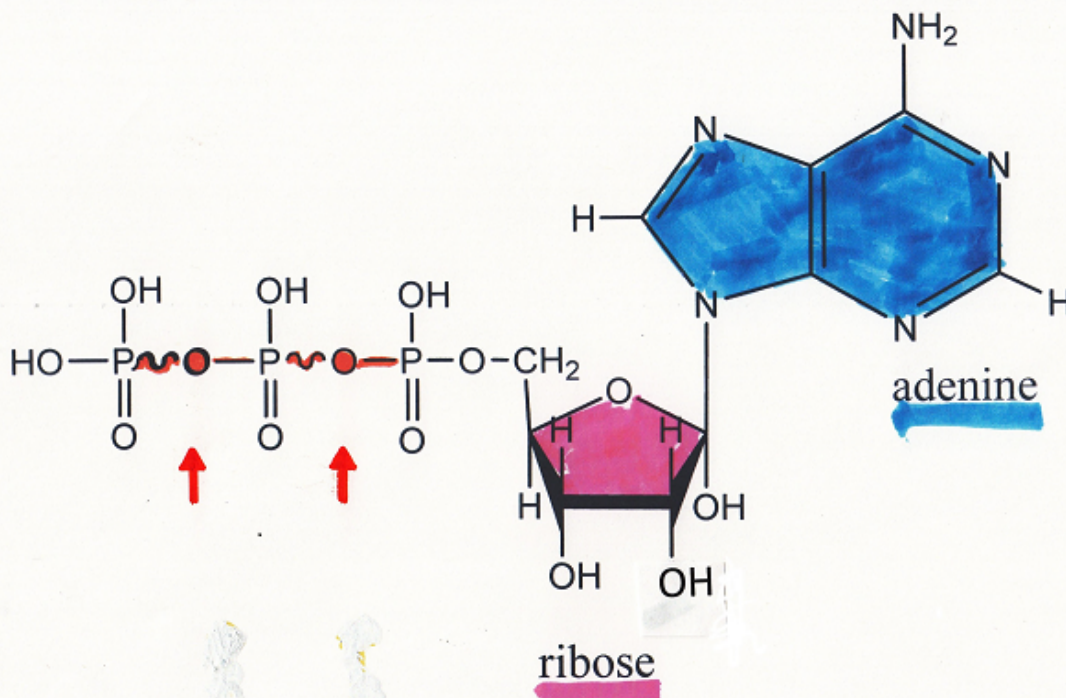
I. Welcome

II. Staying Healthy

A. Context

1. All organisms (including plants and fungi) have defense mechanisms. These are clearly derived from common ancestral forms, currently classified as innate.
2. Vertebrates have an additional particularly effective defense - acquired or adaptive immunity involving antibody production.
3. Insects, the group multicellular animals with the greatest number of species and probably the highest overall biomass, also have a form of immunity that allows for a flexible response.
4. The defenses are energetically expensive. (*Figure 1.1*)

Figure 1.1: ATP



5. These defenses represent a serious threat to your own body, and you

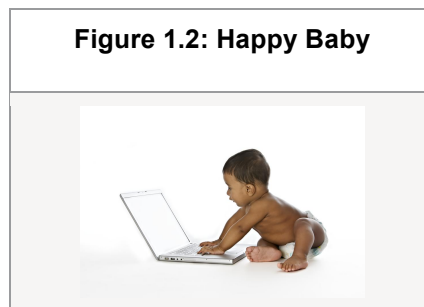
control them to make sure that they don't wind up attacking the wrong cells, which certainly does happen.

B. In Praise of Engineers - How We Stay Healthy

1. clean water
2. proper sewage disposal
3. mosquito (and other insect) discouraging buildings
4. communications and transportation infrastructure - allows delivery of preventive health care and distribution of food.
5. vaccination- OK the engineers didn't give us this one, but without the communications and transportation infrastructure, it's hard to deliver vaccines.

C. Disease Burden

1. Economic costs of being sick and having to tend to sick children.
2. Rates of infections disease and general ill health correlated with lowered IQ. (Figure 1.2)

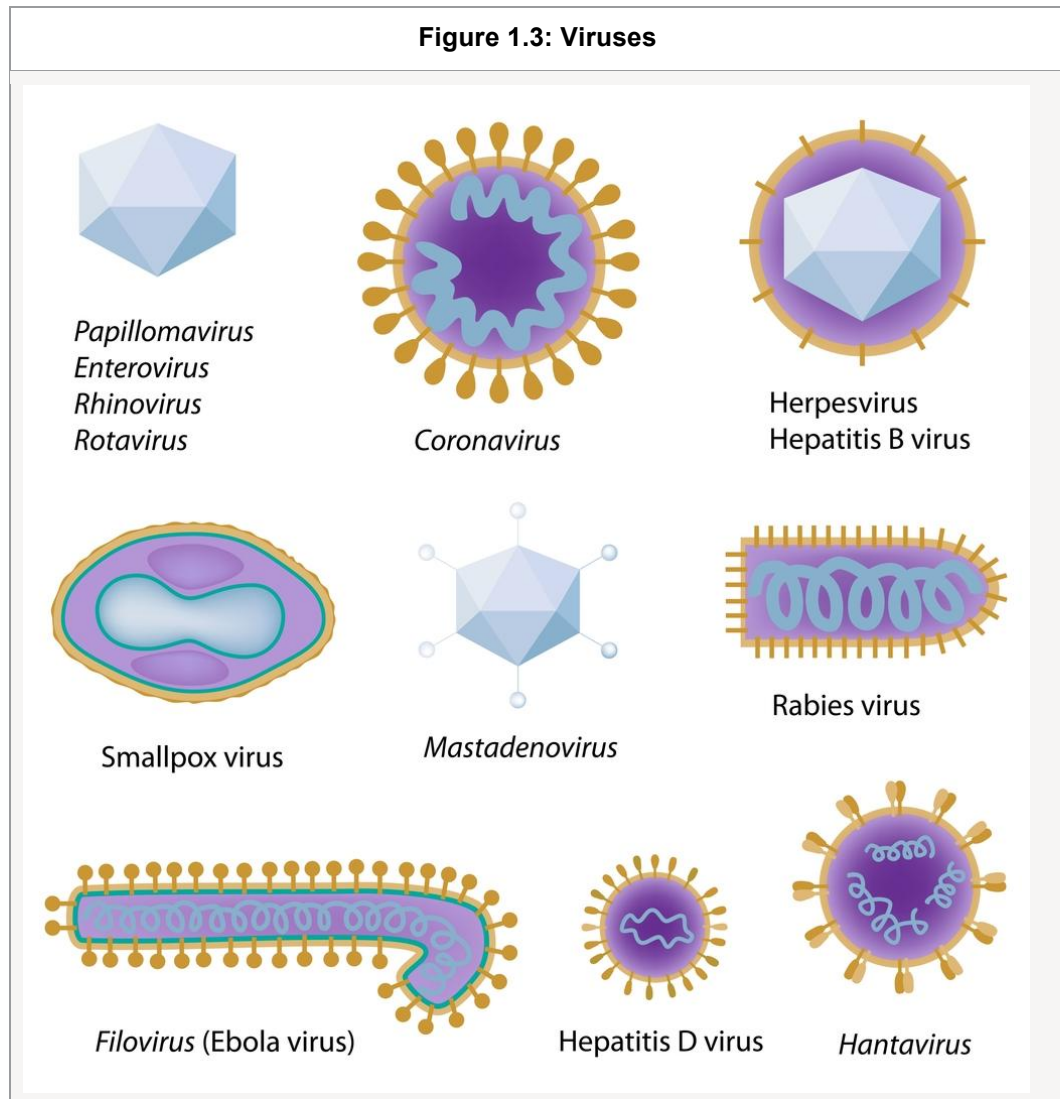


- a) Correlation is about 67%, which suggests that this is not the only factor, but it does provide a possible explanation for the Flynn effect.
- b) Diarrheal diseases rob infant of nutrition at a period of critical brain growth. 87% of the nutritional energy in newborns goes to the brain.
- c) Cerebral malaria can damage the brain directly.

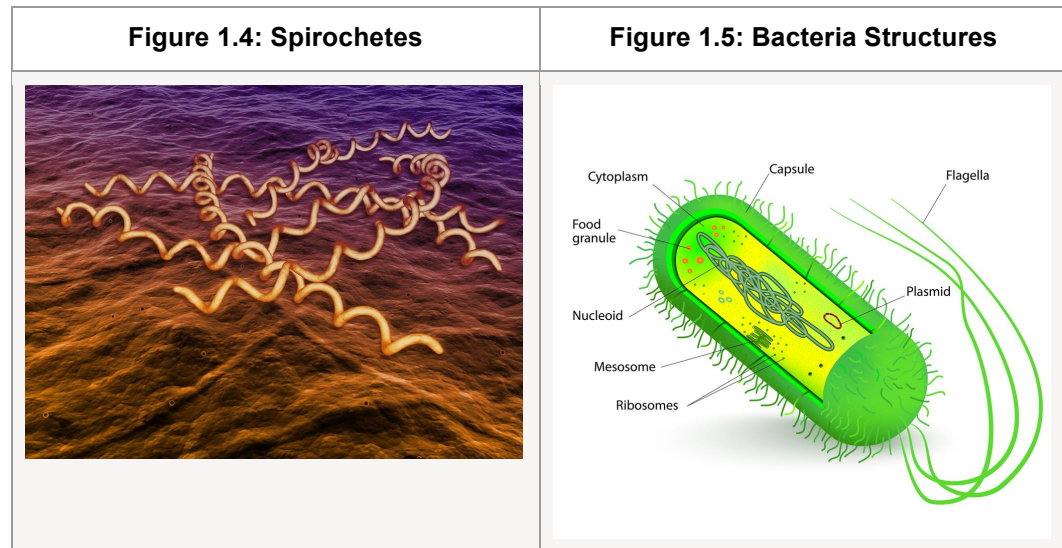
III. Pathogens and Immunity

A. Types, with a few examples

1. Viruses: rhinovirus, flu, small pox, Ebola, polio (*Figure 1.3*)



2. Bacteria: *Mycobacterium tuberculosis* (TB), *E. coli*, anthrax, bubonic plague, strep, *Clostridium difficile* (Figures 1.4-1.7)



*Close up
view*

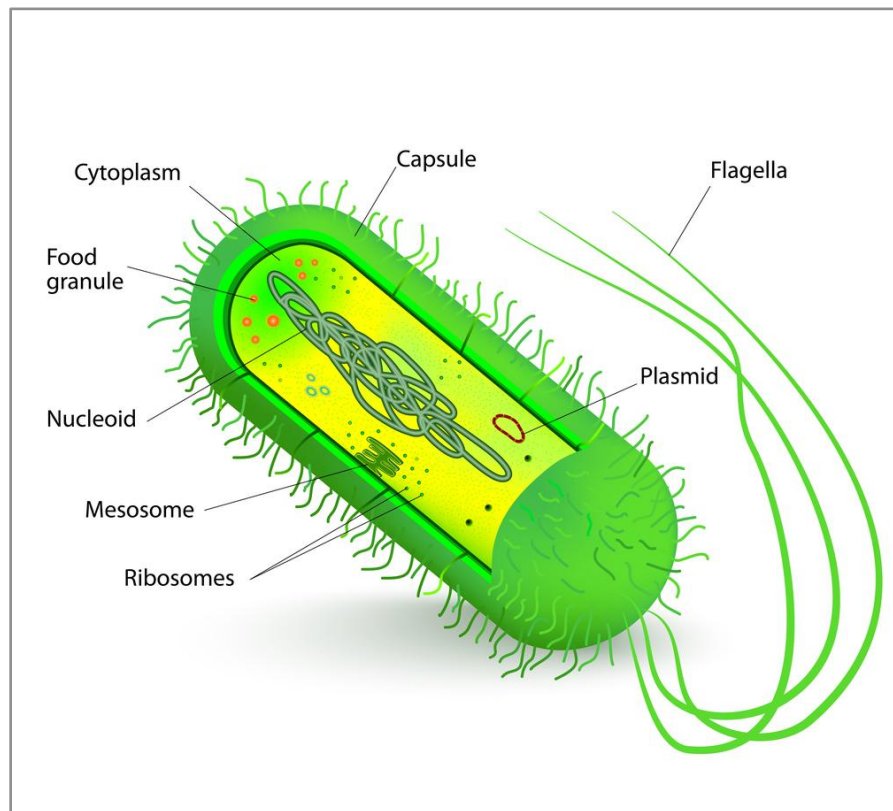


Figure 1.6: Gram Positive

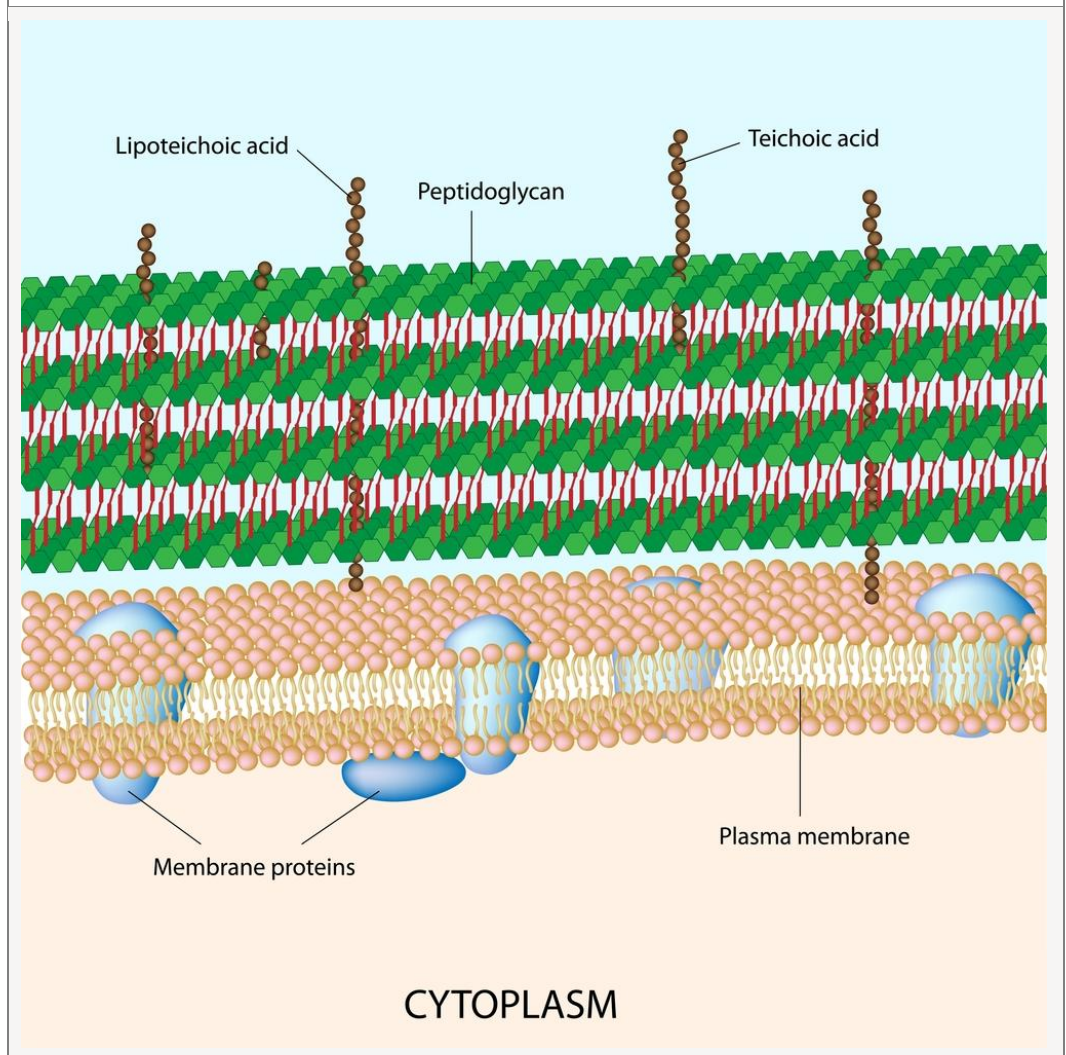
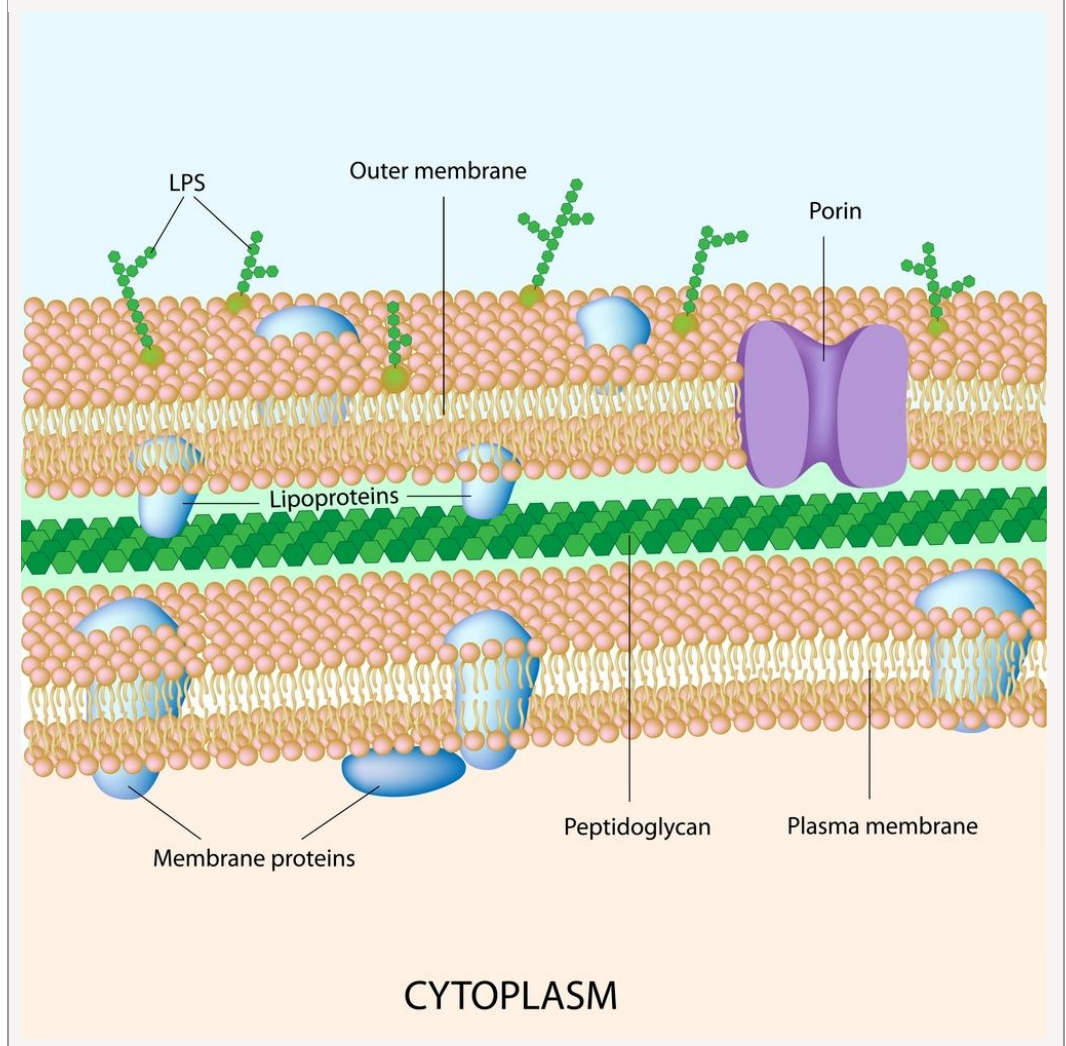
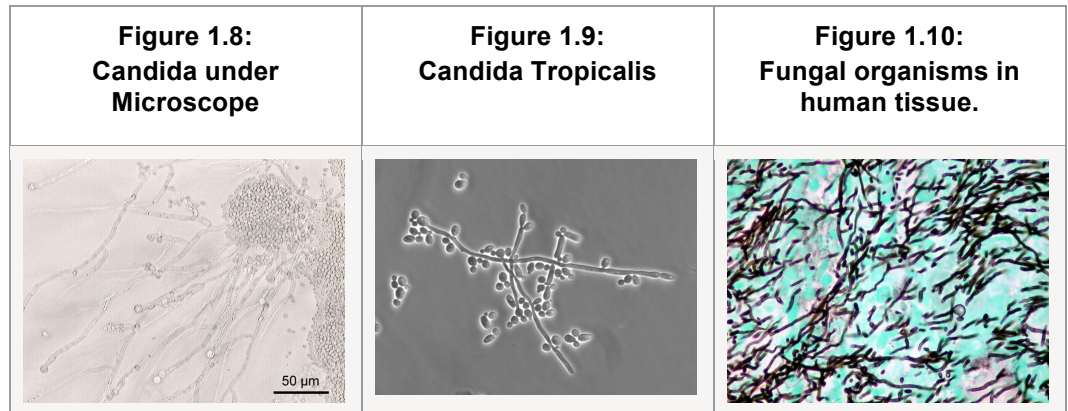


Figure 1.7: Gram Negative



3. Fungi: *Candida albicans* (yeast), athlete's foot, ringworm, *Cryptococcus* (Figure 1.8-1.11)



4. Unicellular eukaryotes: malaria, trypanosomes, amoebae, Giardia, Chagas (Figure 1.11-1.12)

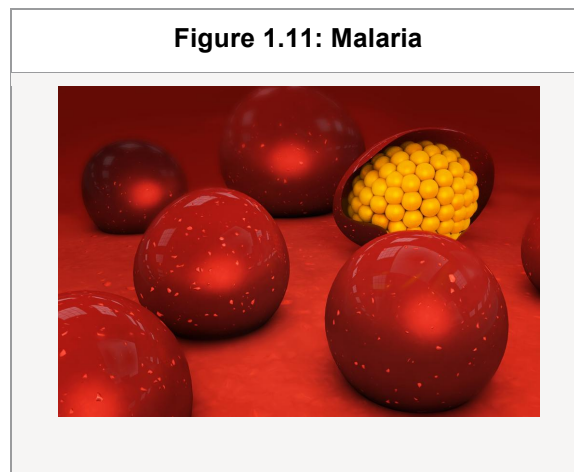
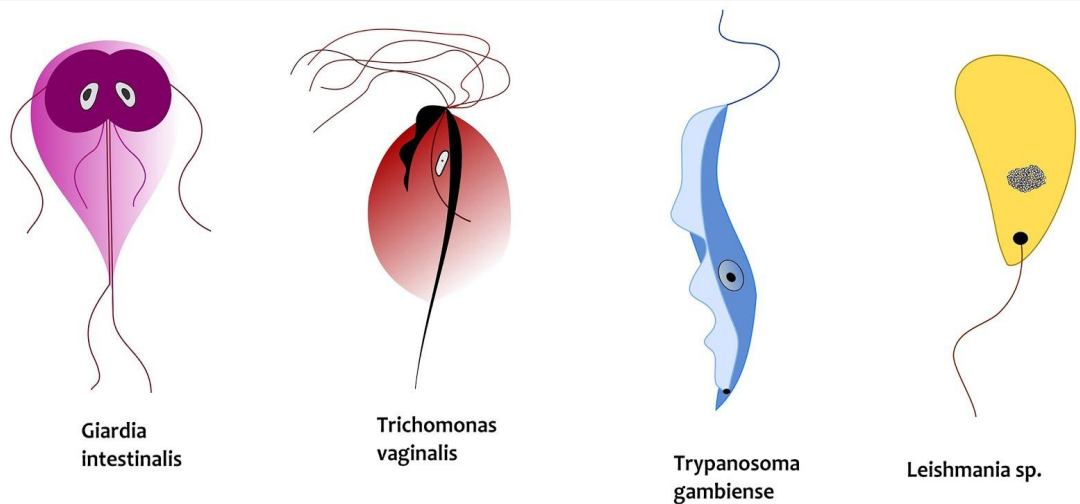
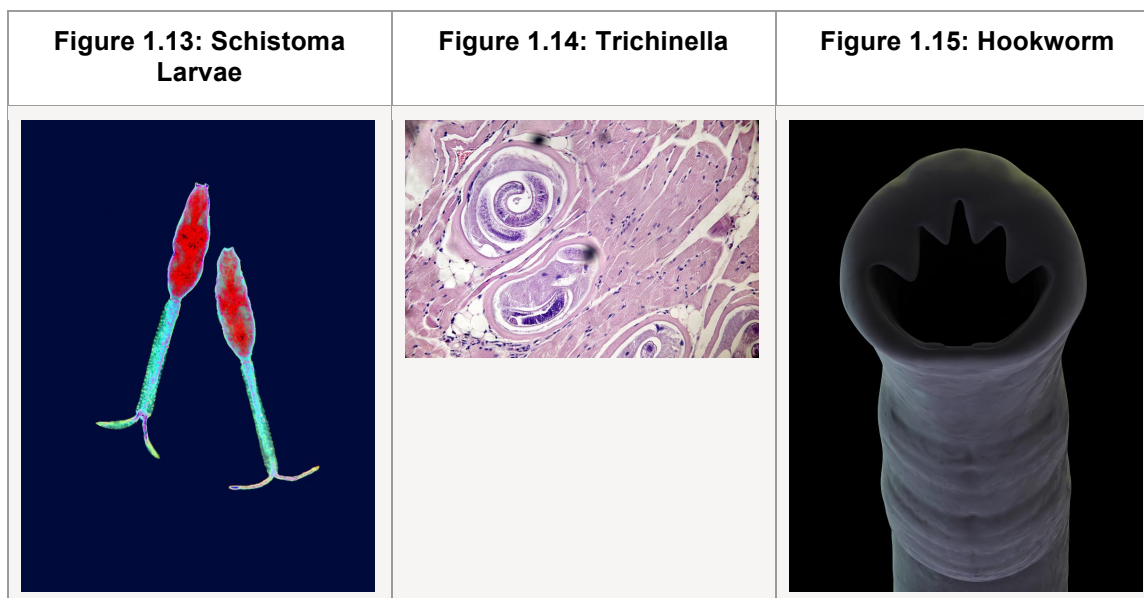


Figure 1.12: Protozoans



5. Parasitic worms (Platyhelminthes and Nematoda): flukes, tapeworms, hookworms, heartworms (*Figure 1.13-1.15*)



B. *Recognition – Two general strategies to identify and neutralize the threat.*

1. Innate recognition: pattern of molecules characteristic of general category of pathogen. It does not require previous exposure to a pathogen.
2. Adaptive recognition: identifies molecules (usually specific proteins) found only in a specific strain of pathogen (like mug shots, fingerprints, DNA fingerprinting, facial recognition, text analysis.) Parallels: both are highly specific, require previous exposure and are more recent innovations.

IV. Words of Advice

A. *Wash your hands*

1. Hands are a big source of contamination.
 - a) Fecal-oral – you pick up a bacterium and transfer it to your own mouth or someone else's food - "employees must wash their hands..."
 - b) colds and flu viruses - picked up by hands and transferred to eyes and nose
 - c) Think about what you touch.
 - d) Rub your eyes with the backs of your fingers.
2. Hand washing effectively prevents this.
 - a) Soap and water

- b) Gel alcohol
- 3. Doctors are a particularly lethal source of infections.
 - a) Ignaz Semmelweis and the prevention of puerperal fever
 - b) Hospital germs are much more likely to have multidrug resistance.
- B. *Think before you have sex.*
- C. *If you're sick, stay in bed.*
 - 1. You'll keep your illness to yourself.
 - 2. You'll force the virus into evolving strains that make everyone less sick.

V. Metaphors if Power Politics

A. *Policing Functions*

- 1. Expensive-Malnutrition is associated with chronic infection.
- 2. Necessary
- 3. Deployed frugally

B. *National Defense and the Defended Body*

- 1. Variety of agents with complex interconnected controls and communication.
- 2. Different levels of defense and hostile engagement.
 - a) All-out war: – T_H1 (destruction) - all-out war. If this response consumes a lot of energy and destroys many of the body's own cells, so be it. The alternative is death and you risk all.
 - b) Cold war: T_H2 (containment) – diplomatic sanctions and trade embargoes. This is the kind of response we make to chronic infections and to many helminth (worm) parasites.
 - c) Non-Hostile or normal relations: T_{reg} (peaceful coexistence): So you want some kind of signaling process that tells you to leave alone harmless bacteria.
- 3. Misdirected Defenses
 - a) Allergy - immune response to non-pathogens
 - b) Inflammatory tissue damage – collateral damage during attack on pathogen
 - c) Autoimmunity- harm by “friendly fire” when the immune system attacks your own tissues.
 - d) Against transplanted tissues

VI. Innate versus Adaptive Immune Response

A. *Innate and Adaptive are parts if a whole*

- 1. Innate evolved first
- 2. Adaptive later, in the vertebrates only
- 3. The two interact, cooperate and exchange information.

B. *Innate Characteristics – ready to go*

- 1. Phagocytes (neutrophils and macrophages) and NK cells

2. Defensive proteins – complement, lysozyme
3. Barriers skin- mucus
4. Pattern recognition molecules – sense general characteristics of pathogens

C. Adaptive Characteristics

1. Requires more time
2. Requires gene rearrangement

Table 1.1: Innate Versus Adaptive

Innate	Fast (minutes)	Always there	Recognizes patterns	Phagocytes, NK cells, proteins & barriers
Adaptive	Slower-weeks initially, 3 or more days subsequently	requires gene rearrangement	Recognizes specific proteins	B and T _H and T _c cells

D. *Interactions in Action*

1. T_H cells (adaptive) are at the heart of the immune response.
2. Antigen presenting cells (innate) provide them with information.
3. T_H cells in turn chemically stimulate innate cells, such as macrophages.

E. *B-cells*

1. T_H cells will also stimulate B cells (adaptive) to develop and produce antibodies.
2. Referred to as humoral immunity.
3. When stimulated they divide (clonal expansion).
4. After maturing, they secrete antibodies.

F. *T_C cells*

1. Stimulated sick cells, which present antigen (on MHC I)
2. Gets OK from T_H cells
3. Begins attacking sick self cells

VII. The More you Know: Optional Resources and Fun stuff (*You don't get tested on this!*)

- A. We cover T cells in the second session of this course. However, I can't discuss B cells without mentioning T cells, so here's a table (see next page) to help you sort out some T cell traits:

Table 1.2 Responding to Foreign Antigen (Inevitably simplified)

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	CD 4	CD 8
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 antigen	phagocytosis	synthesized in cell
Antigen hydrolyzed in	phagolysosome	proteosome
Response	Coordinates immune response	Attacks and kills cell

- B. Also, if you'd like to follow up on some the issues raised in lecture, here's some sources:
1. From a previous student on the death of a doctor who caught Nipah virus from a corpse (There weren't proper handwashing station at the hospital. This is a horribly wasteful and tragic way to die.)
 2. http://wwwnc.cdc.gov/eid/article/19/2/12-0971_article.htm
 3. Importance of sanitation:
<http://abcnews.go.com/Health/GlobalHealth/story?id=2805299&page=1>
 4. Disinfecting patients prevents staph:
http://www.msnbc.msn.com/id/34733342/ns/health-infectious_diseases/
 5. Low national average IQs linked with infectious diseases:
www.physorg.com/news197179291.html and reported in *The Economist*, July 3, 2010, pages 75-76

Lecture L02a

Surveying the Cells & Organs of the Immune System, part a

I can assure you that peace will not be built on poor nutrition and human suffering. - Norman Borlag, 11/19/01 (from talk at Rice University)



I. Orientation to Terminology

A. Analogies- At the end of your lecture outline, you can find a table that summarizes the various cells and attempt to draw a parallel between their function and the function of some element of military or policing defense.

B. Primary Classification Distinctions

1. Primary versus secondary organs:

- a. Primary organs are where cells divide, decide on a developmental fate and, if part of the plan, rearrange genes.
- b. Secondary organs are site of co-ordination of information about pathogens and the subsequent activation of cells.

2. Innate versus adaptive cells: innate cells don't rearrange genes, adaptive ones do.

3. myeloid versus lymphoid cells: two general categories define by an early branching decision early in development. All adaptive cells are lymphoid, but some lymphoid cells (NK cells) are innate. Most of the cells in the lymph are lymphoid, but some of the cells in the blood plasma are lymphoid as well.

C. "Cluster of Differentiation:" The Term from Hell

1. Immune cells differ in their surface markers, which are characteristic proteins extending from the plasma membrane.
2. Different cell surface properties cause a cell to sort differently during a process called flow cytometry.
3. Scientists have a collection of different monoclonal antibodies that attach to and identify these proteins.
4. Proteins are identified by a number preceded by CD, for "cluster of differentiation."
5. Thus the names CD8 or CD25 simply indicate the relative order in which they were identified.

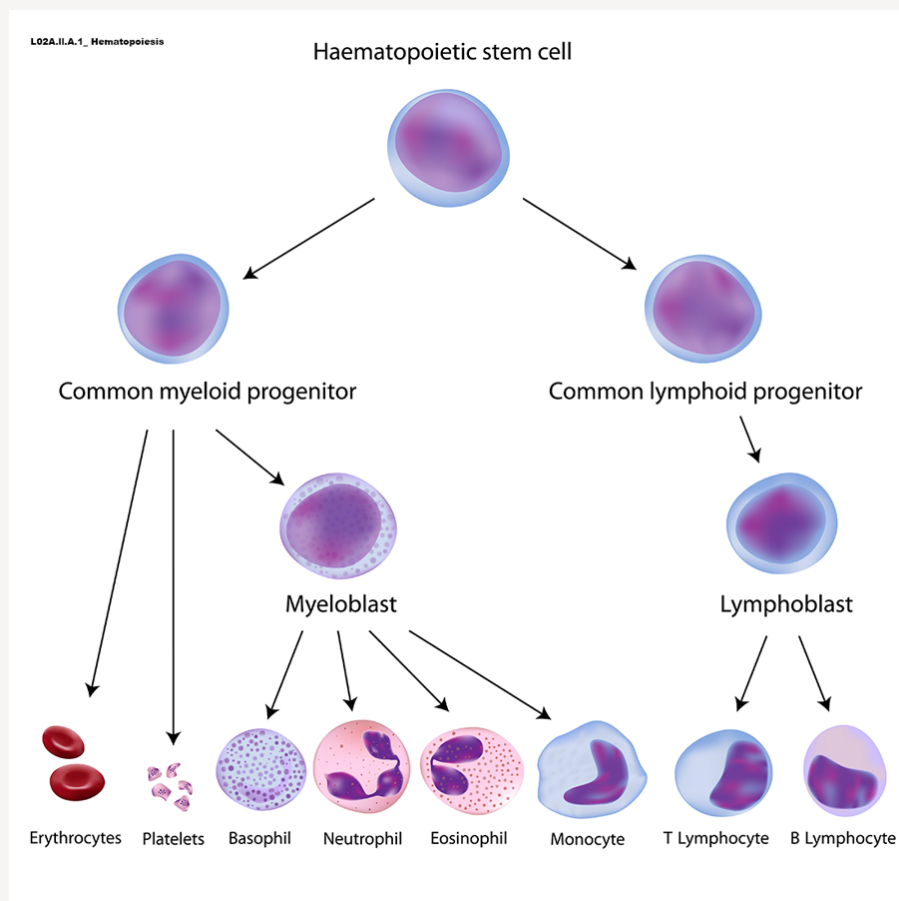
II. The Source of It All – Hematopoiesis

A. Hematopoietic Stem Cell (*Figure 2A.1*)

1. This is a pluripotent stem cell that can give rise to any type of blood cell.
2. It can divide to make more of itself - self-renew – or it can begin to differentiate by making a series of choices that narrows its options.
3. HSCs first form in the yolk sac membrane in the early embryo, migrate to the liver and spleen and most settle in the bone marrow before birth.

4. As few as 100 or so HSCs are enough to completely regenerate the whole hematopoietic system.
5. Isolate *lin*⁻ stem cells from various types of *lin*⁺ cells.

Figure 2A.1: Hematopoietic Stem Cell

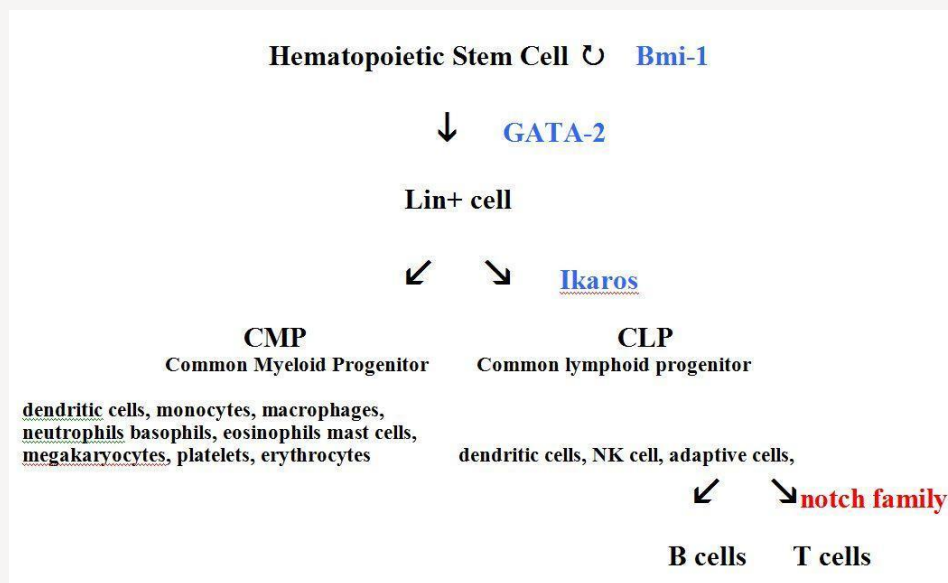


B. Signaling Differentiation (Figure 2A.2)

1. *Bmi-1* (transcription factor) keeps the HSCs undifferentiated and continuing to divide and renew.
2. *GATA-2* (transcription factor) triggers differentiation from the HSC into the general path of division and development into a specialized cell.
3. The first decision or branch point, is whether the cell will go myeloid or lymphoid:

- CMP- If it turns into a common myeloid-erythroid progenitor, it may develop into a huge number of types including different types of white blood cells, red blood cells and platelet-producing megakaryocytes.
- LMP- If it turns into a common lymphoid progenitor, it expresses the transcription factor Ikaros, and can become the adaptive T and B cells, and also the innate NK and dendritic cells. The notch family of transcription factors decides the T- or B-cell choice.
- Dendritic cells (these are not nerve cells) – can arise from either myeloid or lymphoid lineages.

Figure 2A.2: Signaling



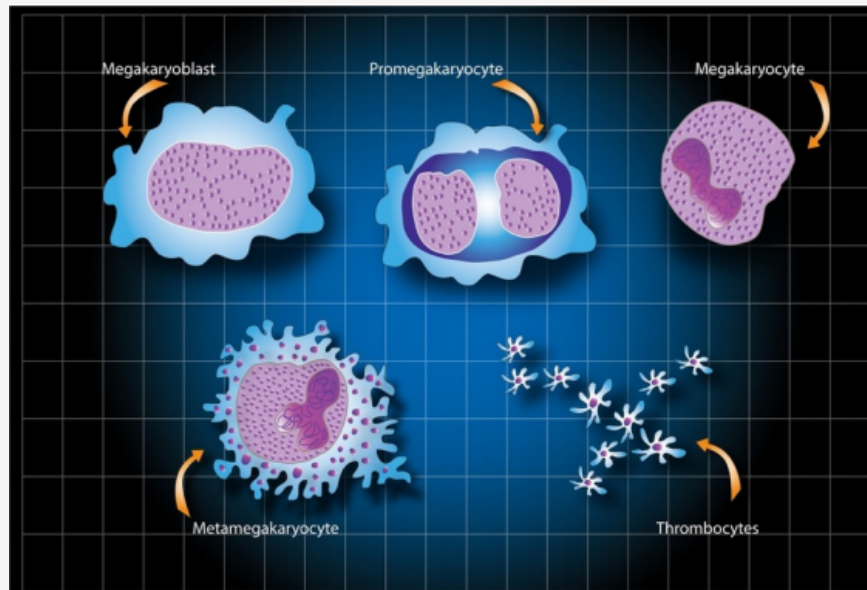
C. Historical Baggage

1. red blood cells, or erythrocytes. (*Figure 2A.3*)
2. platelets, or cell fragments from the megakaryocytes. (*Figure 2A.4*)
3. white blood cells, or leukocytes, which include cells from both the myeloid and lymphoid lineages.

Figure 2A.3: Erythrocyte



Figure 2A.4: Platelet



III. Myeloid Cells

A. Immune Involvement a Secondary Function

1. red blood cells – only extrude nuclei in mammals
2. megakaryocytes – pinch off platelets and cell fragments without nuclei.

B. Granulocytes: These cells all have specific granules that compartmentalize potentially dangerous molecules.

1. neutrophils – the infantry of the system, in the most modern techie sense.
 - a. strongly phagocytic – first responders to infection and population expands if they infection does not rapidly clear. (*Figure 2A.5*)
 - b. typically live for only a day (in some ways, these guy resemble Kamikaze pilots) and remains accumulate in an infected region as pus. (*Figure 2A.6*)
 - c. granules stain with both acidic and basic stains (different granules with different functions).
 - d. nucleus multilobed (sometimes called a polymorphonuclear leukocytes)

Figure 2A.5: Neutrophil

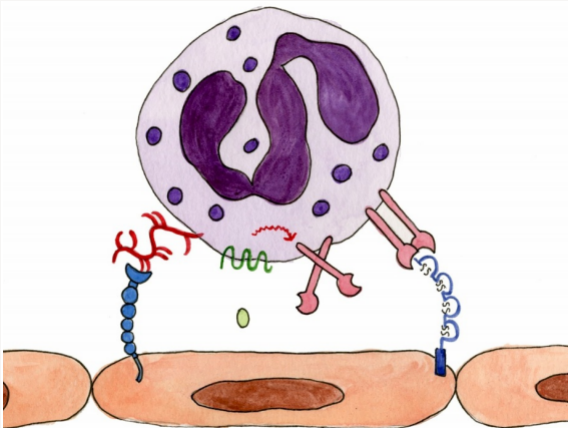
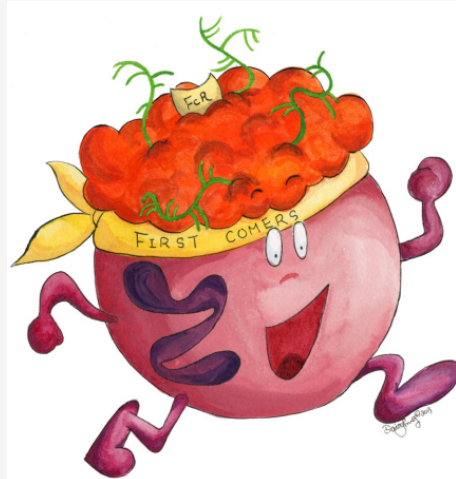
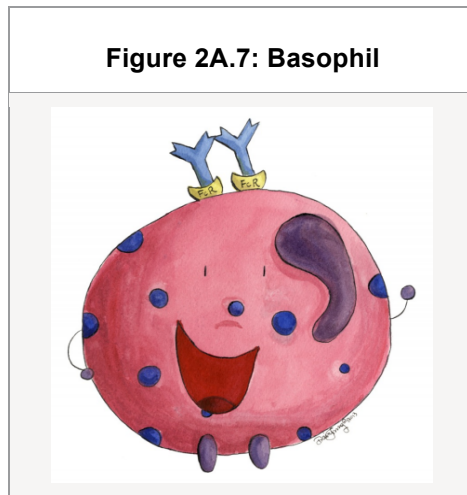


Figure 2A.6: Neutrophil



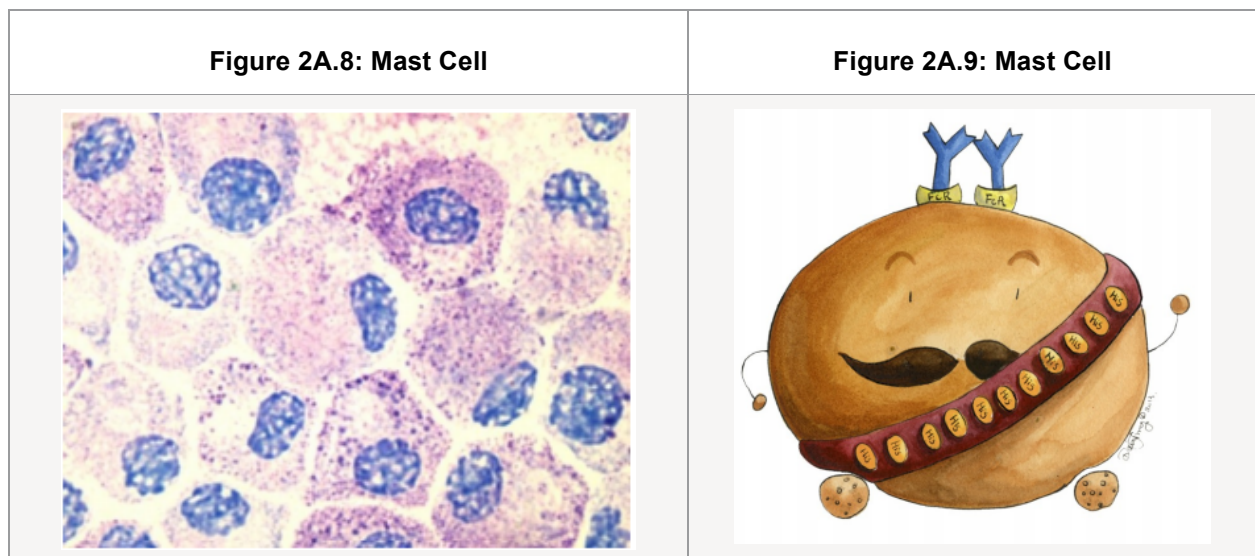
2. basophils

- a. granules with histamines stain with methylene blue, a basic stain
- b. lobed nucleus (*Figure 2A.7*)
- c. not phagocytic
- d. respond to worms



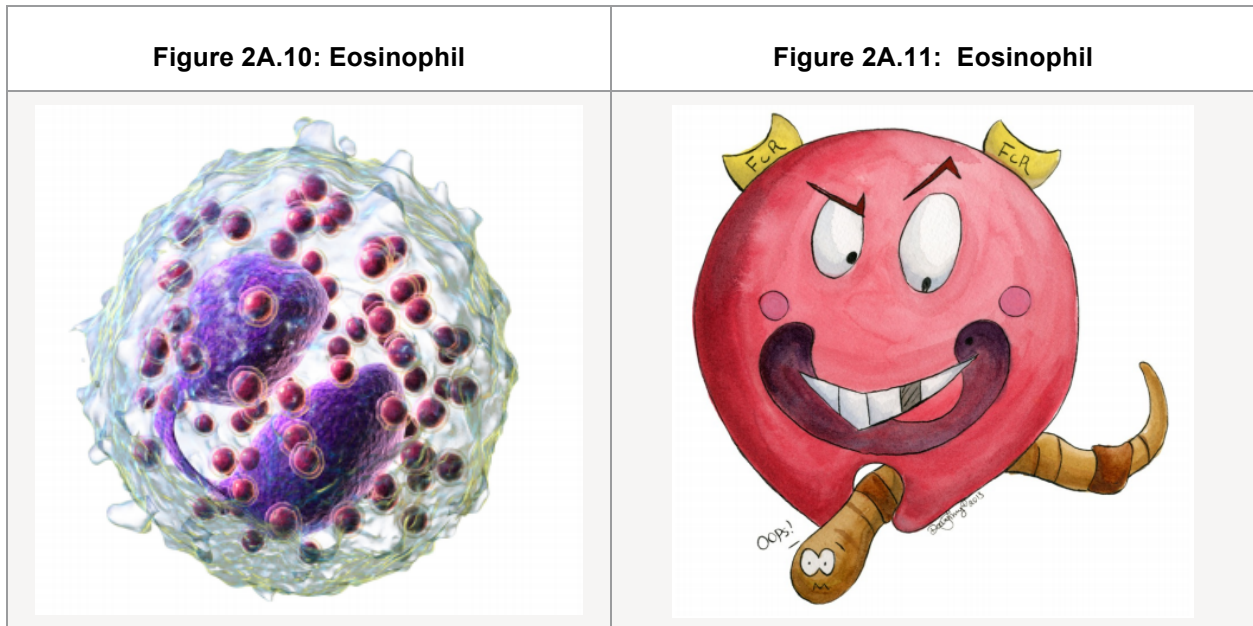
3. mast cells similar to basophils, only they associate with tissues instead of circulating.

- a. also basic granules with histamines (*Figure 2A.8*)
- b. non-lobed nucleus (*Figure 2A.9*)
- c. released as undifferentiated cells, maturing in their tissues.
- d. have other immune regulatory functions



4. eosinophils

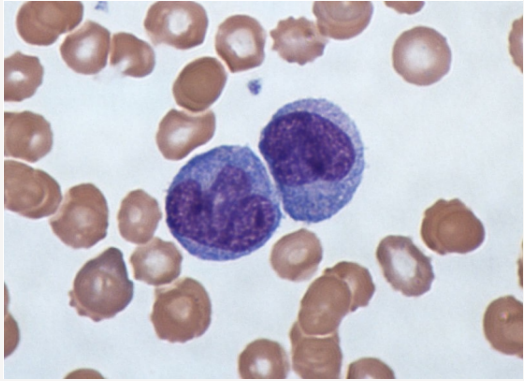
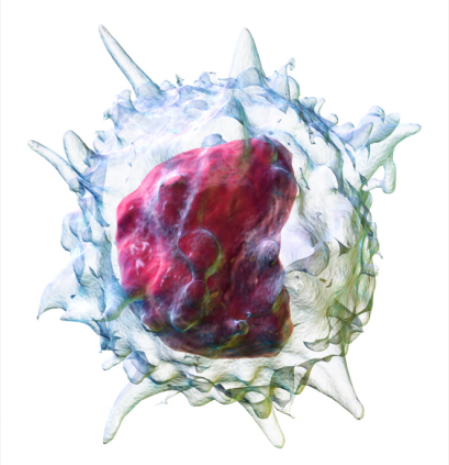
- a. granules stain with eosin red, an acidic stain, have hydrolytic enzymes (*Figure 2A.10*)
- b. bilobed nucleus (*Figure 2A.11*)
- c. phagocytic, though less important
- d. target worms



C. Myeloid Antigen-Presenting Cells, called mononuclear because the nuclei are unlobed and look like proper single nuclei. These cells are a little like cavalry or scouts: They both patrol and report back and may kill bad guys.

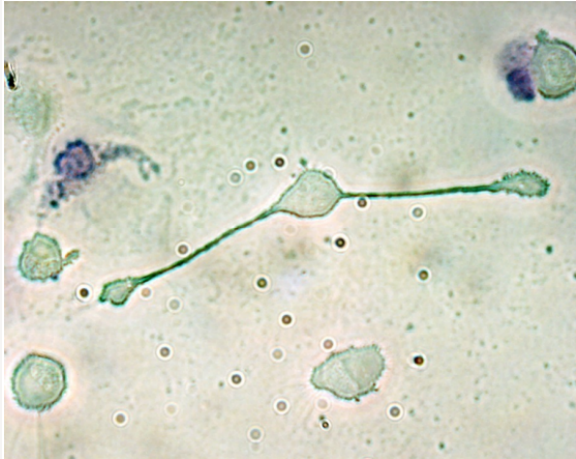

1. monocytes

- a. circulate in blood for about 8 hours (*Figure 2A.12*)
- b. enlarge and give rise to - macrophages (*Figure 2A.13*)

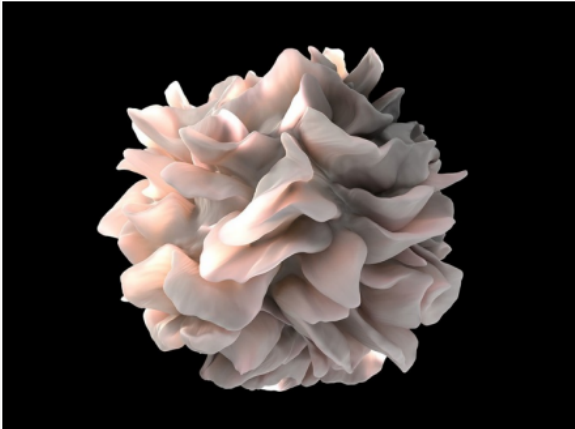
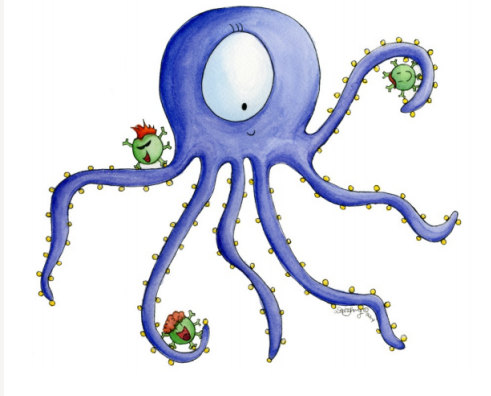
Figure 2A.12: Monocyte	Figure 2A.13: Monocyte
 A light micrograph showing several monocytes. These cells are larger than the surrounding red blood cells, with a characteristic kidney-shaped or bilobed nucleus stained purple. The cytoplasm is light blue.	 A 3D surface model of a monocyte. The cell has a large, irregular, reddish-brown nucleus and a highly convoluted, spiky outer membrane shown in light blue and green.

2. macrophages

- migrate into tissues by amoeboid motion, enlarge five to 10 fold (*Figure 2A.14*)
- phagocytize pathogens and debris from dead cells. Antibodies attached to pathogens make this easier. (*Figure 2A.15*)
- present antigen derived from phagocytosis to T_H cells.
- subtypes guard specific tissues, becoming a fixed part of the structure.

2A.14: Macrophage	2A.15: Macrophage
 A light micrograph of a macrophage. The cell is large and irregularly shaped with a foamy or vacuolated appearance. It has a large, dark, multi-lobed nucleus. Several small, dark-stained granules are visible within the cytoplasm.	 A cartoon illustration of a macrophage as a yellow, blob-like character with a large, open mouth showing a red tongue and white teeth. It has a single eye and a small antenna. A small brown horse is riding on its back, and a blue bat-like creature is flying nearby.

3. sentinel dendritic cells
 - a. NOT related to nerve cells – just have a lot of extensions
 - b. phagocytize pathogens and debris, but also use receptor mediated endocytosis and pinocytosis (*Figures 2A.16-17*)
 - c. hang out in the peripheral tissues and only migrate to secondary lymphoid organs if they sense something suspicious
 - d. present antigen to T_H cells- most effective cell for initiating the immune response
 - e. Some types develop from the lymphoid lineage

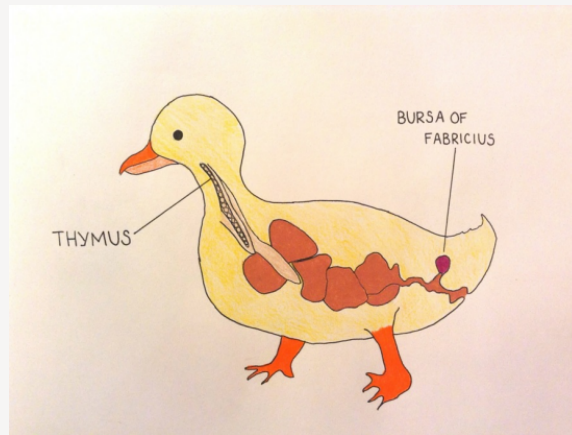
Figure 2A.16: Sentinel Dendritic	Figure 2A.17: Follicular Dendritic
	

IV. Lymphocytes make up 20 to 40% of the circulating leukocytes in the blood and 99% of the cells of the lymph.

A. B Cells (The equivalent of 007's M)

1. The name derives from their origin. They mature in the
 - a. bursa of Fabricius in birds – (which is the dorsal wall of the cloaca) (*Figure 2A.18*)
 - b. bone marrow of most mammals

Figure 2A.18: Bursa of Fabricius



2. B-cell surface markers

- a. Membrane-bound antibody - arms of the Y stick out from the cell
- b. Class II MHC molecules - allow B cells to present antigen to T cells after activation

3. B-cell lineage (*Figure 2A.19*)

- a. small lymphocytes - naïve, unexposed to antigen. Only $6\ \mu$ in diameter and indistinguishable from naïve T cells. Enlarge during differentiation.
- b. plasma cell - loses surface antibody and secretes soluble antibody, and then dies in a couple of weeks by apoptosis. Lots of RER. (*Figure 2A.20*)
- c. memory cell - held in reserve if the infectious agent should reappear.

Figure 2A.19: B-Cell



Figure 2A.20: Plasma Cell



B. T Cells

1. The name derives from the fact that they mature in the thymus.
2. T-cell surface markers include T-cell receptor
 - a. antigen receptors, which differ in structure and function from embedded antibody
 - b. binds antigen bound to a MHC molecule of a presenting cell, infected cell, cancer cell (altered-self)
3. T-cell lineage (*Figures 2A.21-2A.23*)
 - a. small lymphocytes - naïve, unexposed to antigen - visually indistinguishable from B version, but with different surface receptors. Also enlarge as during differentiation.
 - b. T_C (cytotoxic - attack altered-self cells) - receive antigen presented with class I MHC, have CD8 in membrane- the sappers of the immune response, in the sense of blowing things up or possibly the FBI in the sense that they check for misbehaving members of the body's citizenry.

Figure 2A.21: T Cell and Platelet

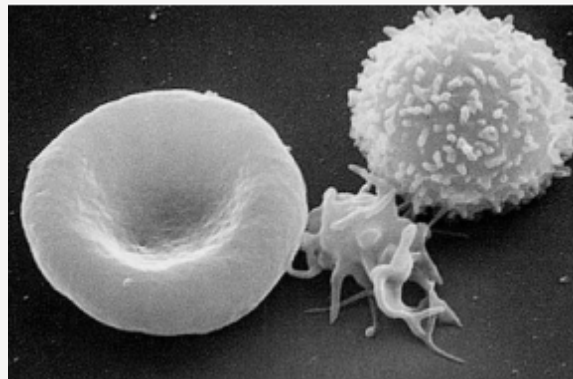
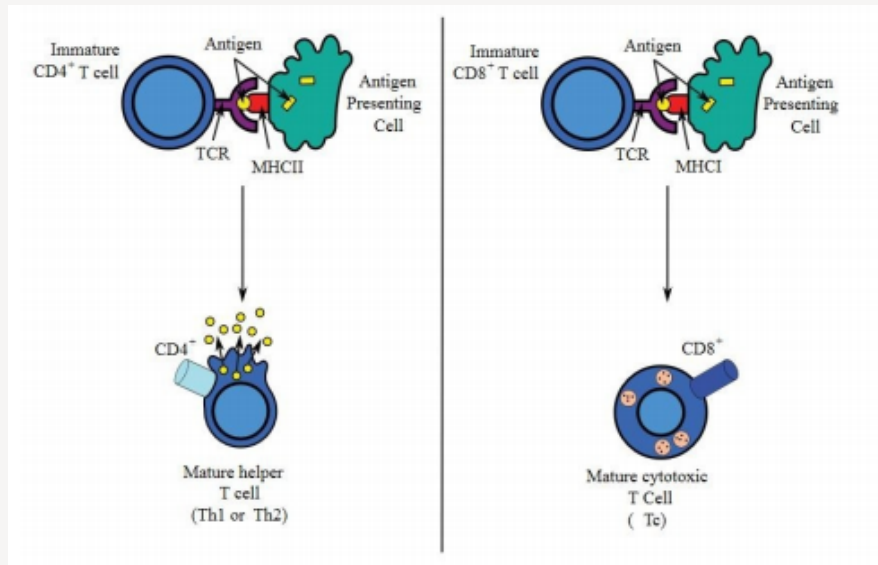
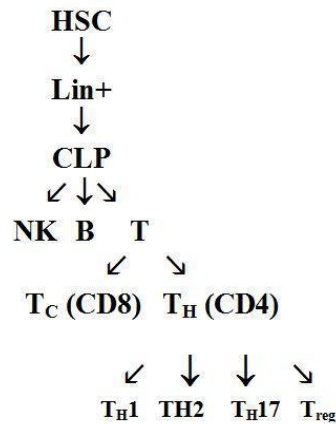


Figure 2A.22: T Cell Interaction



- c. T_H (helper) –the officers of the immune response. They direct immune response by signaling other cells via cytokines. - receive antigen presented with class II MHC, have CD4 in membrane. The officers of the immune response
 - i. T_H1- direct all-out response against intracellular pathogens
 - ii. T_H2- directs restrained containment response against chronic diseases, including many worms.
 - iii. T_H17 – directs response against extracellular pathogens, especially fungi
 - iv. T_{reg} – down-regulated immune response- the diplomatic corps.
- d. memory cells - held in reserve if the infectious agent should reappear.

Figure 2A.23: T Cell Lineage



C. NK (*Figure 2A.24-2A.25*), or Natural Killer cells (the Seals or Green Berets of the immune response) – no T cell receptors, no CD4, no CD 8, but they do have:

1. MHC I receptors. They do not recognize antigen, but rather trigger an attack if a cell doesn't have MHC I.
2. Antibody receptors (CD 16) can also recognize antibodies bound to altered cells, which triggers NK attack.
3. large, with lots of granules containing enzymes and other molecules used to kill aberrant cells, and apoptosis triggers extending from the plasma membrane.
4. released from the bone marrow ready to kill

Figure 2A.24: NK Cell

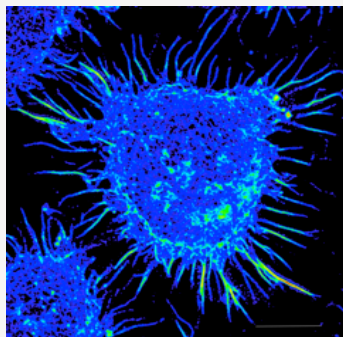


Figure 2A.25: NK Cell



The More You Know: Optional Resources and Fun Stuff
(You don't get tested in this!)

Primary Organs of the Immune System

A. Youtube:

This last for 90 minutes, but it's a different approach to much of the material in the first two lectures. UCTV Dr. Anthony de Franco.

<http://www.youtube.com/watch?v=mFNxXfwIP3A>

B. Leukocytes and Analogies tables. See next pages 32-33

Table 2A.1 Leukocytes

Leukocytes

type		name	function	characteristics	Surface expression	Responds to	releases
lymphoid	adaptive	T _H (helper) lymphocyte	Activates inflammation, macrophages, and B cells	Coordinates immune response	CD4 Receptors for class II MHC plus antigen	Antigen presented on class II MHC	Variety of cytokines
		T _R (regulatory) lymphocyte	Suppresses immune response, generally and of specific lymphocytes	Prevents allergy and autoimmune diseases	CD4 and CD25 when naïve	Mechanism of action still mysterious	Cytokines: IL-10, TGFβ
		T _C lymphocyte or cytotoxic T cell	Destroy altered-self cells	Kills viral infected and malignant cells	CD8 Receptors for class I MHC plus antigen	Antigen presented on class I MHC	Perforin and granzymes
		B lymphocyte	Recognize antigen can present antigen to T cell	Develops into plasma cell	membrane bound antibodies, class II MHC, complement receptors 1 and 2	Circulating antigen, T _H cytokine signals	
		Plasma cell	Secretes antibodies	Mature B cell, lives 1 - 2 weeks	No surface antibodies	T _H cytokine signals	antibodies
	innate	Natural Killer (NK) cell (null)	Destroy altered-self cells	Kills viral infected and malignant cells. Can recognize altered-self on first exposure	CD16 – binds Fc (stem) of antibodies, MHC receptor-when activated, inhibits killing	Antibodies and down-regulated MHC I	Perforin and granzymes

granulocytic myeloid cells	neutrophil	Phagocytosis, part of inflammatory response. Move into infected tissues	picks up both acidic and basic dyes; hydrolytic enzymes in granules	Fc and complement receptors	chemokines	
	basophil	Important in allergy	picks up basis dyes, e.g. methylene blue	Fc receptors for IgE	chemokines	prostaglandins and leukotrienes
	mast cell	Inflammatory response, especially to allergies	Mature cells in skin, mucosal, digestive tract and other first defense tissues.	Fc receptors for IgE		
	eosinophil	defense against parasites	Pick up acidic dyes, e.g. eosin	Fc receptors for IgE	chemokines	anti-helminth agents
antigen-presenting myeloid cells	Sentinel dendritic cell	Process and present antigen to T _H cells	Covered with p.m. extensions, potent antigen presenters	Class II MHC, B7, toll-like receptors		
	monocyte	Macrophage precursors	Circulate in blood for 8 hours, then differentiated into macrophage			
	macrophage	Phagocytosis of microorganisms and debris, present antigen after activation	Move through tissues, differentiated from monocytes	Class II MHC, toll-like receptors	Interferon from T _H	
NOT from hematopoiesis	follicular dendritic cells	feed antibody-antigen complexes to B cells	found in secondary lymphoid organs	receptors to hold antibody –antigen complexes		

Table 2A.2 Analogies

Analogies			
element	function	characteristics	comparison
hypothalamus	Assesses overall states	Decides how much energy the body spends on immunity	Federal government – Congress, executive branch, permanent agencies
T _H (helper) lymphocyte	Activates inflammation, macrophages, and B cells	Coordinates immune response	Military officers – captains, majors and generals
T _R (regulatory) lymphocyte	Suppresses immune response, generally and of specific lymphocytes	Prevents allergy and autoimmune diseases	Diplomatic corps
T _C lymphocyte or cytotoxic T cell	Destroy altered-self cells	Recognizes signatures on MHC I	Sappers, Seabees, military engineers
Natural Killer (NK) cell (null)	Destroy altered-self cells	Can recognize altered-self on first exposure	Navy Seals, Green berets, etc.
B lymphocyte	Recognize antigen can present antigen to T cell	Develops into plasma cell	James Bond's M – the guy who invents
antibodies	Recognize specific pathogenic signatures	Tie up pathogens, direct immune cells to pathogens	Smart bombs with homing devices and signaling units
neutrophil	Phagocytosis, part of inflammatory response.	Move into infected tissues	infantry
basophil eosinophil mast cell	Important defense against parasites	May produce allergic response	Policing function where you want to keep the bad guys contained without harming the citizens.
Sentinel dendritic cell	Process and present antigen to T _H cells	Covered with p.m. extensions, potent antigen presenters	scouts
macrophage	Phagocytosis, present antigen after activation	Move through tissues, differentiated from monocytes	cavalry
Follicular dendritic cells	Present antigen-antibody complexes to B cells	Found in lymph nodes, improve Ig affinity	M's cadre of techies, working to improve defenses
Complement proteins	Attach to pathogens and debris	Kill pathogens and summon immune cells	Land mines
Megakaryocyte: (platelets and fibrinogen)	Recognize mechanical damage	Plug holes in vessels, summon immune cells	City's Public Works department

Lecture L02b

Surveying the Cells & Organs of the Immune System, part b

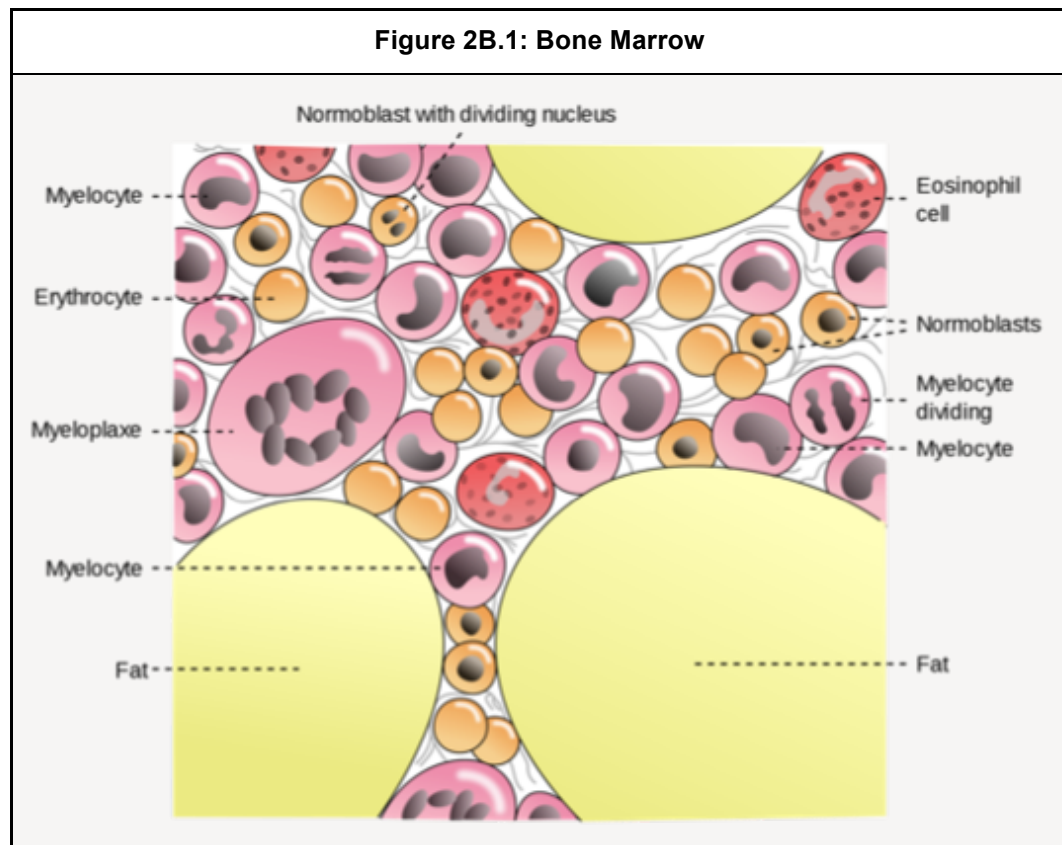
I can assure you that peace will not be built on poor nutrition and human suffering. - Norman Borlag, 11/19/01 (from talk at Rice University)



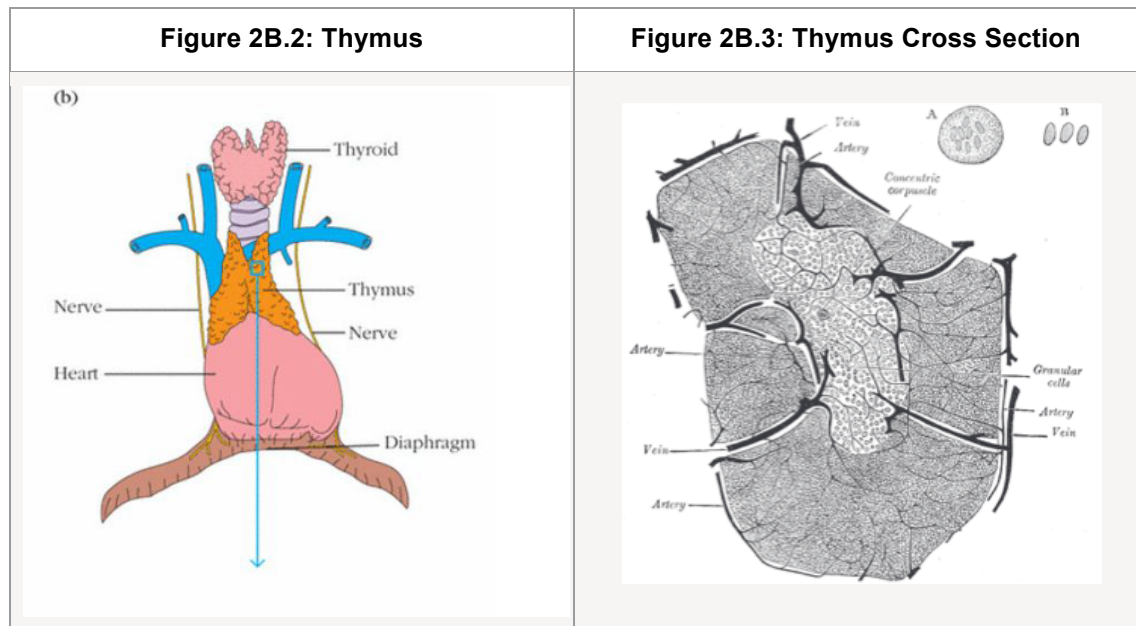
I. Primary Organs of the Immune System

A. Bone Marrow (Figure 2B.1)

1. Location of HSCs, myeloid cell production, and initial division of lymphoid cells.
2. NK cells rise from here and B cells divide and rearrange their genes here.
3. T cells undergo initial commitment here, but then leave for the thymus to finish rearranging genes and determining their specific roles.
4. Marrow of femur, humerus, hip bones and sternum are major sites.



B. Thymus (*Figure 2B.2*)



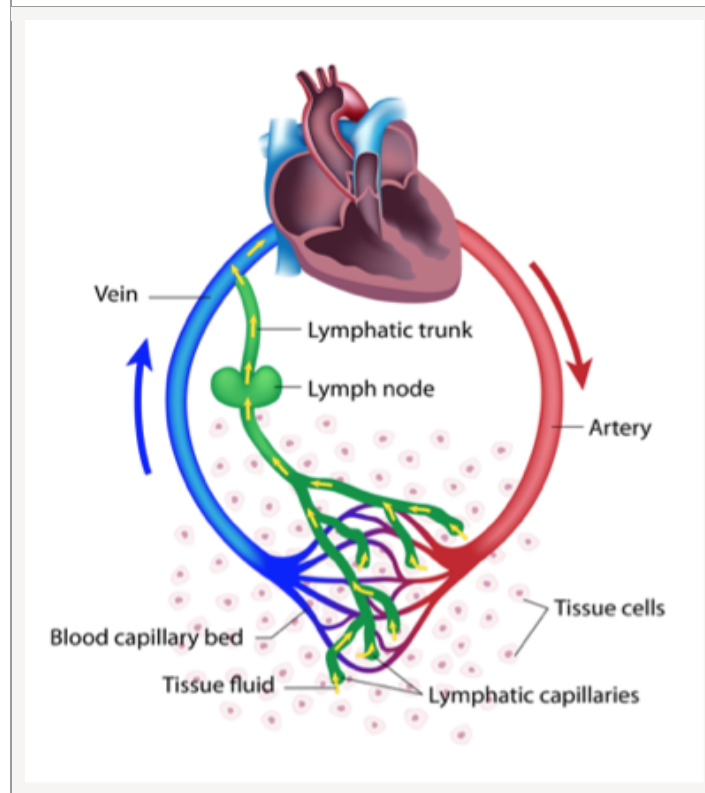
1. located above the heart, below the thyroid and behind the upper part of the sternum.
 - a. cortex (general word meaning outer layer) - Immature T cells (thymocytes) start here.
 - b. medulla – (general word meaning interior) – Final Quality check.
2. Site of T-cell maturation (details of the process later) (*Figure 2B.3*)
 - a. Cells rearrange genes for TCR (the T-cell receptor) in cortex.
 - b. Cells are checked for the ability to recognize antigen on MHC with the correct affinity (positive selection) in cortex.
 - c. Cells that survive selection travel through the medulla and undergo selection to remove self-reactive cells (negative selection).
 - d. Cells that survive enter the circulation.
 - e. Cells that do not (over 95%) undergo apoptosis.

II. Secondary Organs of the Immune System– an interconnected surveillance system, where the immune cells gather and exchange information.

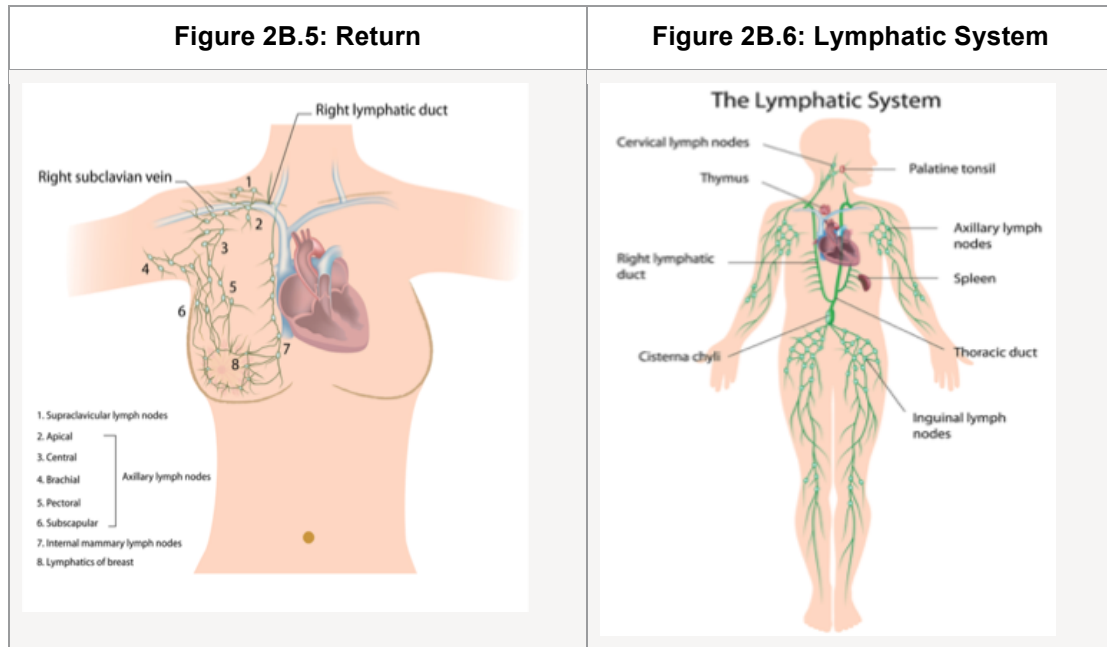
A. Circulation among the organs: lymph makes a one-way trip, while blood makes a round trip.

1. Blood moves immune cells throughout the body (along with erythrocytes) (*Figure 2B.4*)

Figure 2B.4: Blood Movement



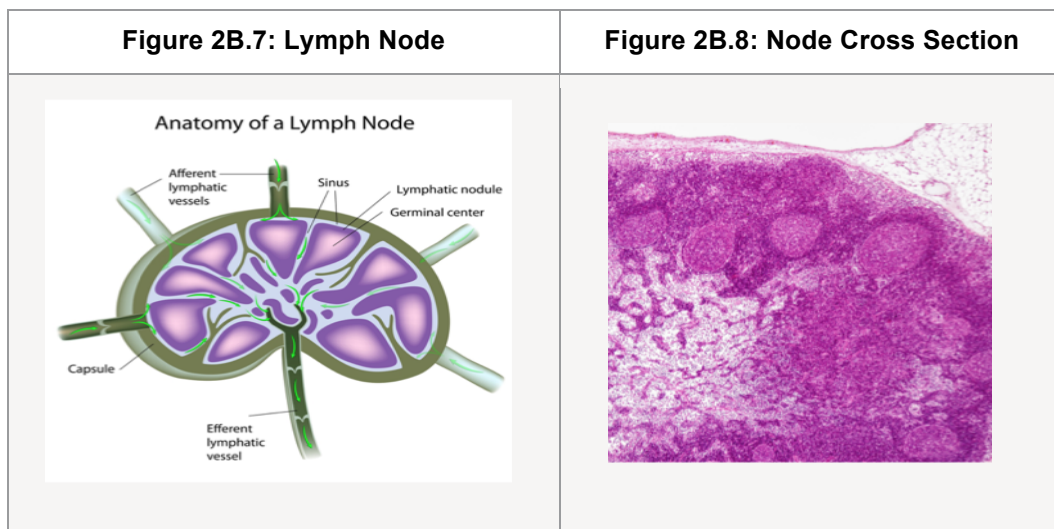
- a. The lining of vessels (endothelium) responds to infections with inflammation and this directs neutrophils and other immune cells to the infected site.
- b. Proteins in blood plasma include antibodies, clotting proteins and complement proteins that attack foreign cells.
- c. Blood filtered by spleen, which recycles aged erythrocyte and picks of antigen and other detritus.
2. Lymph also provides transport of immune cells, primarily lymphocytes, but no erythrocytes (*Figure 2B.5*)
 - b. Drains interstitial fluid from tissues, picking up antigens and white cells (*Figure 2B.6*)
 - c. Lymph (fluid) filtered through lymph nodes, where antigen is trapped and acted on.
 - d. Vessels join into larger ones that empty into the thoracic duct, which in turn empties into left subclavian vein and then enters heart.



B. Lymph nodes - trap antigen and provides sites for the lymphocytes to interact with antigen.

1. Basic structure (*Figure 2B.7-2B.8*)

- cortex receives incoming lymph (afferent)
- follicles embedded in cortex receive and hold B cells
- paracortex (immediately inside) hold T cells
- mature B cells leave through this
- exit out the efferent vessels



2. Cell interactions

- a. B cells activated by antigen migrate to the paracortex to alert T cells. Some get instruction to go forth and make antibodies.
- b. Secondary follicle develops after antigen exposure. Has active germinal center where B cells develop in response to signal from follicular dendritic cells, T_H cells and macrophages.
- c. B cells that have spent time in a secondary follicle learn to make more effective antibodies.

C. Spleen (*Figure 2B.9*)

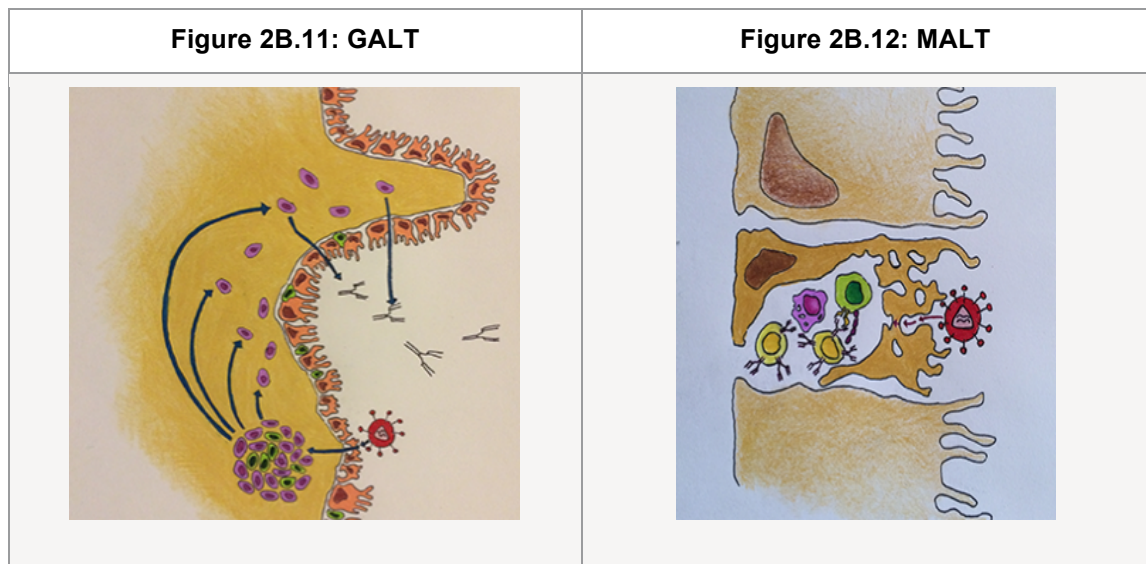
1. in abdomen, next to pancreas
2. filters blood, not lymph (*Figure 2B.10*)
3. red pulp with macrophages that recycle old red blood cells
4. white pulp (PALS) has T cells
5. marginal zone with B cells in follicles - system works like the lymph nodes:
6. Removing the spleen can increase a person's risk for bacterial infections, but there does seem to be some redundancy in the system as a whole.



D. Mucosal-Associated Lymphoid Tissue – MALT (*Figure 2B.11*)

Also gut associated – GALT, and bronchial (lung epithelia) – BALT, nasal – NALT

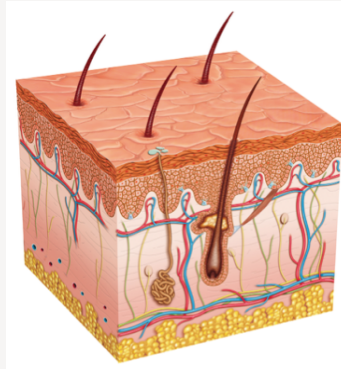
1. The mucosa of the digestive, respiratory, and urogenital systems represents the major site of entry of most pathogens.
2. The epithelia of these systems contain defensive lymphoid tissues.
3. Organized structures present include tonsils, appendix, and Peyr's patches in the intestine (*Figure 2B.12*)
4. Epithelial cells of the mucosa deliver antigen samples from the lumen, delivering them via M cells
5. M cells are large epithelial cells, each with a number of smaller immune cells residing in the basolateral pocket it makes.
6. Antigen crosses the plasma membrane to these. The B cell then migrates to inductive sites.



Skin- the largest organ of the body, not technically a Secondary Lymphoid Organ (*Figure 2B.13*)

1. Important in innate defenses
 - a. epithelial cells (keratinocytes) of the outer layer secrete cytokines
 - b. Also die, leaving behind keratin intermediate filament as a protective barrier.
2. Important in adaptive defenses
 - a. Keratinocytes can express class II MHC and present antigen.
 - b. Langerhans (dendritic) cell phagocytize antigen and carry it to lymph nodes. Also carry class II MHC and activate T_H cells.
 - c. Intraepidermal (a form of intraepithelial) lymphocyte, or IELs, many with specialized T cell receptors) - activated or memory cells.

Figure 2B.13: Skin

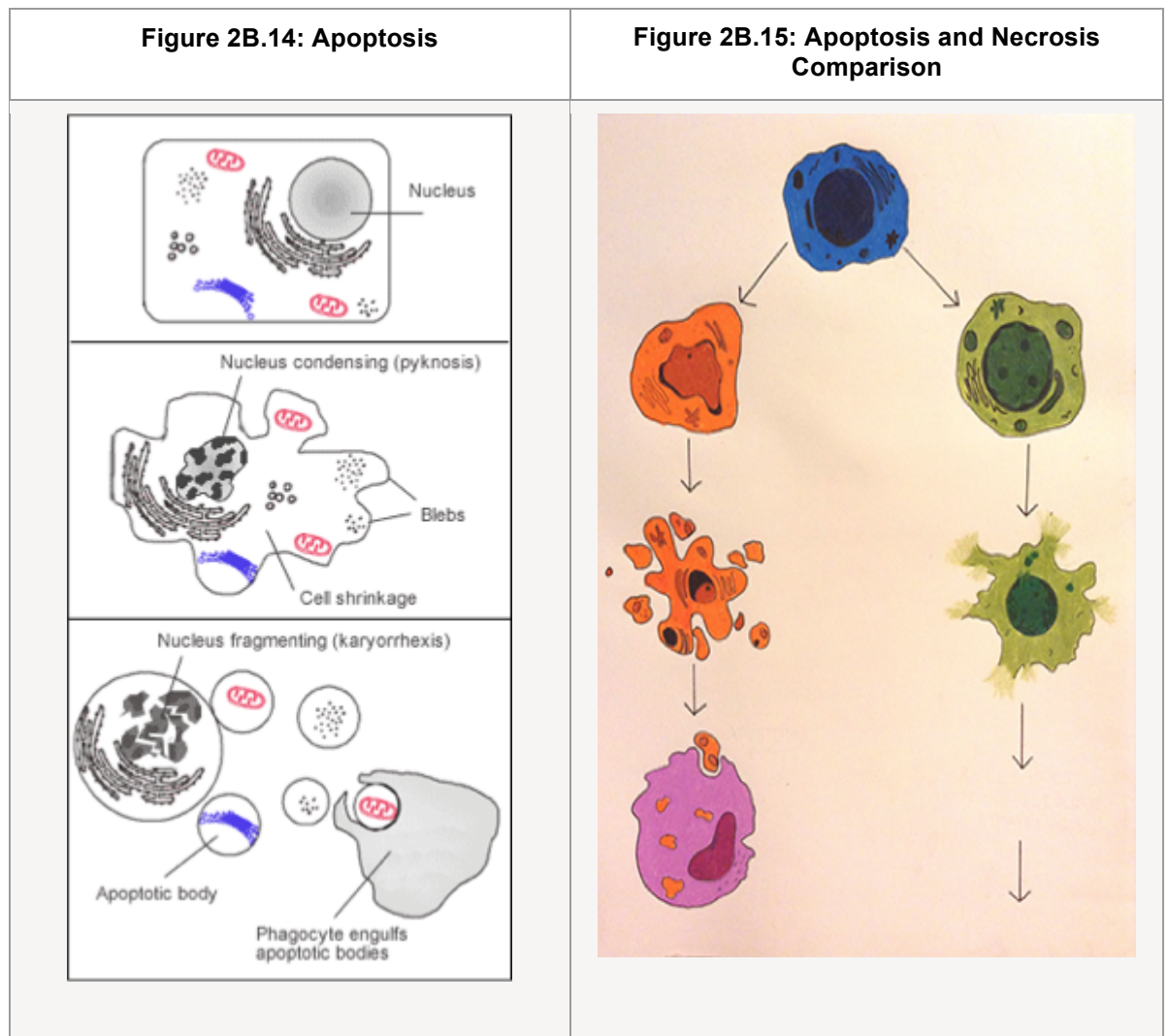


III. Final Issues:

A. Apoptosis (*Figure 2B.14*)

One aspect of the immune system that makes it so energetically expensive is that it produces huge numbers of cells and then gets rid of the great majority of cells before they are even used.

1. Analogous to imploding a building.
 - a. Cell shrinks
 - b. Chromatin condenses
 - c. Membrane blebs
 - d. Cell fragments into intact pieces, easily phagocytized
2. Necrosis – analogous to blowing up a building (*Figure 2B.15*)
 - a. Organelles swell and break down
 - b. Cell disintegrates
 - c. Contents released where they can cause tissue damage and inflammation
 - d. Much harder to clean up after



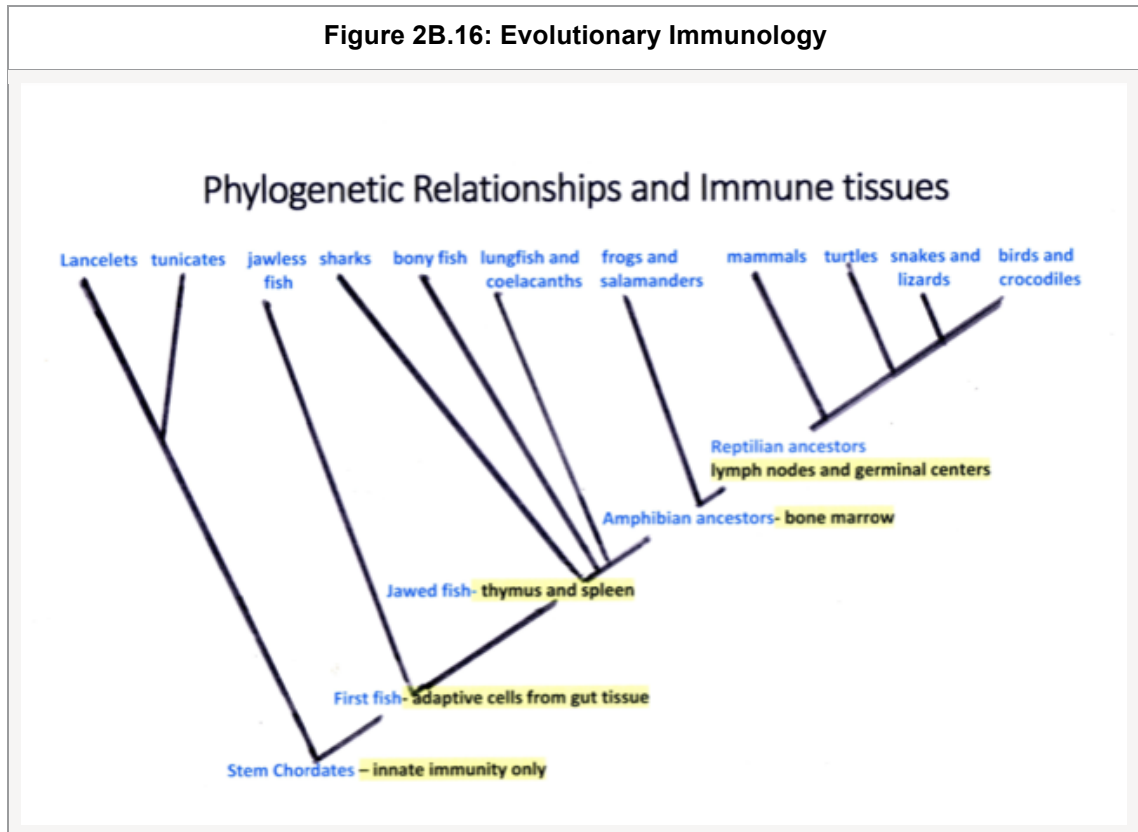
B. Evolution

Ancestral chordates, which gave rise to the vertebrate members of the phylum Chordata, do not have an adaptive immune system.

1. The first fish to evolve were jawless and we have only a few remaining examples of this type, among them the lamprey eel. These eels have B cells, GALT and some thymic tissue with T cells at the tips of their gills.
2. Other fish have immune tissue around the gut, as well as spleens and defined thymic tissue. (Figure 2B.16)

3. Amphibians, reptiles, birds and mammals all have bone marrow, but their B cells mature in a variety of places.
4. So, while it's true that reptiles, bird and mammals have B cells and T cell along with their innate defenses, there is a lot of variety in what gets made where and when.
5. Happily rodents and humans have reasonably similar immune systems, making mice and rats good lab models for the study of the immune response.

Figure 2B.16: Evolutionary Immunology



Lecture L03

Innate Immunity

Nothing in biology makes sense except in the light of evolution.
- Theodosius Dobzhansky



Table 3.1 Immune Response Summary

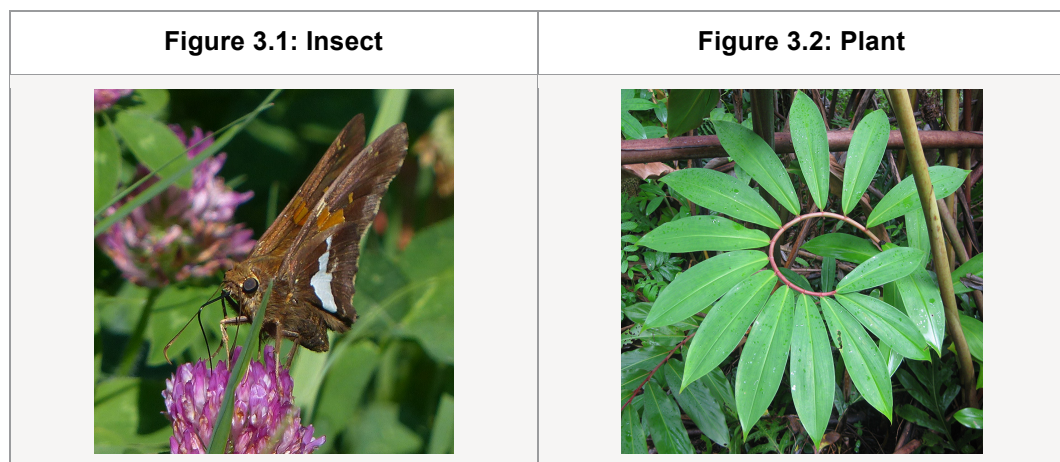
Summary of Distribution of Immune Responses

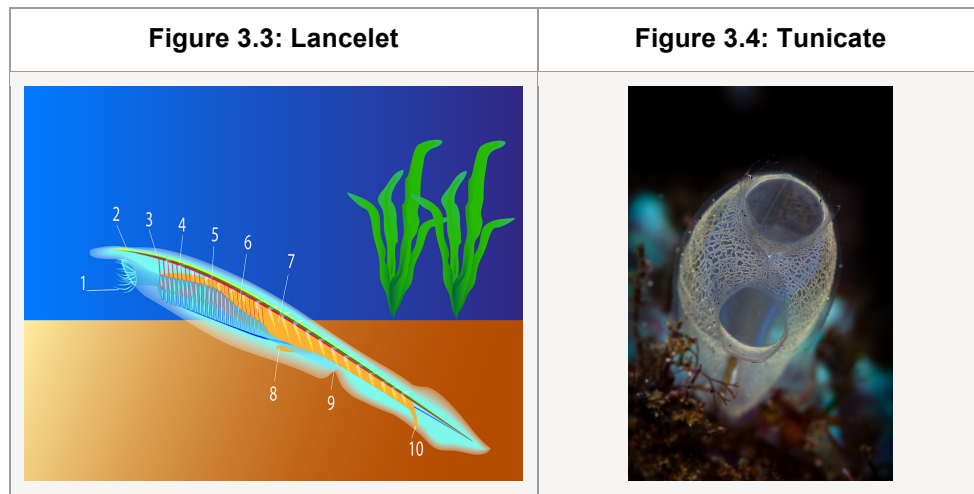
organism	innate defenses (PRR, attack peptides, and more)	phagocytosis	transplant (graft) rejection	adaptive defenses (B and T cells)
plants	yes	no	no	no
sponges	yes	yes	yes	no
arthropods	yes	yes	yes	no
fish	yes	yes	yes	yes
reptiles	yes	yes	yes	yes
birds	yes	yes	yes	yes
mammals	yes	yes	yes	yes

I. Basic Considerations- An innate defense is one that you can produce prior to exposure by a specific pathogen. Doesn't require changes to DNA/genes -- all organisms have a form of innate immunity.

A. Ubiquity

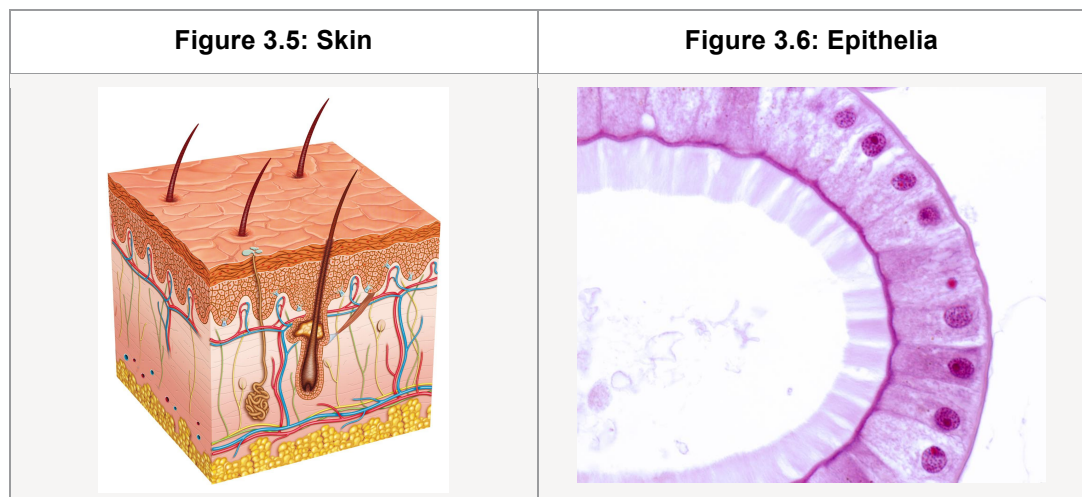
1. Insects have amoeboid cells patrolling their body cavities and can make anti-bacterial or anti-fungal peptides when challenged by specific pathogens. (*Figure 3.1*)
2. Plants have an even wider array of pathogen sensors than do mammals. (*Figure 3.2*)
3. Stem chordates, like the sea squirts (tunicates) and Amphioxus (lancelets), have innate, but not adaptive, defenses. (*Figures 3.3-3.4*)
 - a. Have spinal cord that runs down back, primitive members of our phylum.
 - b. Adaptive immunity only found in vertebrates – fish, mammals, reptiles, birds, amphibians.





B. Anatomic Barriers.

1. Skin (tertiary immune organ)— Few pathogens can penetrate intact skin, relying instead on bites and abrasions. Water-borne schistoma parasites, causing schistosomiasis, or bilharzia, are an important example. (*Figure 3.5*)
 - a. Epidermis - thin layer of dead cells (keratin) over a thinner layer of live regenerative cells over a basement membrane. Oil glands/hair follicles secrete sebum, antibacterial peptides, and psoriacin to kill bad bacteria (like *E. coli*), help promote good bacteria.
 - b. Dermis – deeper thicker layer with blood supply, connective tissue and mesenchymal tissue important in developmental signaling.



2. Mucosa– more vulnerable, typically single layer of epithelial cells over basement membrane over connective tissue. (*Figure 3.6*)
 - a. Secretions: tears, mucus, lysozyme - can tear up bacterial membranes.
 - b. Cilia (lungs and reproductive organs) sweep out pathogens and debris trapped in mucus
3. Additional GI defenses:
 - a. stomach acid (vultures) (*Figure 3.7*)
 - b. enzymes
 - c. competing flora (good bacteria)

Figure 3.7: Vulture



II. Inflammation

A. Fever

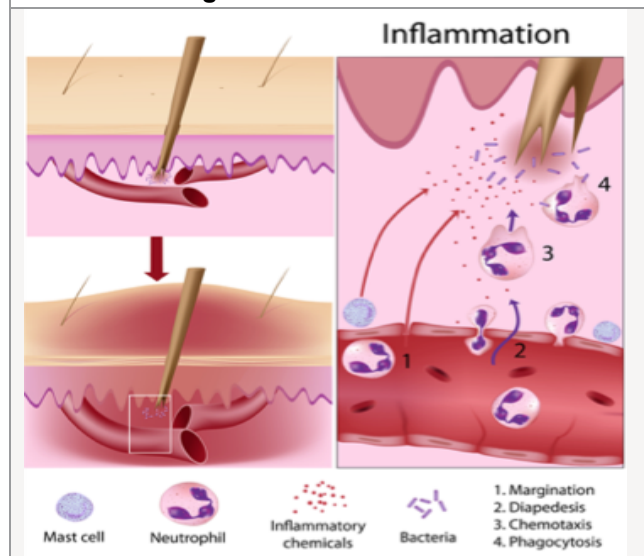
1. Hypothalamus controls the body's temperature set point.
2. Fever provides overt sign of immune up-regulation.
3. Higher body temperature associated with better survival rate, even in reptiles.
Reptiles are cold-blooded; a healthy reptile will want body temp. close to or a little lower than a human's for optimal enzyme functionality. However, if a reptile is sick, the reptile will go to hottest place available until it feels better. If you keep the reptile cold and don't let it get warmer, it has a lower survival rate.

4. Fever induction used with mixed success to treat cancer at the turn of the 20th century (Coley toxins).
5. Inflammation is a primary response that involves marshalling the whole immune system (especially the innate part at first) to fight a new pathogen threat. If you take an antipyretic, you are downregulating this inflammatory response and mitigating its effectiveness. Fever goes hand in hand with the inflammatory response, which is why Coley toxins are effective stimulants of strong immune response.

Figure 3.8: Inflamed Finger



Figure 3.9: Inflammation



B. Local Response: calls in phagocytic cells to the site of infection or injury.

1. Recognized in Roman times (heat, swelling, pain, reddening and loss of function).
TA Note: Sprained ankle won't get infected but definitely will get inflamed. Any type of injury, even internal, triggers local inflammatory response. Involves swelling (tissue leaks), redness (blood flow increased here), heating up, and pain. This helps by preventing you from doing something to exacerbate the injury and allows the body part to heal. (Figures 3.8-3.9)

2. Symptoms produced by vascular changes – capillaries become more permeable so that plasma (edema) and cells (extravasation) enter infected tissue.

3. Leukocyte extravasation is regulated by the changes in surface CAM molecules.

C. Signals and Receptors:

1. White cells and damaged tissues release chemokines, a type of cytokine, also generally up-regulate inflammation, attracting cells to the site of the infection. *(Figure 3.10)*

2. Activation of cells by pattern receptors also activates inflammatory signals.

3. Neutrophils leave the blood stream and respond by changing the conformation of their integrins, which makes them stick to the blood vessels near the problem.
(Figure 3.11)

a. Rolling

TA note: Eventually causes enough internal changes for the next step, activation, to occur. Neutrophils sticking and releasing, bouncing on endothelial surface.

b. Activation

c. Arrest

d. Transendothelial migration

TA Note: On its way to the tissue to instigate inflammatory response.

Figure 3:10: Extravasation

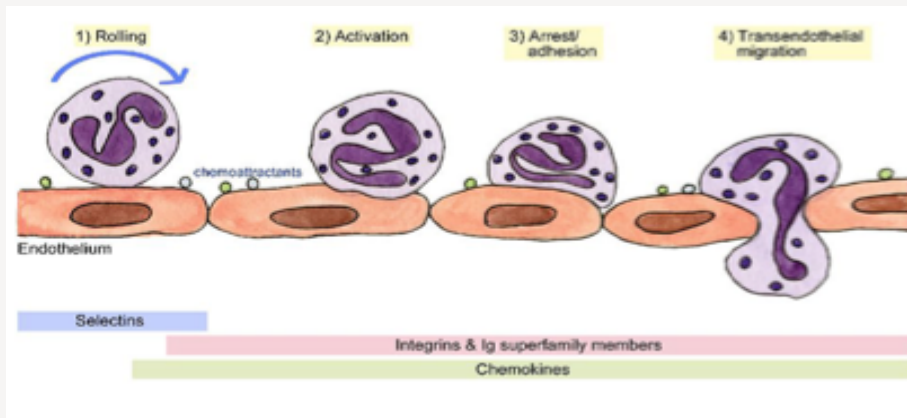
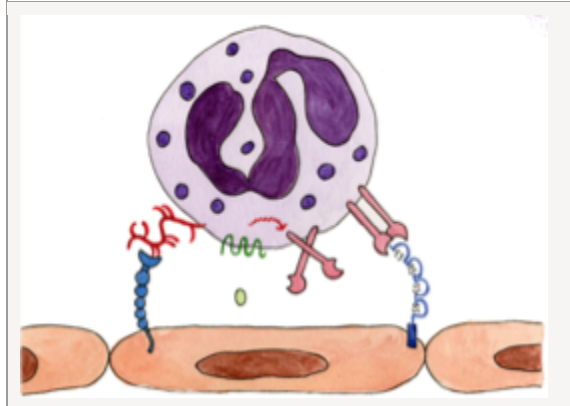


Figure 3.11: Neutrophil



4. The cytokines produced may also regulate the response so that it is most effective for the particular type of pathogen that initiated it.
5. Activated macrophages and dendritic cells then travel to the T_H cells and present antigen, specifically activating the adaptive response.
6. The T_H cells then coordinate an adaptive attack on the infections.

III. Innate Targeting of Pathogens

A. Reviewing the Bad Guys (Figures 3.12-3.17)

TA Note: How the innate immune system recognizes potentially dangerous pathogens. Mainly characteristic cell surface proteins, types of genetic material.

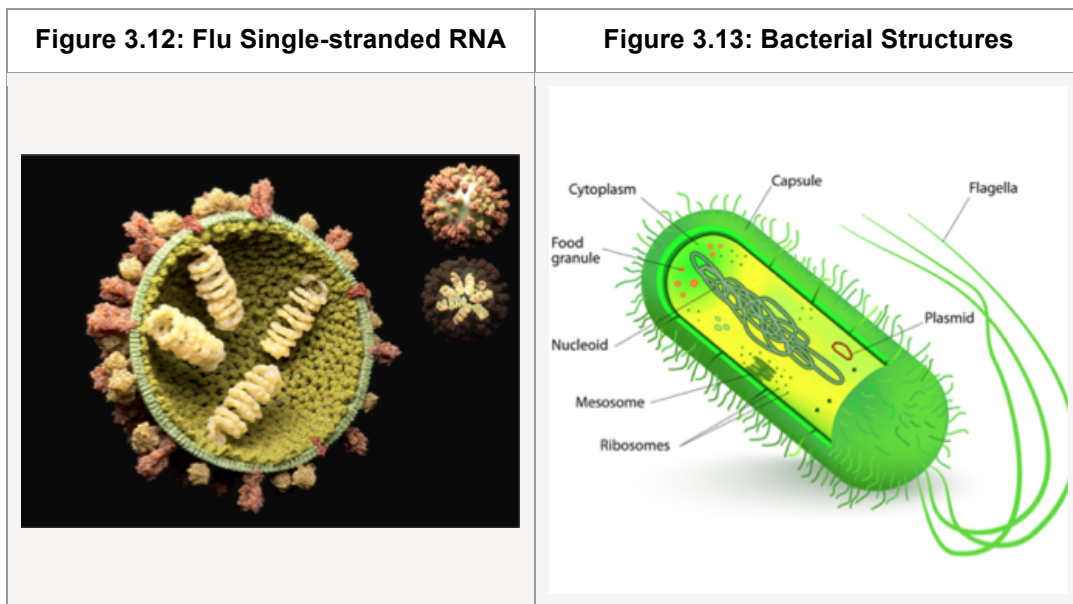


Figure 3.14: Candida (Yeast)

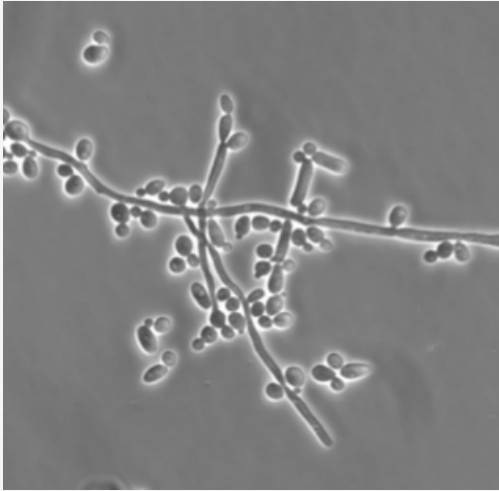


Figure 3.15: Giardia

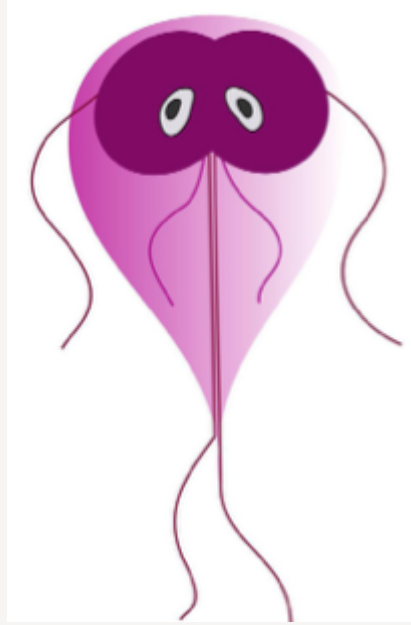


Figure 3.16: Trypanosome

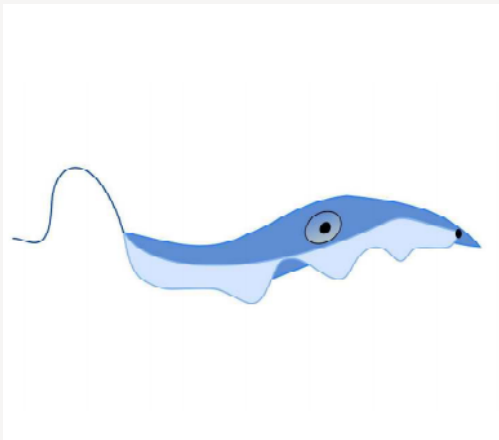


Figure 3.17: Tapeworm

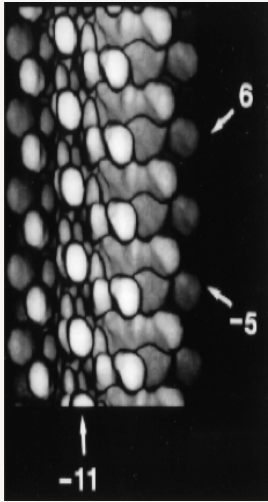
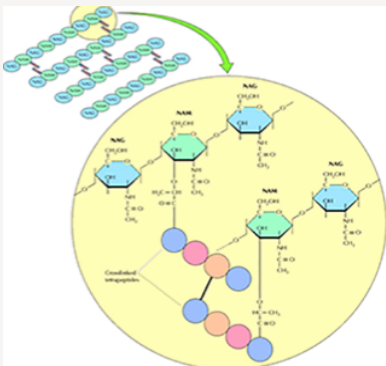
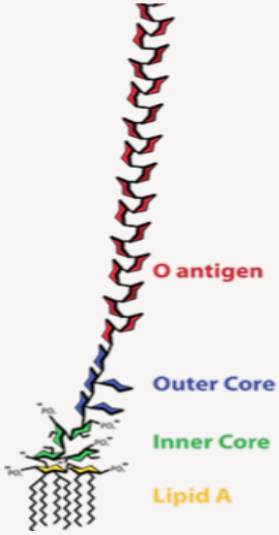


Table 3.2 Pathogen Recognition System

Pathogen	Examples	Factoids	Pattern recognition alerted by:
Viruses	flu, small pox, HIV, polio, Ebola, rhinovirus, hepatitis, measles	Can only reproduce inside cells	Foreign nucleic acids (double stranded RNA, single stranded DNA, foreign methylation patterns), reduced antigen presentation by infected cells
Bacteria	Strep, staph, TB, anthrax, leprosy, bubonic plague, pertussis, diphtheria	Reproduce intracellularly or extracellularly, depending on type	Characteristic surface carbohydrates (peptidoglycan, mannose repeats), flagellar proteins (flagellin), lipids (lipotechoic acid) characteristic DNA methylation patterns. Bacteria can't evolve version of flagella that can't be recognized anytime soon.
Fungi	Candida (thrush) athlete's foot, <i>Cryptococcus</i> , ringworm	Eukaryotic, unicellular, multicellular or multinucleate	Cell wall: zymosan (β 1-3 glucan) and chitin (cellulose with N-acetyl glucosamine instead of just glucose)
Protozoa	malaria, Chagas, sleeping sickness, amebic dysentery, leishmaniasis	Unicellular eukaryotes	Characteristic cell surface proteins (profilin) and lipids (glycosylated phosphatidyl inositols - GPI)
Worms (helminth parasites)	pin worms, hook worms, heartworms, schistosomiasis, flukes, tapeworms	Primarily members of Platyhelminthes (flatworms) and Nematoda (roundworms)	Characteristic cell surface proteins

B. PAMPs (Pathogen Associated Molecular Patterns) Recognized by PRRs – pattern recognition receptors.

1. proteins - So far, we have identified very few proteins that we recognize innately by their overall structure. The ones we've found are:
 - a. Flagellin (figure) – bacteria (*Figure 3.18*)
 - b. profilin – surface protein of protozoan (toxoplasmosis)
2. carbohydrates and glycopeptides
 - a. zymosan – component of fungal cell walls – a vague term because it has not been exactly characterized.
 - b. peptidoglycan – the cell wall component of both gram positive and gram negative bacteria, although gram positive have a much thicker wall. (*Figure 3.19*)
3. lipids, especially attach to signature carbohydrates or peptides. (*Figure 3.20*)
4. nucleic acids
 - a. DNA with specific methylation patterns - recall restriction endonucleases.
 - b. “wrong strandedness” – single stranded DNA and double stranded RNA are usually signs of a potential threat.
 - c. Mice can specifically recognize the 23s component of bacterial ribosomes

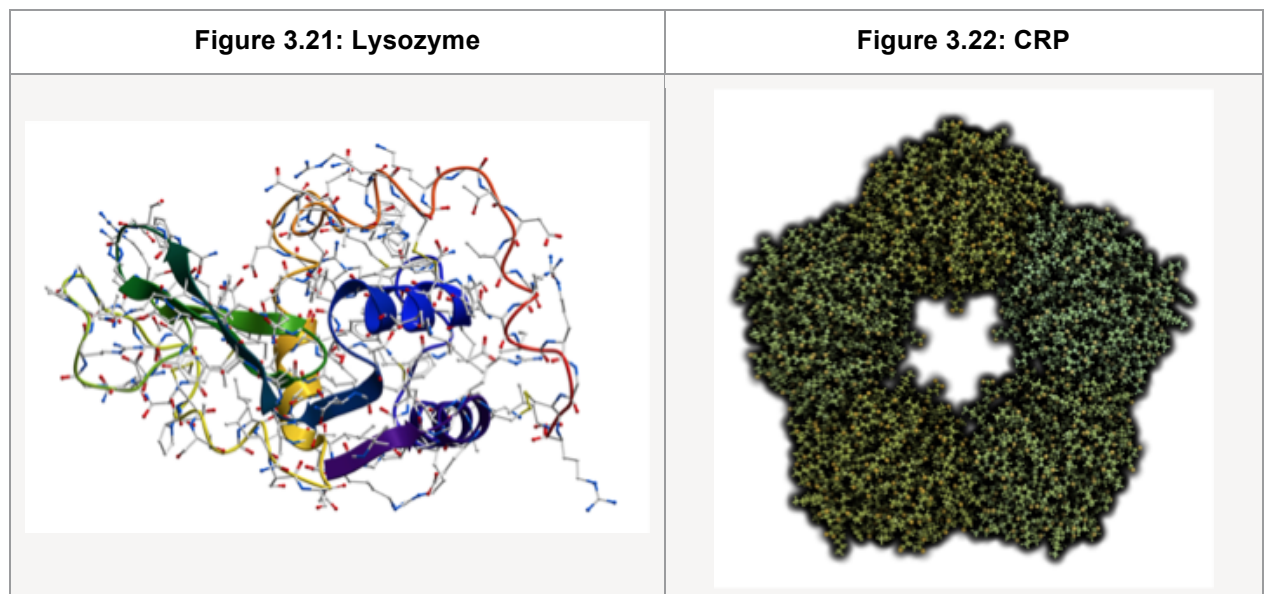
Figure 3.18: Flagellin	Figure 3.19: Peptidoglycan	Figure 3.20: Lipids
		

C. Categories of PRRs: Specific receptors bind to characteristic pathogen molecules.

1. Extracellular

- a. Lysozyme (mucus and tears) – against peptidoglycans (*Figure 3.21*)
- b. Psoriacin (skin) – against *E. coli*. Can be induced by sunlight (UVB) via vitamin D.
- c. AMPs – defensive peptides that typically kill by disrupting bacterial membranes.
- d. Mannose-binding lectin (plasma) – activates complement.
- e. C-reactive protein (CRP) (plasma) also recognizes microbes and damaged self-cells. (*Figure 3.22*)
- f. Lipopolysaccharide binding protein (LBP) specifically recognizes gram negative bacteria.

TA Note: Gram negative bacteria have relatively thin peptidoglycan walls, also have second membrane on outside often covered with lipopolysaccharide -- setting off a HIGHLY inflammatory response – can kill.



2. cytoplasmic - NOD proteins (Nucleotide-binding oligomerization domains)
3. Membrane Bound – the Toll-like receptors (TLRs) (*Figure 3.23*)
 - a. Work singly or in pairs. Singles extend onto the endoplasmic reticulum lumen, pairs from the plasma membrane.

- b. Binding of a molecule characteristic of a pathogen on the outside of the membrane triggers a signal on the opposite side.
- c. The internal signal activates NF- κ B, the major internal inflammation regulator.
- d. NF- κ B turns on the production of cytokines, alerting a variety of other immune cells.
- e. Identify the category of pathogen and set of inflammatory and adaptive response

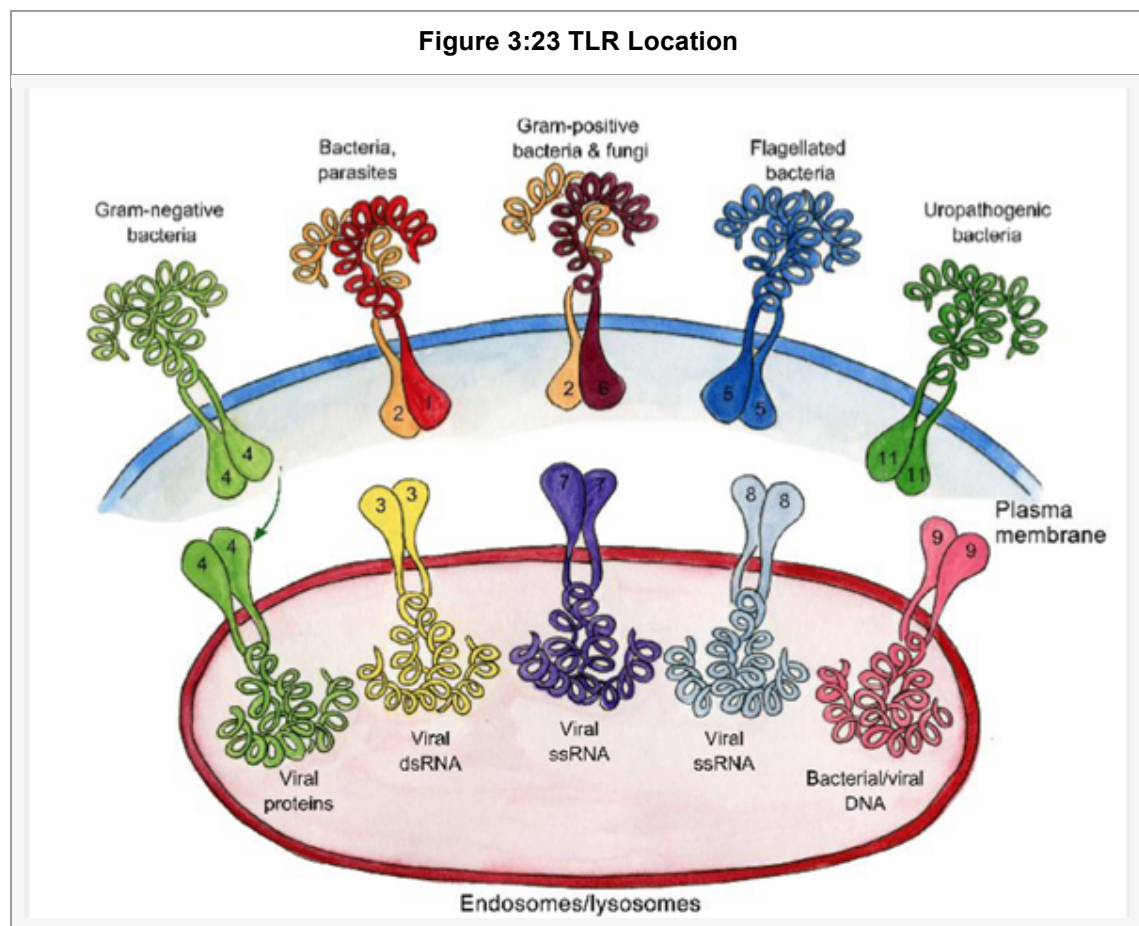
TA Note: Internal TLRs (including 3, 7, 8, 9) largely involved in recognition of bacterial/viral DNA. External (including 1, 2, 4, 5, 6) largely involved in recognition of characteristic cell surface components. Usually paired TLRs on outside and unclear if endomembrane TLRs operate singly or paired. For example, macrophages can use external TLRs to recognize pathogen cell surface molecules during phagocytosis, and after it internalizes the pathogens it can use internal TLRs to recognize internal pathogenic molecules such as their DNA or RNA.

Table 3.3 TLR

TLR	Recognizes	Pathogen	Membrane
1	Triacyl lipopeptides	mycobacteria	plasma
1 & 2	Peptidoglycan component and triacylated lipopeptides lipotechoic acid zymosan (β -glucan cell wall component) mucin	Gram+ bacteria mycobacteria yeasts and fungi trypanosomes	plasma
2 & 6	lipopeptides	Gram+ bacteria and mycoplasma	plasma
4 & 4	lipopolysaccharide (exterior membrane) F-protein	Bacteria, Ni RSV virus	plasma
5 (&5?)	Flagellin	Flagellated bacteria	plasma
6	Diacyl lipopeptides zymosan	Mycobacteria Yeasts and fungi	plasma
10 (Non-functional in mice)	unknown	allergens	plasma
11 (mice only)	Profilin	Eukaryotic parasites (also recognizes bacteria)	plasma
12 (mice only)	profilin	Eukaryotic parasites (especially trypanosomes)	plasma
13 mice only	bacterial 23sRNA	prokaryotes	endomembrane
3	Double-stranded RNA	viruses	endomembrane
7	Single-stranded viral RNA	viruses (HIV)	endomembrane

8	Single-stranded viral RNA	viruses	endomembrane
9	DNA with unmethylated CpG signatures	virus and bacteria	endomembrane

- f. Mechanism for endomembrane TLRs
- g. Negative regulation- you have to be able to turn these off or you get excess inflammation and maybe and autoimmune disease.
- h. Interact with: MD2 at surface for binding LPS (and also certain viral and cancer proteins), MyD88 to initiate internal signal sequence.



IV. Cell Types and Function

- A. *Phagocytosis – Macrophages and Neutrophils – capture pathogens and kill them with a complex toxic brew. (Figures 3.24-3.26)*

Figure 3.24: Macrophage

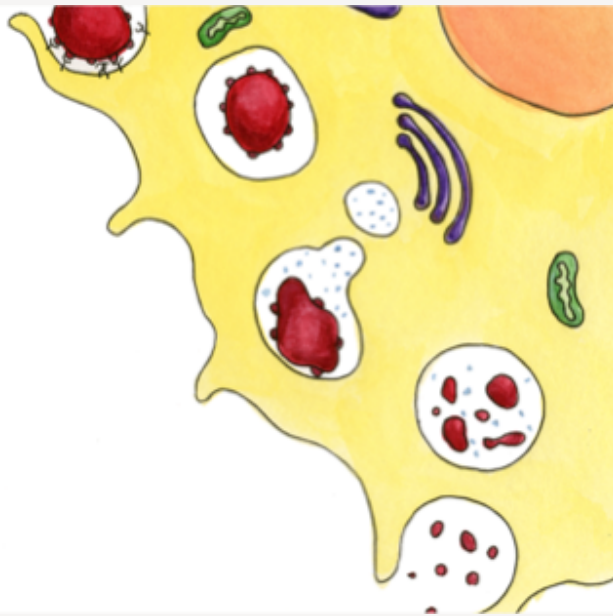
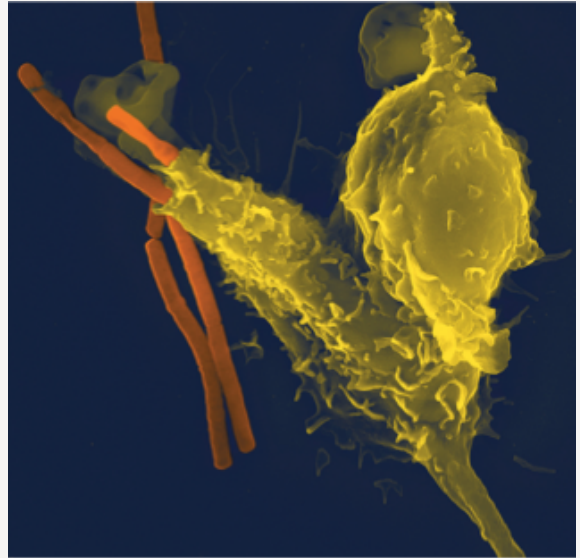


Figure 3.25: Neutrophil Anthrax



1. Capture pathogen in phagosome, promoted by PAMP, complement or antibody on surface of pathogen. - activates membrane pump which
2. Triggers respiratory burst (O_2 uptake) by NADPH phagosome oxidase (phox enzymes) complex. This is not mitochondrial respiration, but rather the direct uptake of oxygen by enzymes that use it to create toxic reactive oxygen species (ROS). (*Figure 3.27-3.28*)
 - a. superoxide radicals ($O_2^{\cdot-}$)
 - b. hydrogen peroxide (H_2O_2)
 - c. HOCL (hypochlorous acid or bleach)
 - d. Can also produce reactive nitrogen species (RNS) including nitric oxide.
3. Superoxide radicals also generate reactive nitrogen species (RNS) including NO, also toxic (*Figure 3.29*)
4. Oxidation is coupled to K^+ transport, rendering the interior hypertonic. K^+ enters to compensate for the negative charges, resulting in a rise in pH (8.5). Once the pH reaches this, further neutralization is done by transporting H^+ .
5. Change in K^+ and tonicity dispersed protein granules. These release
 - a. hydrolytic enzymes
 - b. peptides that poke holes in the bacterial plasma membrane (BPI or defensin) (*Figure 3.30*)

6. Microfilaments package the exterior of the vacuole, preventing swelling (which would normally occur after influx of K^+ and subsequent increase in osmotic pressure) and maintaining the concentration of toxic compounds inside.

Figure 3:26: Phagocytosis



Figure 3.27: NADPH

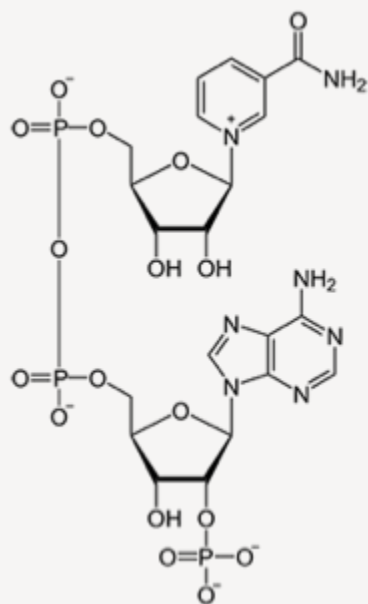


Figure 3.28: Phox Complex

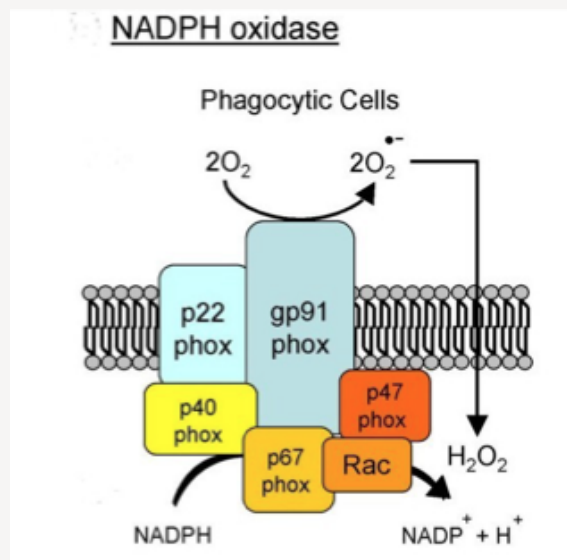


Figure 3.29: RNS, ROS

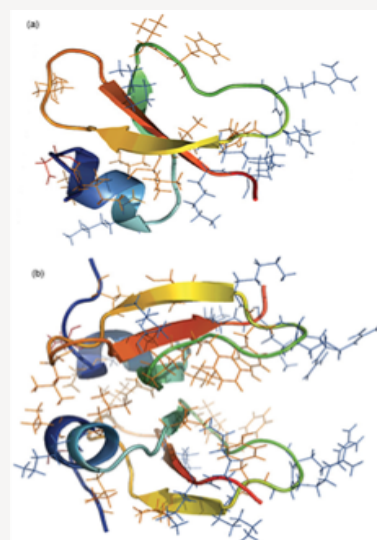
Reactive Oxygen Species (ROS)

$O_2^{\cdot-}$ superoxide radicals
 H_2O_2 hydrogen peroxide
 $HOCl$ hypochlorous acid or bleach

Reactive Nitrogen Species (RNS)

NO^{\cdot} nitric oxide
 $RNSO$ RNS thiols (contain sulfur)
 $ONOOH$ peroxynitrous oxide

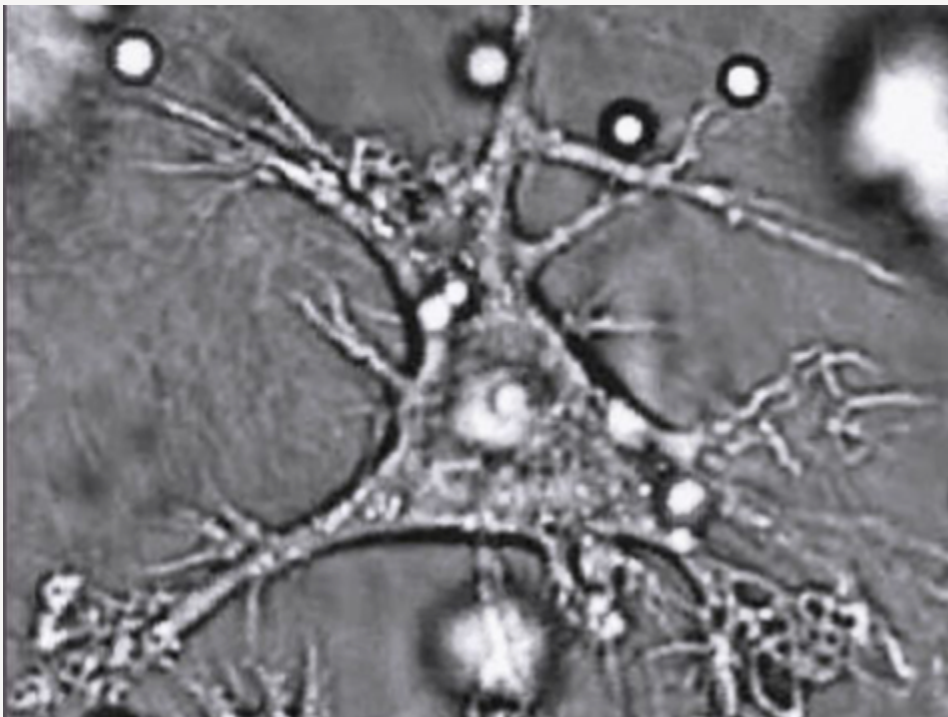
Figure 3.30: Defensin



B. Cells

1. Neutrophils - the infantry in the modern sense, and the first on-site defenders. Along with macrophages, most important phagocytes.
2. Macrophages - the cavalry of the outfit. Phagocytosis causes them to secrete IL-1, IL-6 and TNF α , all inflammation activators. Always on the lookout for pathogens, and after phagocytizing, they relay info about the pathogen to the Th cells (unlike neutrophils).
3. Dendritic cells - the scouts and patrols. Most important initial trigger of the adaptive response. They phagocytize primarily in order to sample pathogens and activate inflammation on the adaptive response. Specifically designed to activate Th cells and therefore phagocytes, secrete a lot of cytokines and coordinate with Th cells. (*Figure 3.31*)
4. NK Cells - function by pattern recognition making them part of innate defenses. Principally attack rogue-self by inducing apoptosis. Innate, but lymphoid cell that recognizes self-cells acting out -- often because they have been commandeered by pathogens. Also helps activate macrophages, which go on to activate Th cells.

Figure 3.31 Dendritic Cell



V. Summary

Table 3. 4 Innate and Adaptive Immunity Compared

Characteristic	Innate	Adaptive
speed	response within minutes	first response: 2 weeks second response: 3 days
recognizes	molecules characteristic of non-self usu. pattern recognition	(much greater specificity) most proteins, many carbohydrates and a few lipids
diversity	hundreds of types, soluble and membrane bound including TLRs	VAST number of types, soluble and membrane bound, including every possible antibody.
gene rearrangement needed	no	yes
may attack self	no	yes
memory	no	yes, prior exposure speeds response.
Cells	myeloid cells, especially phagocytes, NK cells (lymphocytes)	B cells, T cells (instructed by myeloid antigen-presenting cells)
found in	all living organisms (in various forms) including bacteria	vertebrates only

Table 3. 5 Innate Examples

secretions across skin	secretion across mucus membranes	plasma, interstitial fluid	cell membranes	cell cytoplasm (lumen of endomembrane system)
Sebum	Mucus	complement (MBL), LBP	TLRs	NOD2, defensins, hydrolytic enzymes
antimicrobial peptides (psoriacin and cathelicidin)	lysozyme (hydrolytic enzyme attacking peptidoglycan) defensins	C-reactive protein (bind microbial surfaces) also used as a blood test for heart attacks.		ROS/ RNS
pH adjustment (slightly acid) but nothing like the stomach	acids (slight to strong)			pH adjustment (basic)

Lecture L04

Antigens & Antibodies

All models are wrong, but some are useful – statistician George Box, 1978



I. Context

A. A Riff on Models

1. Examples of Models

- Physical – aircraft carriers, the Mississippi river, antibodies, T-cell receptors, MHC molecules and Toll-like receptors.
- Computer – important in epidemiology
- Maps, house plans, circuit diagrams, flow charts showing signaling pathways
- Model organisms: bacteria, *Dictyostelium*, yeast, *C. elegans*, *Drosophila*, *Arabidopsis*, zebrafish, mice (*Figures 4.1-4.8*)
- We try to use the simplest organism we can that still is similar enough to us to imitate the complex processes we're investigating.

Figure 4.1: Dictyostelium

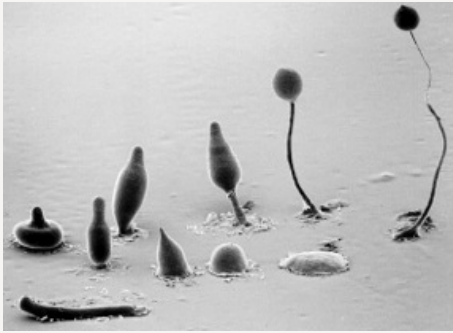


Figure 4.2: Yeast

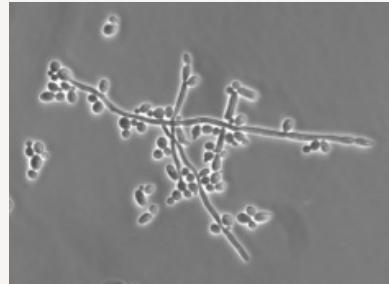


Figure 4.3: DNA

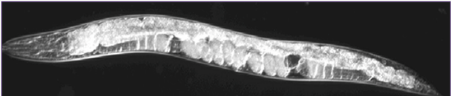




Figure 4.4: Arabidopsis



Figure 4.5: Drosophila



Figure 4.6: <i>C. elegans</i>	Figure 4.7: Mouse	Figure 4.8: Zebrafish
		

2. A Good Model -

- preserves the essential logical relationships or information pertinent to the problem.
- removes any details superfluous to the problem.
- presents problem at a comprehensible scale.
- allows you to manipulate, play and make mistakes at low cost.
- may be quite different from the real thing.

B. Transferring Information

1. typical pathway (**Mousetrap model**)

- Cell A secretes small protein (**signal**)
- The small protein diffuses to the surface of Cell B, where it binds a largish protein embedded in cell B's membrane, extending into the cytosol (**receptor**).
- Binding involves weak interactions
- Upon binding, the receptor shifts shape, and transmits the change to its cytosolic region (**transduction**).
- The change at the inside sets off a cascade of changes: activates enzymes, brings different molecules together, changes binding properties, etc.
TA Note: Ultimately the goal is often to influence the proteins being churned out of cells. Transcription factors are often at the end of the cascade, resulting in changes in the transcription pattern of DNA, upregulating some genes and downregulating others.
- Cell B responds (subroutine in TTSP when a minor trigger leads to dropping a whole piano.)

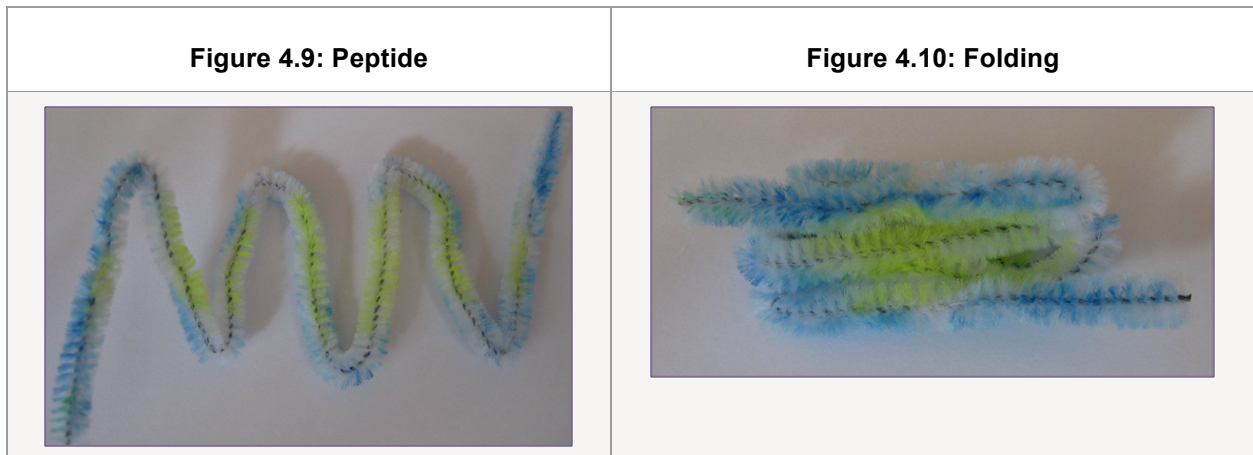
2. Variations

- a. signal may be not be protein.
- b. signal may not come from one of your own cells (pathogen or the environment)
- c. It may take more than one signal (repeats or two different ones at the same time).
- d. Paths may branch, inhibit other paths, and turn themselves off.
- e. The response may involve a physiological change, a change in gene expression or an overall increase in cell division.

II. The Immunoglobulin Superfamily, pipe cleaner models

A. What makes a protein a family member?

1. The molecule has at least one “immunoglobulin domain.” The domain is the compact lump, packed together and stabilized.
2. In this domain, the peptide fan-folds into a compact lump. (*Figures 4.9-4.10*)



3. Hydrogen bonds hold these switchbacks into β pleated sheets
4. Disulfide linkages further stabilize the domain. They form by covalent joining of two cysteine R groups. (*Figure 4.11*)
5. Blue regions are hydrophilic amino acid side chains and yellow regions are hydrophobic, causing the peptide to fold into β pleated sheets to avoid unfavorable interactions.
6. You can refer to the whole domain as a “bread and butter sandwich,” because the hydrophobic amino acid side chains wind up at the interior of the structure (butter) the hydrophilic at the exterior (bread) and the disulfide bond function like a toothpick in nailing everything together. (*Figure 4.12*)
7. Often represented by a structure looking like a capital C with the ends joined by disulfide link. (*Figure 4.13*)
8. Most of these proteins extend from the plasma membrane, nailed there by membrane-spanning regions. Antibodies are a rare exception.

Figure 4.11: Disulfide Linkages



Figure 4.12: Sandwich

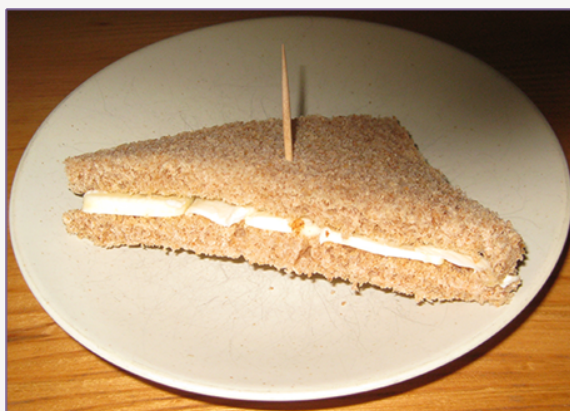
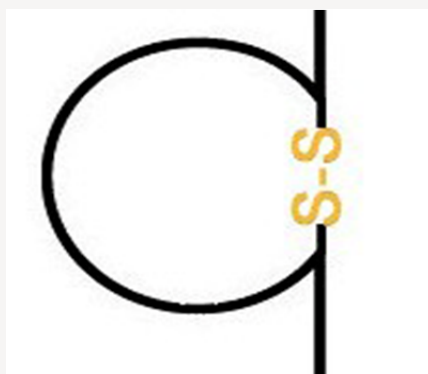


Figure 4.13: Domain Representation



B. Tell me a story.

1. 650 million years ago, the oceans froze solid to a depth of a mile. Liquid water remained on land around hot springs, and life also clung to the thermal vents in the depths of the oceans.
2. At this time, organisms were small and simple in structure.
3. 600 million years ago, the earth warmed and melted.
4. Life multiplied, spread and evolved, using these molecules to construct complex structures.
5. Animals used immunoglobulins to tag nerve cells and serve as signal receptors during development. Cells of primitive organisms, such as sponges, initially used immunoglobulin cell recognition molecules to reform after trauma. Immunoglobulin molecules were thus initially employed in recognizing self-cell molecules spatially and functionally.
6. Eventually animals began using immunoglobulins to recognize not-self, which is how they came to be involved in immune responses.

III. The Structure of Immunoglobulin Receptors (BCR) and Antibodies.

Basically an Ig receptor (or B-cell receptor) is an antibody with a membrane-spanning and cytosolic domain at the end (C-terminal). Thus the antibody is soluble and secreted from the cell and the receptor version is stuck in the cell membrane with the business end facing outside the cell.

A. Terminology

1. In the 1960s, chemists classified proteins as fibrous (silk, collagen) versus globular proteins (most proteins, actually). Globular basically meant soluble in plasma.
2. immunoglobulins: the protein fraction in the plasma involved in fighting disease.
3. gamma (high mobility) fraction
4. Scientist purified this fraction and used it to study the structure of the antibody.
*TA Note: Putting the blood plasma into the gel electrophoresis will give you 5 peaks (the first is much larger than others).
The peaks are albumins (small blood proteins to help carry things around and keep osmotic pressure under control), alpha 1, alpha 2, beta, gamma (heavy one). Majority of gamma globulins were identified as antibodies, conferring the vast majority of "immune protection" among the plasma proteins.*

B. Analytical History – Porter and Edelman

1. Gerald Edelman - treated antibodies with mercaptoethanol (*Figure 4.14*)
 - a. This treatment reduces the disulfide bond, thus breaking the covalent bond that stabilizes the antibody.
 - b. The antibodies separated into two peptides.
 - c. We now know these are the intact light and heavy chains. You can this separate them and study each in isolation. Heavy chains separated by gel electrophoresis, about twice as massive.
2. Rodney Porter - cleaved antibodies with brief exposure to proteolytic enzymes (*Figures 4.15-4.16*)
 - a. This treatment breaks up the peptide bonds between amino acids, targeting the most accessible bonds first.
 - b. Very brief treatment with pepsin: cleaves preferentially at hinge between arms and stem, separating the Fc (stem) section) from the top half, called Fab(ab')₂.
 - c. Mix Fab(ab')₂ with their antigen and they will precipitate. This has both heavy chain parts and light chains -- still held together by disulfide bonds. Fab 2 fragments are the entire top half and retain both antigen binding sites. This lets them bind an antigen on each arm and connect together to form a chain, which readily precipitates out. This is only possible because Fab₂(TWO) fragments have TWO arms, and Fab fragments just have 1, allowing several to bind a single antigen without forming a chain.
 - d. Brief treatment with papain produces FAB fragments, which are isolated arms.
 - e. These can bind antigen, but will not precipitate because they cannot cross-link one antigen to two fragments.

Figure 4.14: Edelman

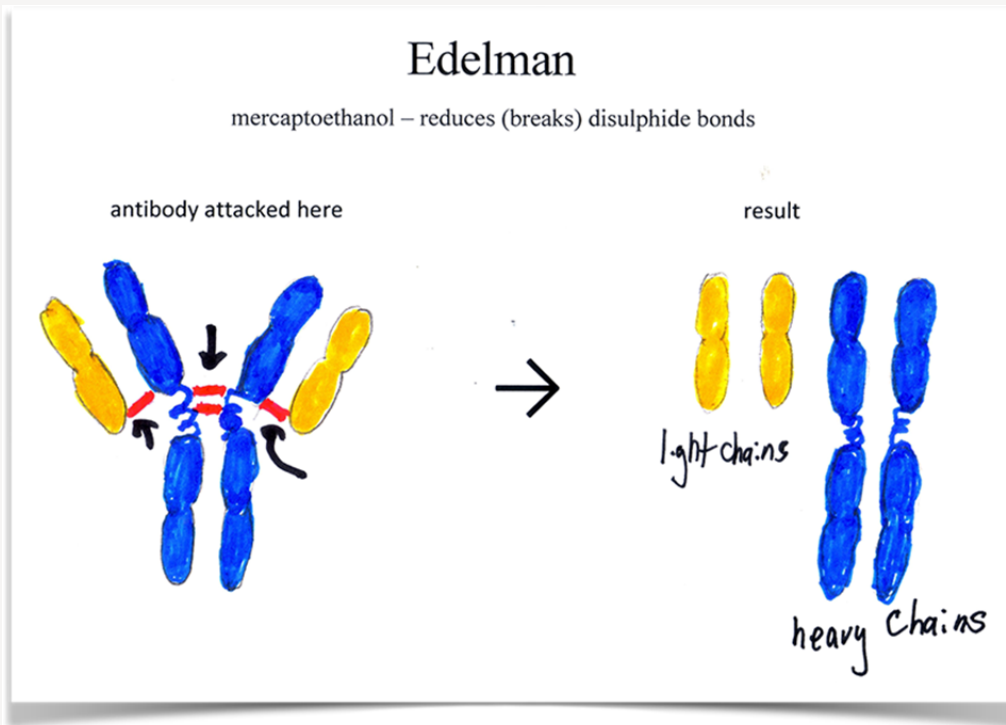


Figure 4.15: Porter - Pepsin

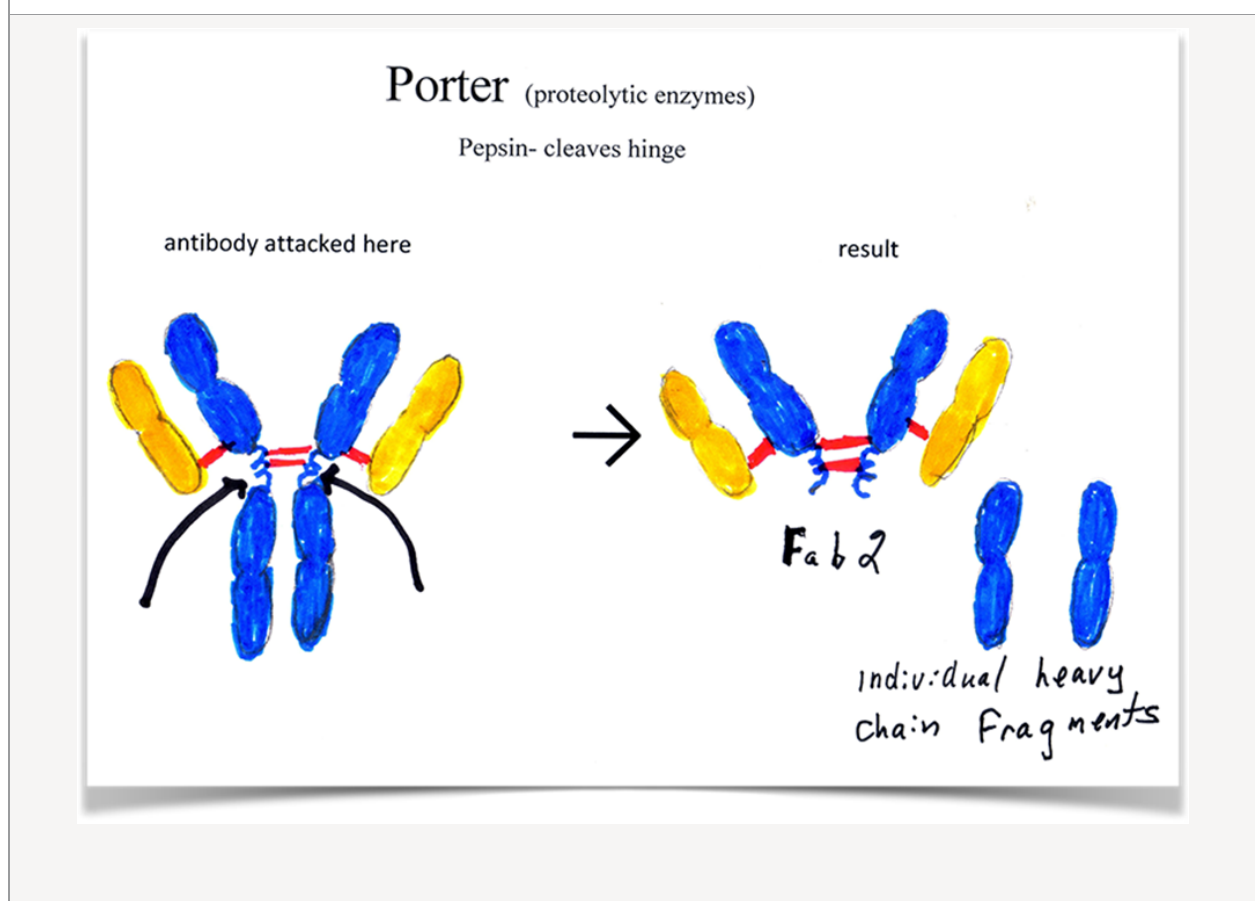
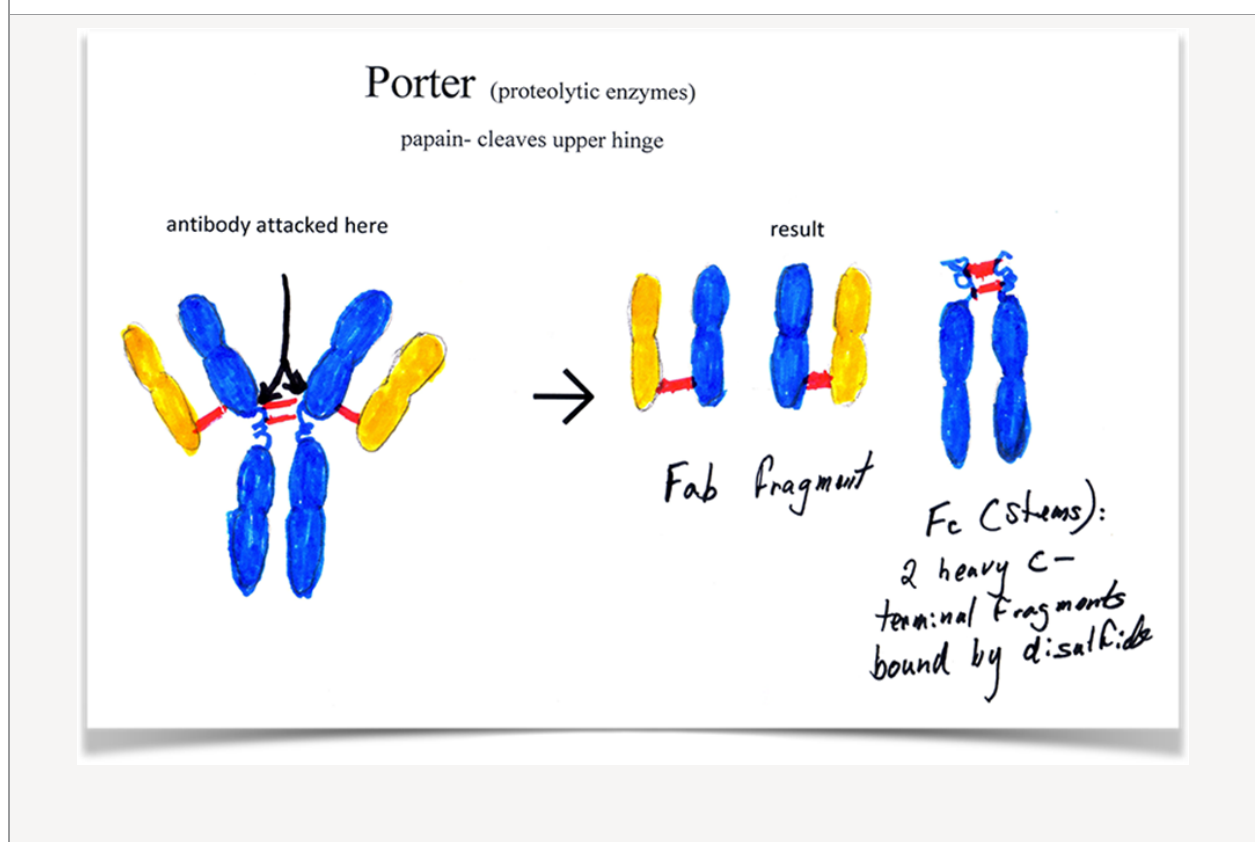


Figure 4.16: Porter - Papain



C. Form and Function (foam board model) (*Figure 4.17*)

1. Two light (L) chains (~25,000 MW; MW 50,000 total for both), identical to each other, composed of 2 immunoglobulin domains, variable and constant. Chains = peptide. The bottom left and right blue and pink regions compose light chains. The top right and left dark blue and pink regions leading all the way down into the Fc stem are heavy chain polypeptides. These 4 polypeptides are associated at the quaternary level in antibodies.. Sequins indicate disulfide bonds.
2. Two heavy (H) chains (~50,000 MW; MW 100,000 for both), identical to each other, composed of 4 or 5 immunoglobulin domains, one variable and 3 or 4 constant.
3. Overall molecular weight: ~150,000
4. The amino (NH₂) end of the heavy chain joins to the light to form the Y arm.
5. The other ends (carboxyl or COOH) of the heavy chains join together to form the Y base or stem.
6. Both L-H and H-H linkages involve weak interactions and covalent disulfide bonds.
7. The amino ends of both L and H peptides (the part found at the tips of the Y arms) vary greatly from one antibody to the other. (*Figure 4.18*)
8. This (the loops at the ends of the arms) is the region that interacts with the antigen
9. An oligosaccharide (small carbohydrate) attaches to the second immunoglobulin domain from the end, pushing open the Fc stem. This is the white fluffy part between the Fc stem on the antibody diagram; it tells the immune system how to treat what antibody is attached to.

Figure 4.17: Foam Board Model

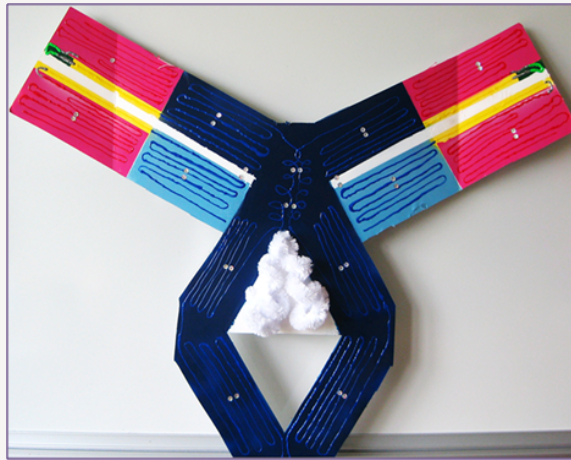
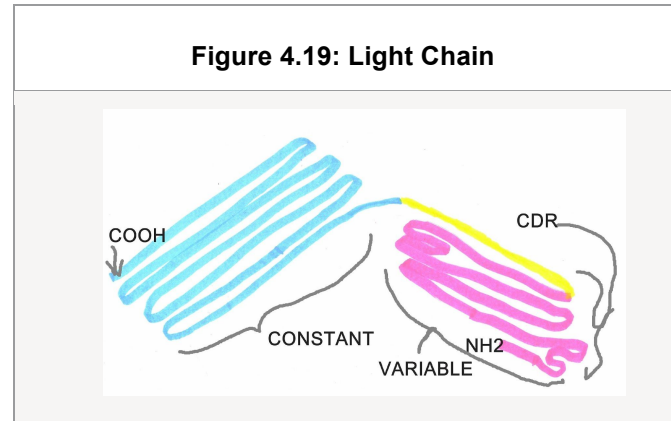


Figure 4.18: CDR



D. Details of the Structure of the Light Chains

Two basic parts or domains (Figure 4.19)



1. Constant Region (C_L)

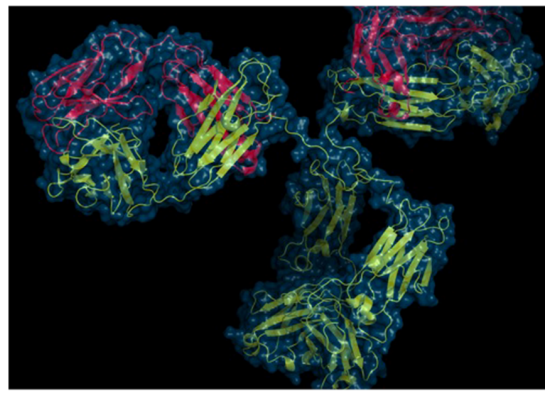
- a. Typical Ig domain: β pleated stabilized by disulfide linkages, hydrophobic side chains to the interior, hydrophilic to the exterior.
- b. Forms part that connect to hinge/bend to base of Y
- c. Two versions: κ for kappa and λ for lambda, differing in the constant region.
- d. In humans, each lambda (λ) gene has five different versions of the constant region.
- e. Either kappa (κ) or lambda (λ) can be in any immunoglobulin class and all versions have very similar overall structures.

2. Variable Region (V_L)

- a. Most of variable domain is Ig domain and is actually pretty constant.
- b. 3 loops that stick out at the end comprise hypervariable region composed of three non-contiguous amino acid sequences (15 to 20% of the domain).
- c. The rest of the domain is the framework region that basically holds the loops in place.

IV. Immunoglobulin Classes (*Figure 4.20*)

Figure 4.20: Antibody Ribbon Model

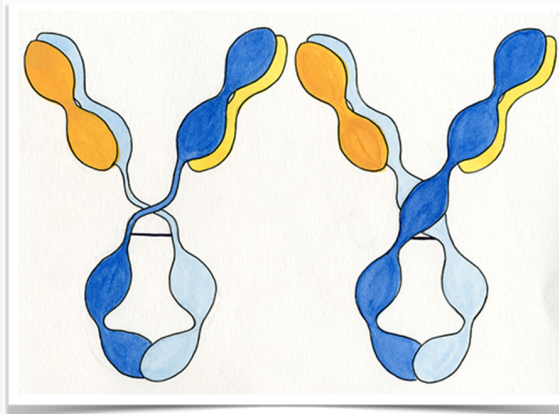


There are 5 classes of antibodies, which differ in function and in the exact amino acid sequence and conformation of the stem part of the Y. In all cases, the basic unit has two heavy and two light chains and the light can be either κ or λ .

A. Categorization by Heavy Chain Structure

1. flexible hinge or rigid bend – number of constant C Ig domains 3 versus 4 (*Figure 4.21*)

Figure 4.21: Comparison



2. oligosaccharide – small carbohydrate added to second domain from the C terminal- varies, depending on exact type.
3. J chain – compound antibodies that can cross epithelia
4. subclasses (different version of related Ig types)

Table 4. 1 Antibody Classes

Ab	Flexible hinge or rigid bend?	Forms complexes	J chain	Subclasses	Timing	Membrane-spanning Ig receptor?	Role
M	rigid	yes	yes	no	first class produced in maturing B cells.	yes, naïve and memory	general
D	hinge	no	no	no	Produced as Ig receptor on mature but naïve B cells	on naïve cells, rarely soluble or memory	aids naïve B cell activation?
G	hinge	no	no	4	after class switching in activated B cells	memory cells	specific responses to acute infections
A	hinge	yes	yes	2	after class switching in activated B cells	memory cells	crosses epithelia, protects boundaries
E	bend	no	no	1	after class switching in activated B cells	memory cells	T _H 2 response: allergies, pollutants, chronic infections

B. Travelling Down the Heavy Chain

1. Variable Region

- a. also composed of β pleated sheets, with switchbacks
- b. also "bread and butter sandwich" structure
- c. 3 loops that stick out at the end show variation, framework region much less

- d. Hypervariable region (coupled with corresponding hypervariable region on the light chain) composes the complementarity-determining regions (CDRs).
 - e. Thus each CDR is composed of 6 loops at the tip of the Y, 3 H and 3 L.
2. First Constant Domain
3. Hinge-Bend Region
- a. M and E (μ and ϵ heavy chains) – rigid bend at C_2 between C_1 and two constant stem domains, C_3 and C_4 . C_2 replaced by hinge in G, A and D.
 - b. G, A and D (γ , α and δ heavy chains) has a longish sequence rich in proline and secured with disulfide linkages that makes this region especially bendable. This connects C_1 and C_2 , occupying the same place in the antibody as C_2 in the μ and ϵ heavy chains.
4. Next Constant Domain (C_2 of γ , α , and δ and C_3 of μ and ϵ)
- a. also "bread and butter sandwich" structure
 - b. site of oligosaccharide attachment (added after protein synthesis)
 - c. opens these domains to the aqueous environment
 - d. interacts with complement (more later)
5. Carboxy-terminal Constant Domain (C_3 of γ , α , and δ and C_4 of μ and ϵ)
- a. Crucial function in determining whether or not the antibody is membrane-bound or secreted.
 - b. Secreted version ends with short hydrophilic sequence.
 - c. Membrane-bound version ends with hydrophobic and then hydrophilic sequence.
 - d. Membrane bound version are expressed first in naïve cells, secreted versions after maturation to plasma cells, and membrane-bound versions in memory cells.

C. Specific Types

1. IgM - μ heavy chain – rigid bend (*Figure 4.22*)
 - a. rigid bend – 4 constant domains
 - b. Function- general purpose - First class expressed in plasma.
 - c. Monomeric form (actually 2H +2L) expressed as a membrane-bound antibody on the naïve B cell.
 - d. Secreted form occurs as pentamer, looking like 5 IgG's stuck together, stems in, 10 antigen-binding sites out.

- e. Held together by an additional peptide, the J chain. The J chain binds to a secretory component, a peptide that allows structure to be secreted into mucus, etc. (*Figure 4.23*)
- f. Very good at binding large complex structures and activating complement (to kill foreign cells).

Figure 4.22: IgM Pentamer

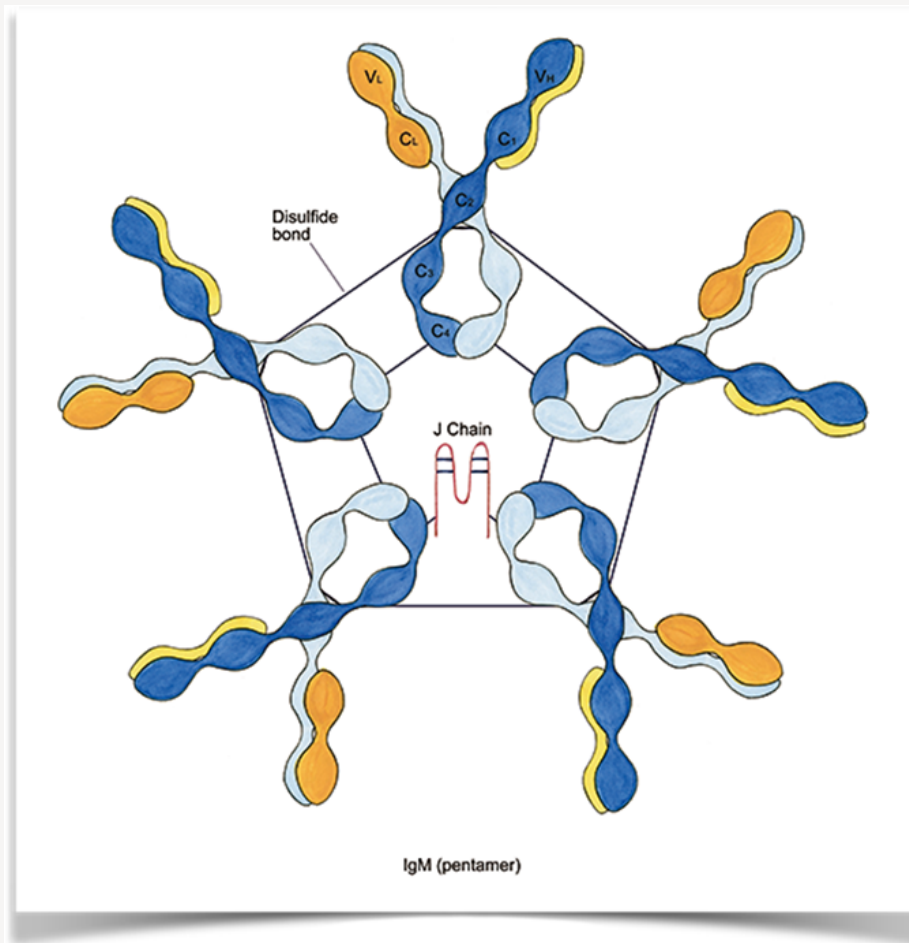
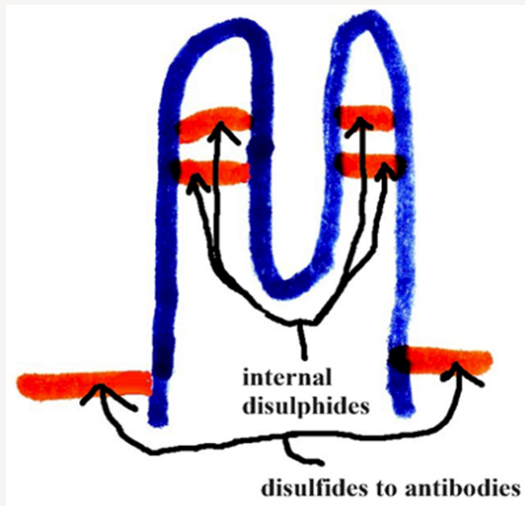


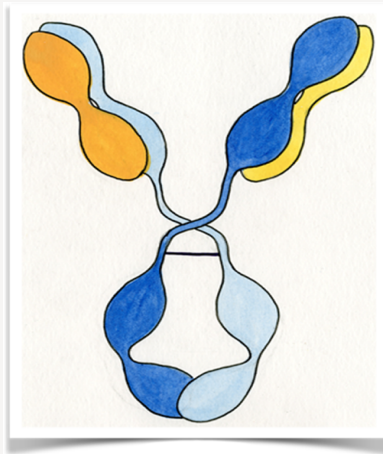
Figure 4.23: J Chain



2. IgD - δ heavy chain – flexible hinge (*Figure 4.24*)

- a. Function- aids recognition by naïve B cell.
- b. Primarily found (with IgM) as a membrane-bound receptor in naïve B cells. While M class antibodies also function in plasma, D class rarely does.
- c. Rarely found in plasma (0.2% of total serum immunoglobulins)
- d. superficially resembles IgG (different amino acid sequence).

Figure 4.24: IgD



3. IgG - γ heavy chain (*Figure 4.25*)

- a. flexible hinge, 3 constant domains
- b. standard secreted antibody defending against bacterial and viral pathogens
- c. comes in four versions, numbered 1 to 4, varying in biological specificity:

IgG1 – activates complement, Fc receptors bind tightly

IgG2 – weakly activates complement, Fc receptors bind weakly

IgG3- strongly activates complement and binds tightly to Fc (lots disulfides)

IgG4 – does not activate complement, binds weakly to Fc.

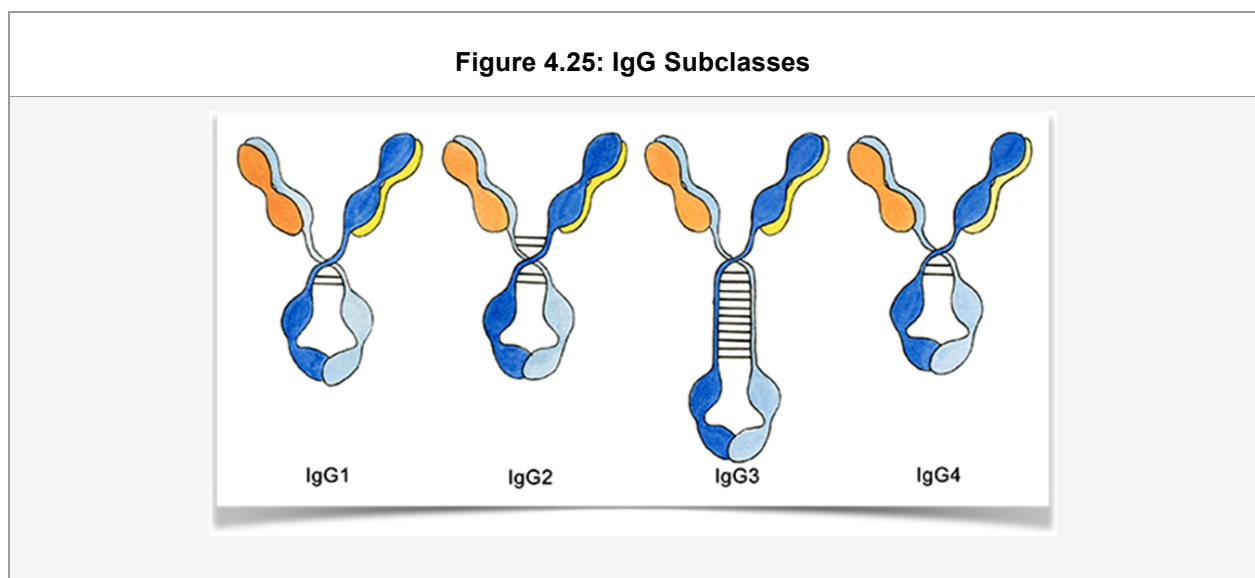


Table 4.2 G-class Antibodies

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)

4. IgA - α heavy chain – flexible hinge (*Figure 4.26*)
 - a. Function- primarily epithelial patrol.
 - b. Also occurs in serum as a monomer.
 - c. The secreted form occurs as dimer, looking like 2 IgG's stuck together, stems in, 4 antigen-binding sites out, and may occur as trimer or even tetramers
 - d. Two subclasses (1 and 2)
 - e. Also held together by an additional peptide, the J chain (binds secretory peptide) which is identical to the one in IgM
 - f. Secreted into mucus, tears, saliva, and breast milk- up to 15 grams per day!
 - g. Plasma cells that secrete this tend to home in on various epithelial linings.
 - h. Unfortunately these same pathogens often produce proteases that specifically target the vulnerable hinge regions of this antibody.

5. IgE - ϵ heavy chain, – rigid bend (*Figure 4.27*)
 - a. Function – defense against worm parasites.
 - b. Monomer superficially resembles IgM (somewhat different amino acid sequence)
 - c. rare, but potent
 - d. involved in allergic response
 - e. binds to F_C (stem) receptors on mast cells and basophils, which causes them to trigger the allergic reaction.

Figure 4.26: IgA

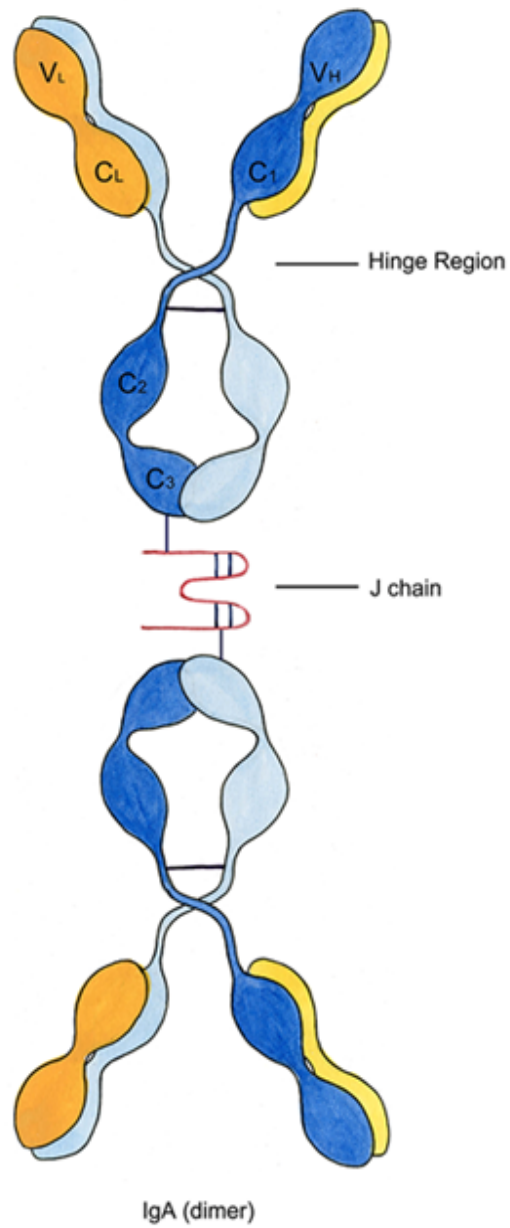
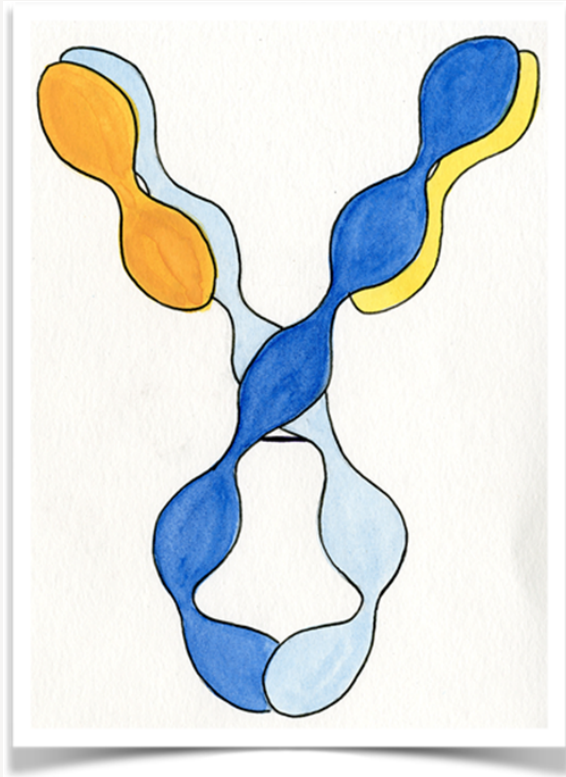


Figure 4.27: IgE



V. Immunoglobulins in Action

A. Antigen Binding (*Figure 4.28*)

1. Antigen bound to the 6 loops at the tips of the Y arms by the same weak interactions that produce enzyme-substrate interactions.
2. As with enzyme-substrate interaction, the binding can involve induced fit, distortion in both structure of antibody and antigen.
3. usually proteins
 - a. B-cell epitopes are found at the surface of a protein often parts of the protein that stick out.
 - b. Tertiary structure (shape) is important. Denatured proteins won't work.
 - c. Quaternary structure (association between two separate peptides) can be important. An antibody may recognize the junction of two different proteins.
 - d. B-cell epitopes can therefore be formed by sequential or non-sequential sequences of amino acids.

Figure 4.28: Antigen Bonding Model

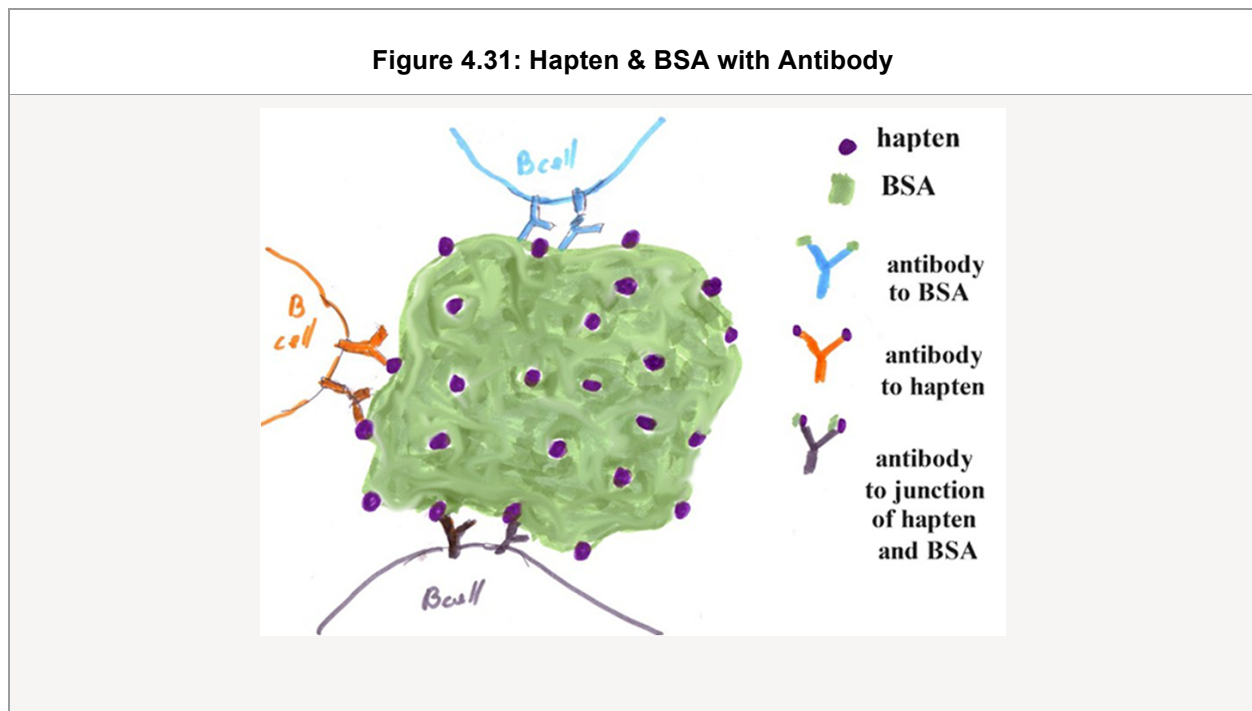
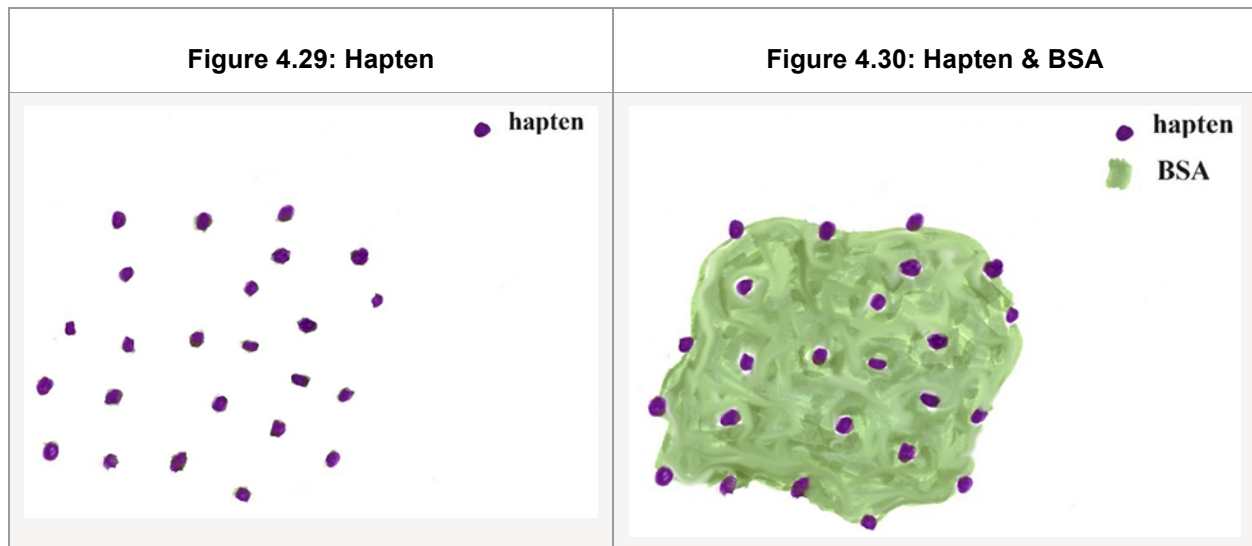


B. Prompting Immunogenicity in B cells: (Adaptive response)

1. differs from self (foreign)
2. big enough to crosslink two receptors
3. arrives with danger signal (activates PRR of the B cell) – vaccine adjuvant, e.g. alum which activate NOD receptors.
4. nutritional status and age

C. Manipulating Immunogenic Responses (*Figures 4.29-4.31*)

1. haptens are not inherently immunogenic
2. Can trigger an immune response is given after incorporation into large protein BSA, making antibodies to:
 - a. BSA
 - b. Junction of BSA and hapten
 - c. Haptens - such as steroids hormones, drugs, pollutants or poisons.



D. Biological Activity – Antibody Signaling – property of the Fc, or stem, region of the antibody.

1. Allergic responses – eosinophils, basophils and mast cells have receptors (FcRs) for IgE. Binding triggers degranulation by these cells.
2. Signal for Phagocytosis: opsonization by macrophages and neutrophils.
 - a. Macrophages and neutrophils have cell surface receptors (FcRs) for IgG Fcs.

- b. A bunch of F_C s sticking out of a bacterium or cluster of viruses will bind to a number of F_C Rs on the surface of a phagocytic cell.
 - c. The binding triggers a transmembrane response that leads to the particle being phagocytized
- 3. Signal to Plasma Proteins: Activation of Complement (landmines in the plasma)
 - a. Activated by IgM and IgG when they are bound to a cell surface.
 - b. This sets off a cascade of activation, leading to the attack version.
 - c. Also opsonizes pathogen, improving phagocytosis by neutrophils and macrophages.
- 4. Antibody Dependent Cell Mediated Cytotoxicity (ADCC)
 - a. When you are infected by a virus, your cells will display foreign antigen.
 - b. Antibodies against this antigen will bind to the surface of your cells.
 - c. This complex activates NK (natural killer) cells, which trigger apoptosis
- 5. Signal to Epithelia - Transcytosis - transfer of antibody across epithelia.
 - a. Mostly IgA, although IgM is transported in small amounts
 - b. Involves secretion into mucus, tears, and breast milk.
 - c. Also, IgG is transported across the placenta to the fetus of mice and humans
- E. Yet More Terms – It is possible to make antibodies to antibodies.
 - 1. Isotype - constant region determinants. IgG3 is a different isotype from IgG4, although they may recognize the same epitope.
 - 2. Idiotype - refers to differences arising from variable domains. IgG and IgM that recognize the same antigenic epitope are the same idiotype, but different isotypes.
- F. B-cell Receptor (*Figure 4.32*)
 - 1. If the antibody is stuck in the membrane, sticking out, it's a receptor.
 - 2. B-cells recognize foreign antigen when two neighboring receptors bind to it and cross-link. (*Figure 4.33*)
 - 3. The signal is transduced by the associated heterodimer of $I\alpha/I\beta$, both of which have long cytoplasmic tails. (*Figure 4.34*)
 - 4. Naïve B cells have M or D class receptors.
 - 5. Memory B cells can have receptors of any class.

Figure 4.32: Ig Receptor

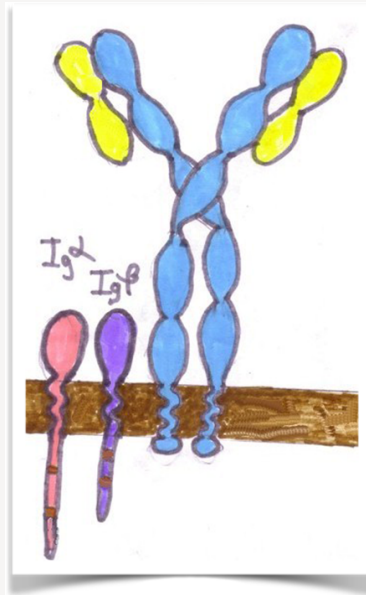


Figure 4.33: Neighboring Receptors

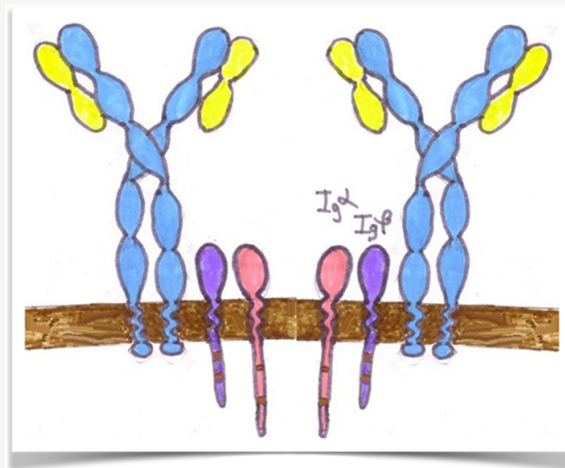


Figure 4.34: Igα/Igβ



VI. Monoclonal Antibodies

A. Very Big Deal

It's incredibly powerful to be able to make a large amount of pure, defined antibodies. You can target cells and proteins, and there are therapies and assays (ELISA) based on this asset. While this lecture concentrates on the use of monoclonal antibodies to identify, locate and quantify substances, these antibodies function in many of the cutting edge therapeutics, especially for cancer.

1. Definition - Monoclonal antibodies are fractions of antibodies with an identical defined specificity (CDR region) for a particular antigen.
2. The cells are **clone** from a **single** cell, a B-lineage cell, all of whose descendants chuck out identical antibody. Ordinarily, you respond to an infection or other immune challenge by making a number of cell lines, each producing antigen to a different epitope.
3. Once you have a cell line producing a pure fraction of antibody to a particular protein you can:
 - a. Use the antibodies to measure the presence and concentration of that protein or antigen.
 - b. Label the antibodies with something fluorescent and localize the protein in the cell.

- c. Label the antibodies with something radioactive toxic and localize the protein in the body. Particularly helpful in tracing metastatic cancer cells.
- d. Use the antibodies to specifically shut down signaling pathways leading to cell division in cancers.

B. Hybridomas

The trick is to get a single cell line that will endlessly crank out a pure stream of antibodies for you.

1. Challenge an organism with the antigen to which you want to make the antibodies.
2. Isolate an activated plasma (B) cell producing an antibody to one of the antigen epitopes. Sadly, this will only live a few weeks on its own
3. Fuse the normal B cell with a myeloma cell. Myeloma cells live indefinitely. The fusion is done by mixing the cells with polyethylene glycol.
4. The cells fuse randomly. There is no guarantee that one myeloma cell will fuse with one normal B cell.
 - a. Myeloma plus B cell – desired result
 - b. B plus B – dies out
 - c. Myeloma plus myeloma (or unfused myeloma) – lives forever. You must get rid of these!
5. There are mutant lines of myelomas that lack the ability to make a component necessary for growth. The typical tool is a myeloma missing HGPRT and thymidine kinase, used to salvage nucleotides. These are OK as long as they can use the regular *de novo* synthesis pathway.

Table 4.3 Plasma and Myeloma Cells Contracted

Plasma (B) Cell	Myeloma (B cancer) cell
Makes desired antibody	Selected line makes no antibody
Can synthesize nucleotides by <i>de novo</i> pathway	Can synthesize nucleotides by <i>de novo</i> pathway
Can synthesize nucleotides by salvage pathway	Can NOT synthesize nucleotides salvage pathway
Divides for only a couple of weeks	Divides indefinitely

6. Grow the mixture of fused cells on HAT medium. This has
 - a. aminopterin, which blocks *de novo* synthesis of nucleotides.
 - b. Hypoxanthine and thymidine, which supplies the salvage pathway for nucleotide synthesis.
7. The B cells making the desired antibody have both nucleotide pathways intact (although the *de novo* will not work in HAT with aminopterin), and any myeloma cells that fuse with them will be able to make nucleotides and grow (hybrid cells). Myelomas that don't fuse with B cells lack the ability to salvage and die out.
8. So, after fusing, the B cell brings in the ability to make a particular antibody along with the salvage enzymes and the myeloma confers immortality.
9. Once you have a line going you have to check and make sure it's making the desired antibody.
10. Once you've got the right antibody, you may need to prod the line into secreting the antibody more effectively.

You have to go through this process every time you want a new antibody against a different protein, but once you've done it, the cells will divide and you can grow large cultures and share or sell them or share or cell the antibodies.

The More You Know: Optional Resources and Fun Stuff (You don't get tested in this!)

A. YouTube:

Rube Goldberg at its finest: This Too Shall Pass:

<http://www.youtube.com/watch?v=qybUFnY7Y8w>

Lecture L05

Organization & Expression of Immunoglobulin Genes

Although we are always taught about astonishing specificity in biology, many mechanisms are only as specific as they need to be. - Gerhart, John, and Marc Kirschner, 1997



I. Uniqueness of the System

A. *The Problem* -

1. The number of possible antibodies that an individual can produce is vast.
 - a. Vast is a good term here. Technically there can't be an infinite number.
 - b. Estimates range from 10^8 to 10^{11} , but frankly no one really knows.
2. Any one antibody-producing plasma cell synthesizes antibodies with one and only one CDR (complementarity determining region or antigen binding region).
3. Any one antibody-producing plasma cell can make more than one class of antibodies, each with the same CDR.

B. *The Solution*

1. Mixing instructions for chains (heavy and light peptides).
2. Mixing instructions for domains within chains: Dryer and Bennet (in 1965).
3. Evidence for gene rearrangement, Tonegawa and Hozumi (1976)

C. **THIS IS A VERY BIG DEAL.**

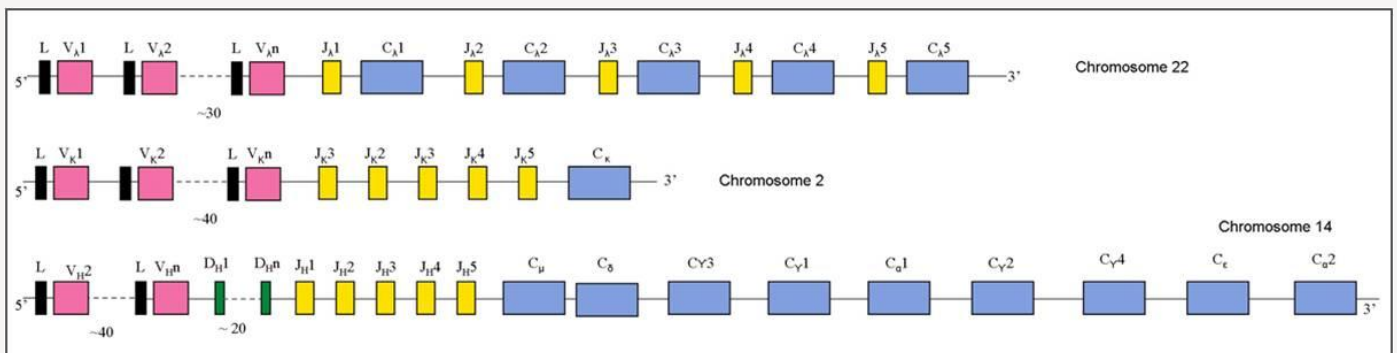
1. Other Systems with Changes to the Germline DNA.
 - a. Chromosome Diminution. This is a loss that accompanies the decision to become a somatic as opposed to a germ cell, and does not seem to be involved in the regulation of genes involving differentiated state.
 - b. Gene amplification. There are a number of systems in which cells make extra copies of particular genes (rRNA for example).
 - c. McClintock's jumping genes. She thought they were important in developmental regulation, but they turned out just to be examples of DNA parasitism
 - d. Neuron development. In mice, at least, as neurons in certain regions of the brain develop, they relax their controls on transposable elements. Such elements then move around randomly in the nuclei.
2. What Specifically Makes the Adaptive Immune System Unique
 - a. Differentiation of B (and T) cells involves clipping DNA out of specific regions of the immunoglobulin genes.
 - b. The clipping is not precise within those regions.
 - c. The clipping takes place at different parts of the regions in different cells.
 - d. The clipping does not take place in regions other than those for immune proteins.
 - e. The clipping results in different cells (and their progeny) ultimately having different versions of the immunoglobulin genes.
 - f. These site-specific DNA rearrangements are unique to the vertebrate adaptive immune system.

II. Gene to RNA to Protein

A. *Orientation: I'm going to show the human version. Many texts show versions from the mouse, which differ from the human. (Figure 5.1)*

1. There are two different genes for the light chain, and one for the heavy, all on different chromosomes.
2. Since we are diploid, there is a pair of each chromosome and therefore each of the following arrangements may occur twice in the genome.
3. Many of the elements of these genes are repeated variations of interchangeable parts.
4. When DNA elements occurs as tandem repeats, individual often vary in the number of these repeats.
5. In this lecture, I refer to the Ig genes as genes and the subparts of the genes are gene regions.
6. Do not confuse the terms "region" with "exon."

Figure 5.1: Human Version IgG Genes



B. Lambda (λ) Gene Expression (Figure 5.2)

1. Gene family includes a series of leader variable regions followed by a series of J-C regions.
2. One leader-variable and one J-C pair come together at random.
3. This activates the promoter of the selected leader only, enabling transcription from the beginning of that leader only.
4. RNA polymerase transcribes the VL and JC into a primary transcript. (Figure 5.3)

Figure 5.2: Lambda (λ) Gene Expression

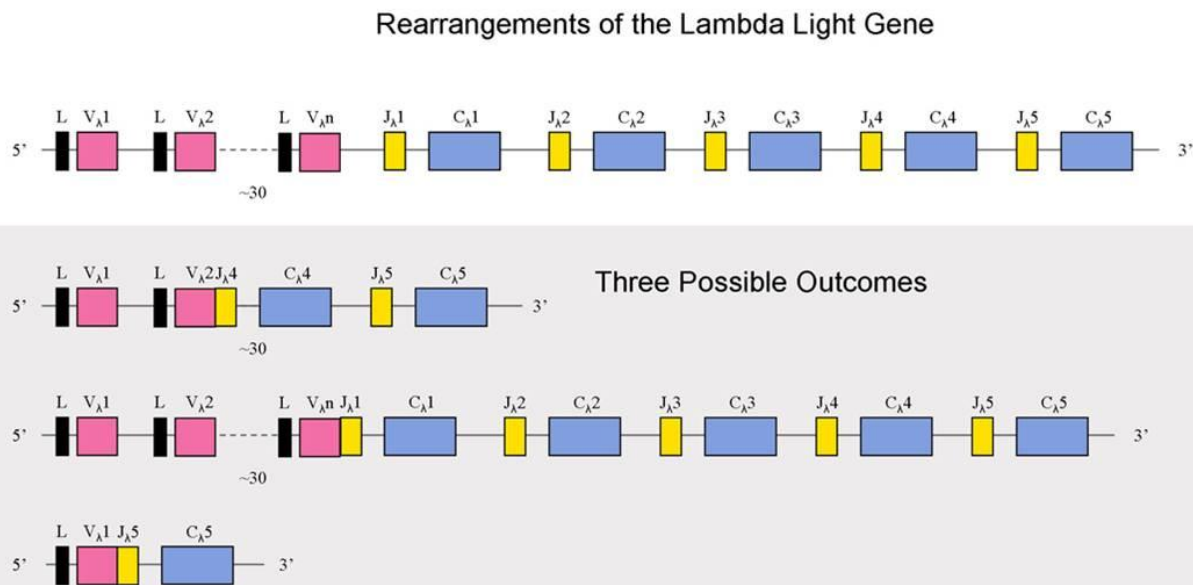
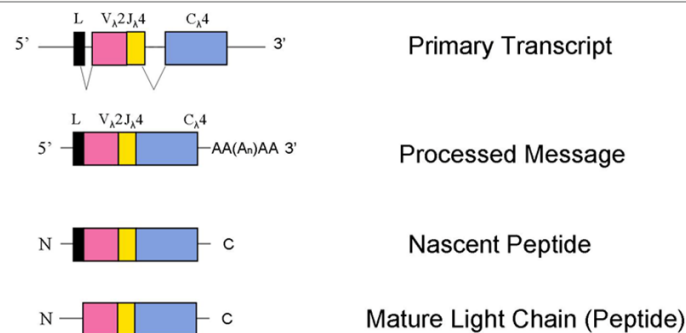
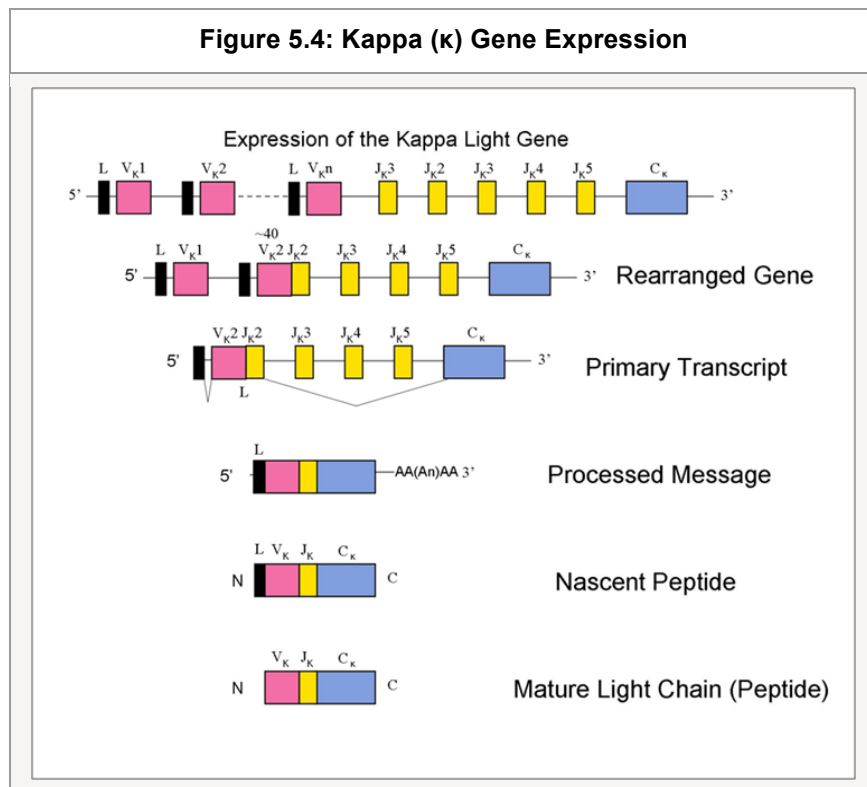


Figure 5.3: Primary Transcription of Lambda (λ) Gene



5. Message processing removes the introns from between the V-L and the J-C, adds a poly A tail and 5' cap (not shown).
6. The ribosome attaches to the message, begins translating, attaches to the RER, and pushes the nascent peptide into the ER lumen.
7. Enzymes clip off the leader, leaving a light chain with a variable domain and a constant domain.

C. Kappa (κ) Gene Expression (Figure 5.4)



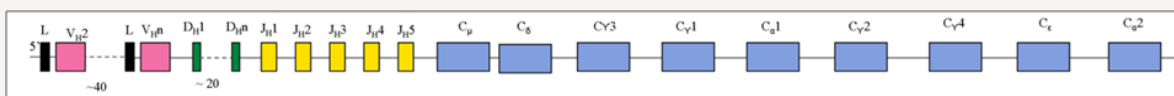
1. Gene family in humans includes a series of about 40 V_{λ} (leader-variable) regions, 5 functional J_{κ} (joining) regions, and 1 C_{κ} constant region.
2. Gene rearrangement places 1 VL next to 1 J gene region, again activating only the promoter of the selected VL.
3. RNA polymerase transcribes a message precursor with one VL, the selected J, the constant region and any remaining Js and introns between them.

4. During processing, introns and extra Js get clipped out, leaving a message with the same structure as that of the lambda.
5. Translation proceeds as above, producing a light chain with the same overall structure

D. Heavy Gene Expression (Figure 5.5-5.9)

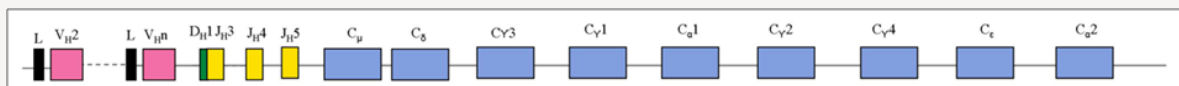
Figure 5.5: Series of Constant Regions

The order seen below (in blue constant regions) is: μ , δ , $\gamma 3$, $\gamma 1$, $\alpha 1$, $\gamma 2$, $\gamma 4$, ϵ , and $\alpha 2$



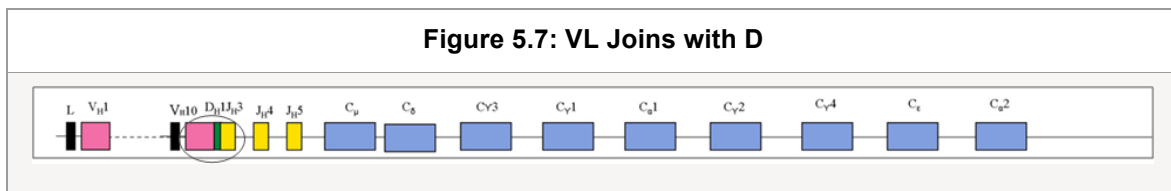
1. As with the kappa light, family begins with sequence of about 40 V-Ls.
2. Next is a series of about 20 short D (diversity) segments, each coding for 3 amino acids.
3. In humans, 5 or 6 J regions follow.
4. Finally there is a series of constant regions, 1 μ , 1 δ , 4 different γ s, 1 ϵ , and 2 α s. The order is μ , δ , $\gamma 3$, $\gamma 1$, $\alpha 1$, $\gamma 2$, $\gamma 4$, ϵ , and $\alpha 2$.
5. First a D region joins with a J, cutting out all the extra downstream Ds and upstream Js between them, but leaving any downstream Js.

Figure 5.6: D Regions Join with a J

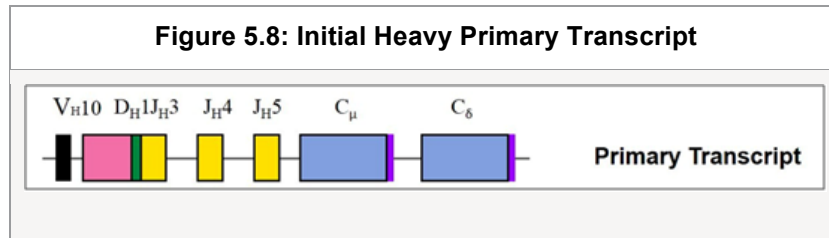


6. Then one of the VLs joins with a D, removing all the extra downstream VLs and up-stream Ds.

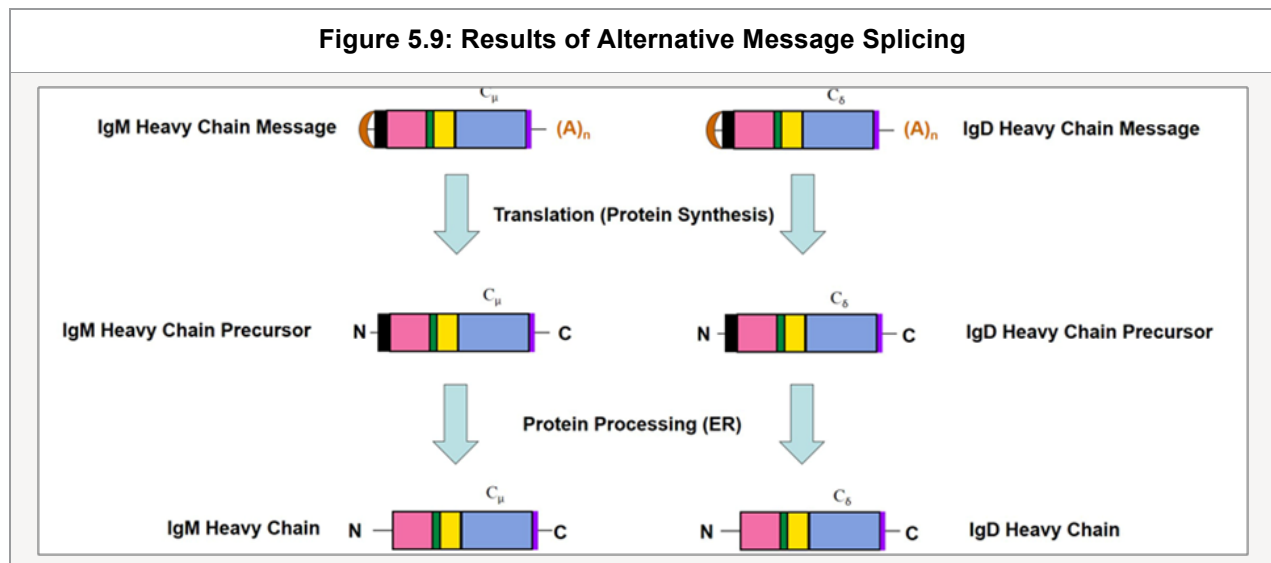
Figure 5.7: VL Joins with D



- The initial primary transcript starts with this LVDJ regions, continues through any remaining J's and introns, and then copies through the and constant regions and stops.



- The transcript now undergoes alternative message splicing to include **either** the μ or the δ constant exons, but not both.

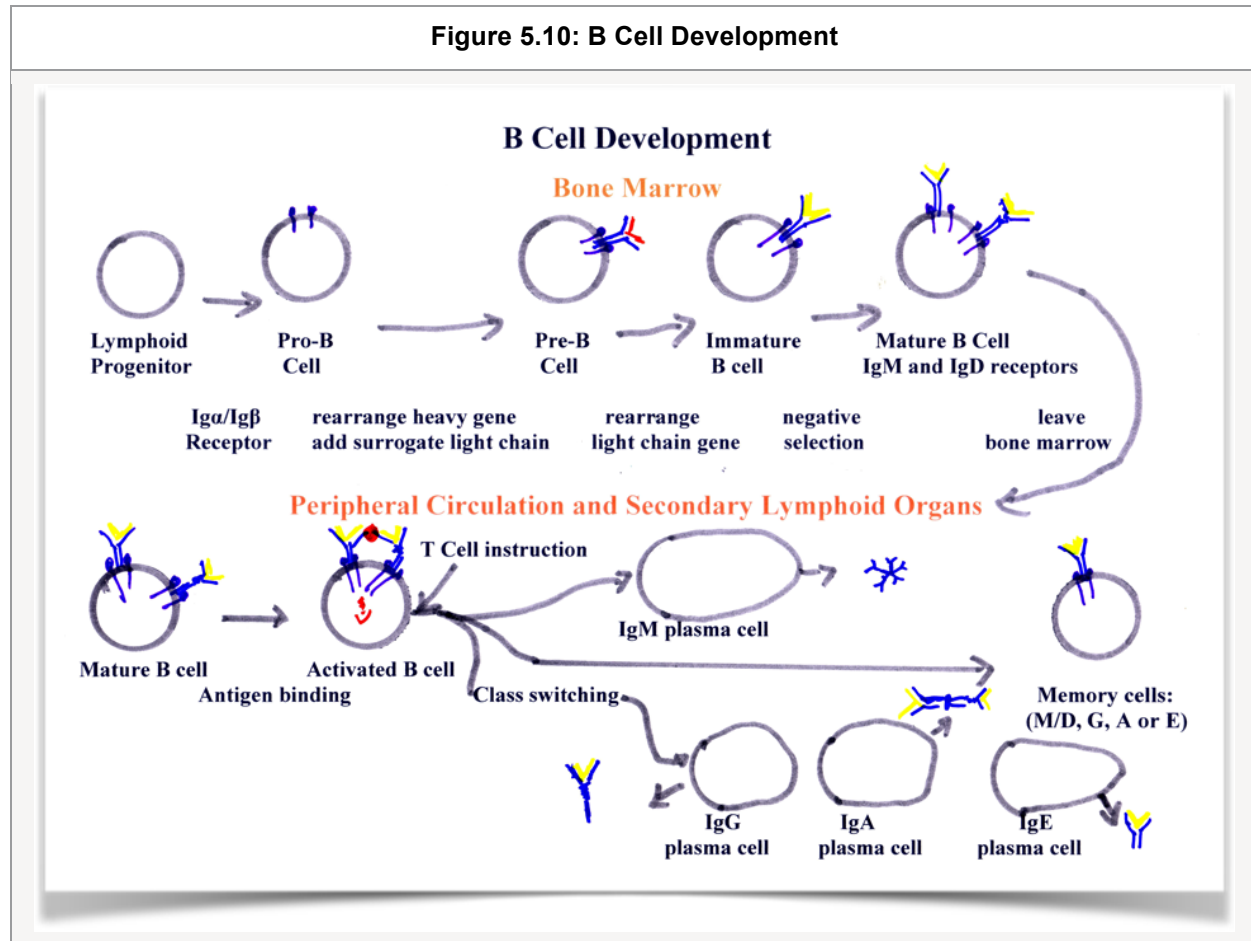


- Translation proceeds as with the other messages.

III. Rearrangement in Developmental Context – in the bone marrow.

A. Variable Region Rearrangements (Figure 5.10)

Figure 5.10: B Cell Development



1. First, the developing B cell rearranges a heavy chain gene. If you get a functioning gene, fine, you express it and shut down the other heavy chain gene.
2. If not you try the other heavy chain gene, if that doesn't work, you proceed to the light gene. If not, the cell undergoes apoptosis.
3. First you rearrange one kappa gene and then, if that does work, the other, again turning off the unused genes.
4. If neither works, you proceed to the lambda, first one then the other.
5. Only if all four genes prove to be duds does the cell apoptose.

B. Comments on the Final Peptides

1. Whether you start with a lambda or kappa gene, you wind up coding for peptides that superficially look the same.
2. The peptides for these genes NEVER have membrane-spanning regions.
3. Once you have decided on a heavy chain gene the RNA from this gene can be alternatively spliced into four **different** peptides.

μ (M class) with M1 and M2

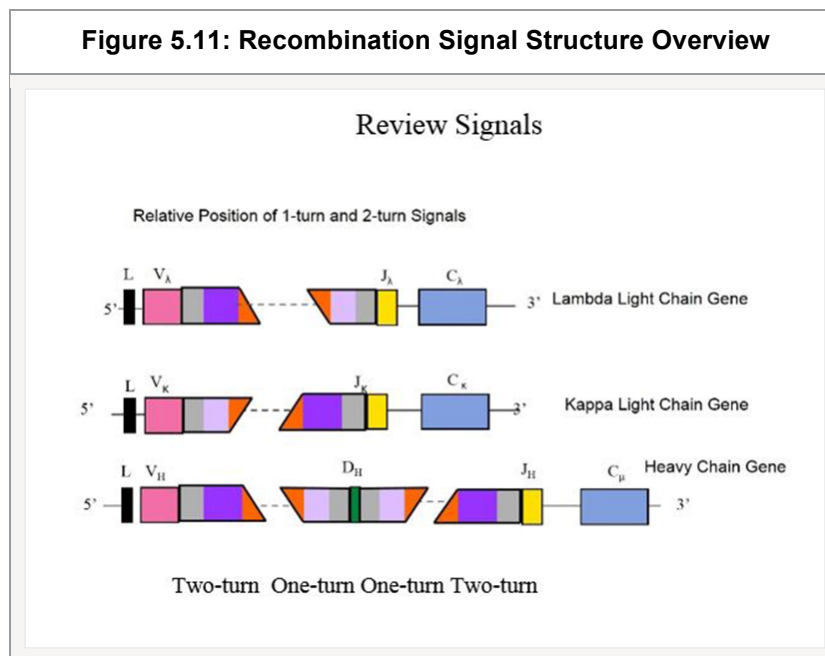
δ (D class) with M1 and M2

μ (M class) soluble

δ (D class) soluble

- a. Initially, a developing cell only makes the μ version with membrane-spanning exons.
- b. Upon maturity, the cell now makes both μ and δ peptides in the same cell.
- c. Once stimulated to produce antibody, the cell makes primarily μ peptides, but without the membrane spanning region. Thus they will make soluble M-class antibodies.
- d. A cell rarely makes δ peptides without the membrane-spanning regions.

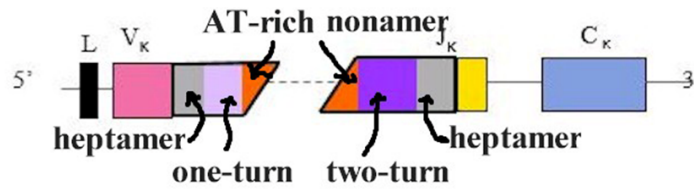
IV. Mechanism Details: Breaking and Joining (Figure 5.11)



A. Signal Structure

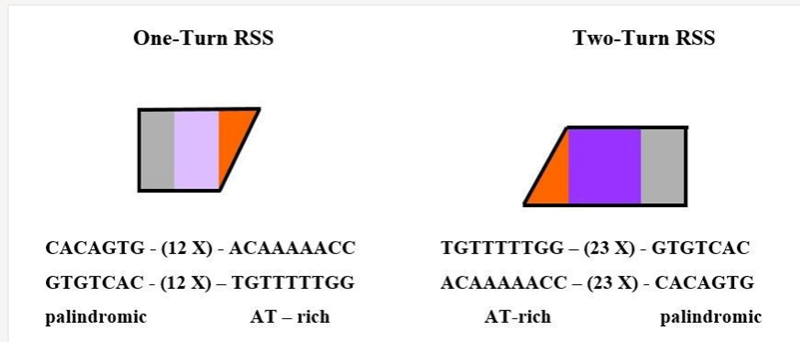
1. Recombination signal sequences (RSSs) flank the V, D, and J segments
 - a. 3' end of V
 - b. both sides of the D
 - c. 5' end of the J
2. Each RSS has (Figure 5.12)

Figure 5.12: Recombination Signal Structure Detail



- a. 7 nucleotide palindrome (reads the same forward and back). This region will participate in the ligation of adjacent V(D)J segments.
- b. spacer, seems to be important in lining up the adjacent V(D)J regions so that they join properly:
 - i. κ 12 base (1 turn of helix - V) or 23 base (2 turns of helix - J)
 - ii. λ 23 base (2 turns of helix - V) or 12 base (1 turn of helix - J)
 - iii. heavy chain, V and J, both are two turn, D is one turn
- c. 9 nucleotides rich in AT – aid in attachment to the multienzyme complex that performs the whole complex function. (Figure 5.13)

Figure 5.13: Recombination Signal Structure Nucleotide Sequence



3. Signal sequences with one-turn spacers can only join with those with two-turn spacers, so this protects against misjoining.
4. The enzyme complex responsible for joining is called the V(D)J recombinase.

B. Mechanism of Rearrangement

1. The genes to be arranged moved to and specific location in the nucleus and open up, detaching from the nucleosomes.
2. First the processing complex grabs one of the RSS signals, than it grabs the complement.
3. One-turn signal juxtaposes to two-turn signal.

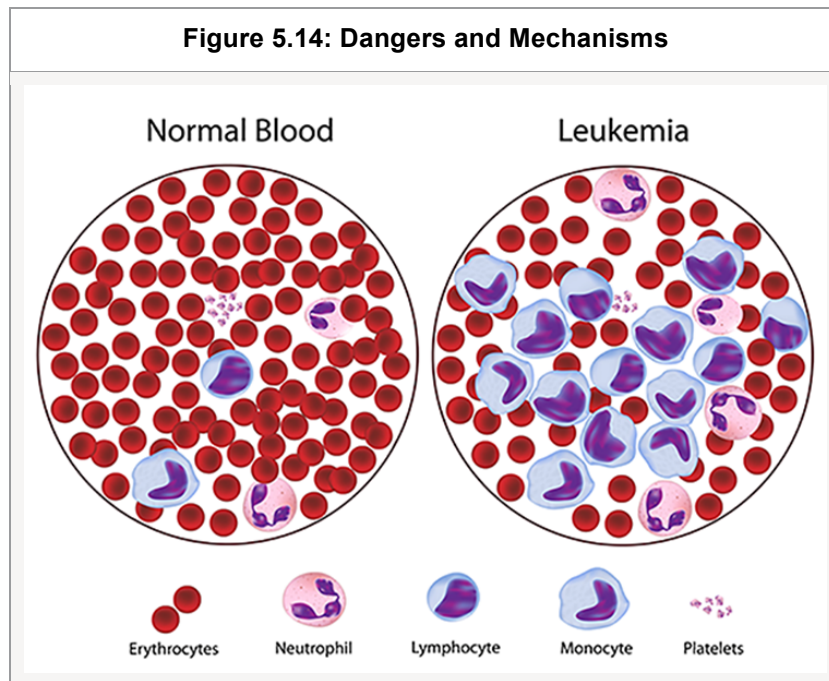
4. One strand of the DNA between the coding and signal sequence cleaves.
5. The 5'OH end of the cut strand attacks the opposite side of the uncut strand of the same DNA molecule.
6. This produces a hairpin loop at the downstream end of the V and the upstream end of the J (light chains).
 - a. The nucleotides within the loop were originally part of the palindromic sequences.
 - b. The one- and two-turn sequences, along with the AT region signal end in a flush cut with a 5' phosphorylated end.
7. Enzymes clip the hairpin loop. Now the gene regions end in a double strand of DNA with an open end, the clip site used to join the V(D)J and add variability at the junction.
 - a. A few nucleotides get trimmed off.
 - b. The ends of two of the single strands are ligated.
 - c. Nucleotides are added to fill in the unmatched single stranded regions (P-nucleotide addition), matching the ends generated by the cut.
 - d. For heavy chains only, up to 15 additional nucleotides can be added at random in the junction
8. Repair and ligation of the coding joint, with release and digestion of the signal sequences (which are retained if there an inversional joining.)

9. At a later stage of development, the parts of the genes coding for the hypervariable region can mutate, introducing further variation (much more later).

C. Commitment to Producing a Single Functional CDR (Antigen Binding Region)

1. Because the joining process between V_L and J_L and V_H , D_H , and J_H regions contains so many random elements, about 2/3 of the time, any one junction will produce a frame shift.
2. If any one junction in any part of the gene gets out of phase, this produces a non-productive rearrangement.
3. If the message remains in phase, this usually produces a productive rearrangement and the message for a working peptide. N and P addition can also throw in random stop codons, even if the message as a whole remains in frame.
4. Recall that these cells are diploid and that these rearrangements will take place on both homologous chromosomes.
5. However you must get both a good heavy and a good light of some kind or the cell dies by apoptosis. Only about 8% make it through this.
6. Allelic Exclusion - you only get one good productively rearranged gene.
 - a. The heavy rearranges first. Once there's a good heavy chain, this shuts off rearrangement of the other heavy gene.
 - b. The light rearranges next. First it tries a κ , and if one works, the cell shuts down rearrangement.
 - c. If the κ doesn't work, it tries a λ . Once one of them works, the cell shuts down rearrangement.
 - d. If nothing works, the cell apoptoses.
7. Once a cell has a working heavy gene and a working light, then it shuts off its recombination enzyme genes.

D. Dangers and Mechanisms (Figure 5.14)

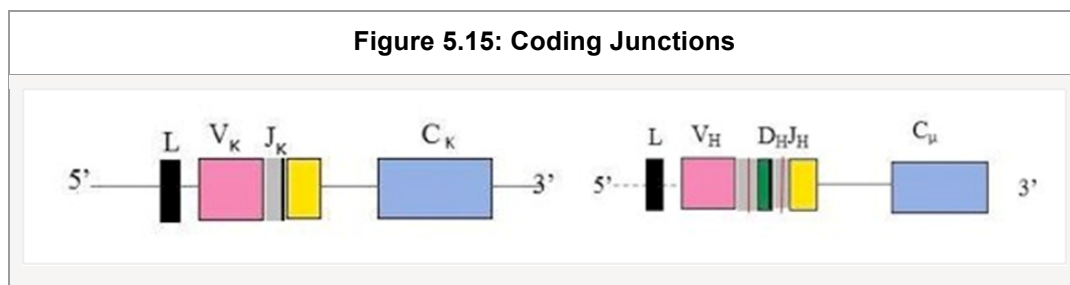


V. Reviewing the Generation of Antibody Diversity.

A. Mixing and Matching Germ Line Genes

1. Recall that both mice and humans have multiple V_H , D_H , J_H , V_K , D_K , J_K , and J_λ regions on homologous chromosomes.
2. You can put any heavy chain with any light.
3. So just from recombinatorial options in the germ line, there's a lot of potential variability.

B. Fooling Around at the Junction (Figure 5.15)



1. Coding junctions do not always join precisely.
2. P-nucleotide addition. Generates a palindrome when it matches the nucleotides opened at the clipping of the hairpin loop.

3. N-nucleotides- those optionally inserted in the middle of this.
4. The region of the protein coded by the junction winds up in the third CDR loop of the variable region.

C. Somatic Hypermutation (Figure 5.16-5.17)

Figure 5.16: Somatic Hypermutation Light Chain

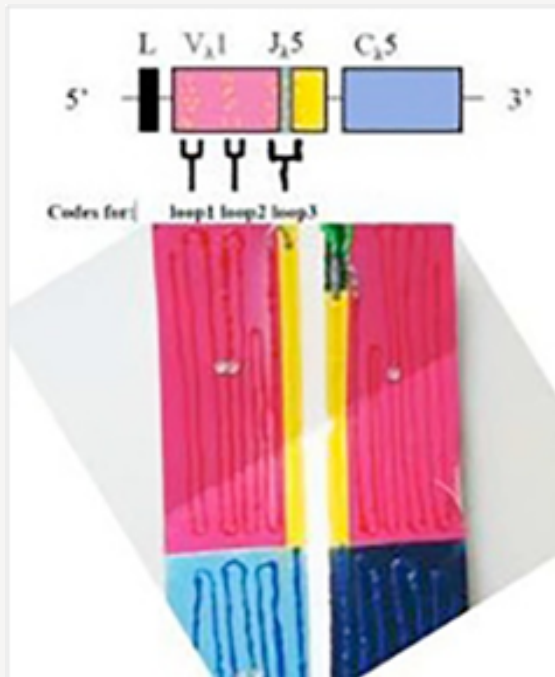
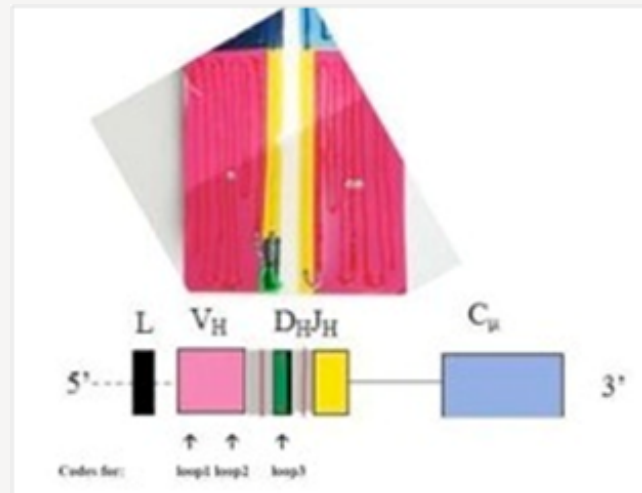


Figure 5.17: Somatic Hypermutation Heavy Chain



1. The naive B cell leaves the bone marrow able to make one and only one kind of CDR (although it can add this to the different classes of antibody heavy C chains).
2. However, it can still change or mutate the region coding for the hypervariable loops.
3. This occurs later in the process of antibody stimulation, maturation, and selection outside the bone marrow.

VI. Class Switching

A. Where and When

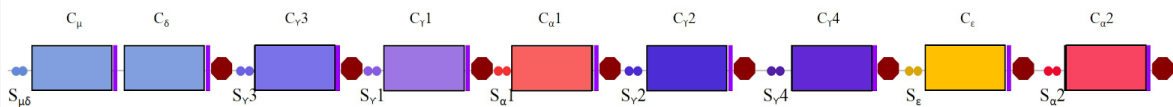
1. Class switching typically occurs in the lymph nodes after exposure to antigen.
2. Class switching takes place after the system has been producing antibodies for a week or more.
3. Thus the first antibodies produced in response to an infection are Class M and you don't start to see G (or other classes) until later in the infection.

B. How

1. Class switching involves further rearrangements to the DNA, but these are brought about by a separate set of enzymes. (*Figure 5.18*)
2. T_H cells synapse with the B cells and signal the specific switch.
3. Recombination sites are called switch regions, and are designated by 2-3 kb sequences of DNA with multiple copies of short consensus sequences.
4. Before switching, the cell expresses the μ and δ constant regions.
5. Switching involves removing these and whatever other constant gene region(s) stand(s) between the VDJ recombined region and the constant to be expressed.

These may be removed in sequence as a cell class-switches down its options.

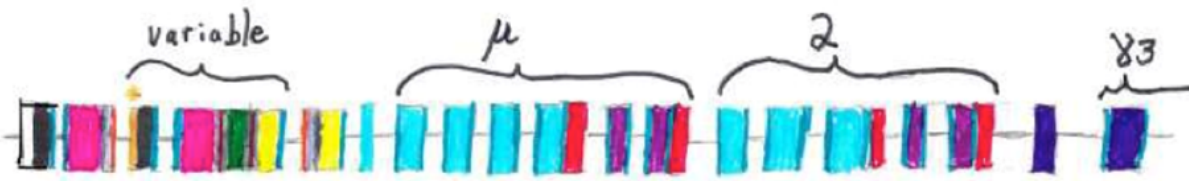
Figure 5.18: Heavy Chain Constant Regions



VII. Synthesis and Secretion

A. A Farewell Look at the Complexity of the Heavy Chain Gene (Figure 5.19)

Figure 5.19: Substructure of the 5' and Heavy Chain Gene



1. Our first look showed the different gene regions, but not the internal structure of the constant region or most of the signals.
2. If we enlarge a portion of the rearranged gene showing just one upstream VL and the downstream region into the first gamma exon, we can show more detail.
3. At a first pass you can see that the turquoise lines indicate splice signals, which will allow introns to be processed from the message.
4. Focus on the part of the gene that codes for the variable domain, which is flanked by an unused VL and an unused J, both of which still have the complete RSSs.
5. Locate the functional parts of the instructions for the variable domain:
 - a. The activated promoter
 - b. The VDJ in use
 - c. The rearrangement joints with the location of the N-nucleotide addition
6. Now focus on the constant chain instructions
7. The C μ and C δ domains are coded for by separate exons for each constant domain with intervening introns.
 - a. The sequence is preceded by a switch signal.
 - b. Thus there are four C μ exons and three C δ , the D class having the hinge region and therefore greater flexibility.
 - c. Both complex regions have two more exons (M1 and 2) downstream from the constant exons.
 - d. Both C μ and C δ have a polyA splicing option in both the last Ig exon and the second membrane spanning exon.
 - e. This explains the four possible options (M or D antibody, M or D Ig receptor) that a message from this region could specify.
8. Beware of confusing terms! Class M refers to the whole antibody, IgM, whether soluble or membrane bound. This class has the μ constant region. If it is membrane-bound then the μ has M 1 and 2 at the end.
9. This drawing does not show most of the rest of the constant instruction, but you can see the switch site and first exon for the $\gamma 3$ gene region.
10. All other gene regions have the two membrane-spanning exons, expressed only in memory cells.

B. Processing in the GERL (Golgi-ER-Lysosome)

1. L and H transcripts exit the nucleus separately, attach to ribosomes, and begin peptide synthesis.
2. The initial signal sequence (leader) causes the ribosome to attach to the RER and the insert the L and H peptide separately into the lumen.
3. Only if the message for the H chain ends on a membrane-spanning region, the peptide will remain anchored in the membrane.
4. In the RER lumen, enzymes clip off the leader and being to add oligosaccharides to the peptides.
5. Assembly and processing occurs in the primarily in the lumen of the RER and the product is then sent to the Golgi.
6. First the heavy chains are put together, then the Ls are added (for the G class first one heavy and one light associate).
7. After assembly, enzymes oxidize the disulfide bonds, nail the structure into position, and adjust the oligosaccharide into the version characteristic of the antibody.

The More You Know: Optional Resources and Fun Stuff

(You don't get tested in this!)

Resource:

VIBE- Virtual Interdisciplinary Biology Education

http://bcs.whfreeman.com/immunology6e/content/cat_070/Stanford%20VIBE/index.html

Lecture 6 (L06)

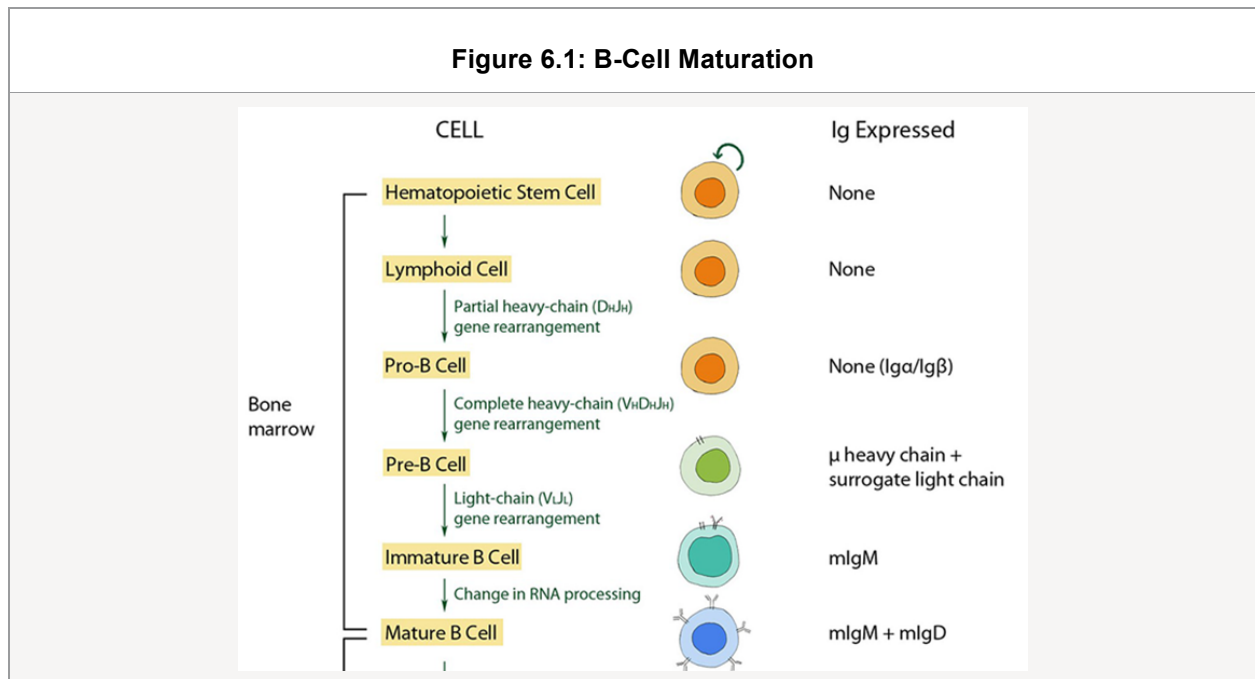
Development of B Cells

Most of what we once thought we knew about global health has been proved wrong by the relentless advances of HIV/AIDS, tuberculosis and malaria.....There can be no more urgent cause facing us today. In Africa, the enemy is already among us. In Asia, the enemy is at the gates.”

– Richard G. A. Feachem, Houston Chronicle, 1/21/03.

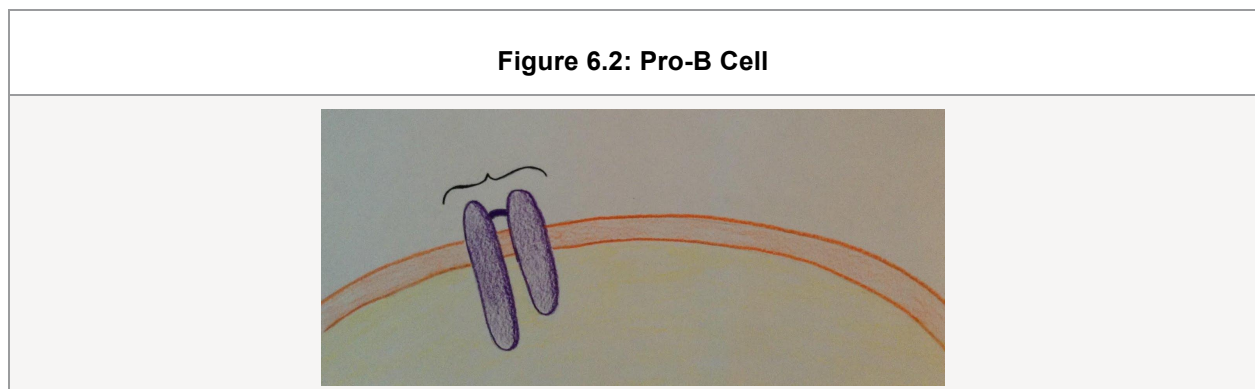


I. B-Cell Maturation (*Figure 6.1*)



A. Initial Steps (Bone Marrow)

1. Stem cell commits to lymphoid line.
2. Lymphoid progenitor commits to progenitor B cell, or pro-B cell, first developing signaling ability using Igα/Igβ transmembrane proteins with ITAMs (*Figure 6.2*)

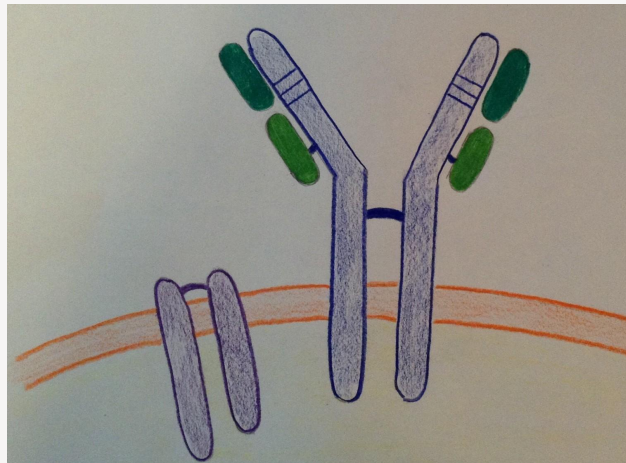


3. Pro-B cells begin gene rearrangement and differentiate into pre-B cells upon stimulation by the stromal cells

B. Differentiation and Gene Rearrangement (bone marrow), review

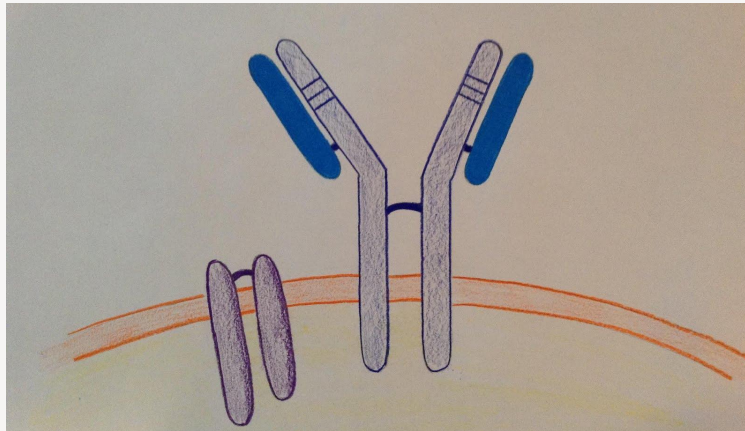
1. Pro-B cells bring D_H and J_H together
2. Then they add leader plus V_H to D_HJ_H , and do the P and N nucleotide additions.
3. If this produces a non-productive (frame shifted) gene rearrangement, then they try the other allele.
4. If the rearrangement is productive, then the heavy chain is put into the membrane with a surrogate light chain, composed of the products of two genes that can function without rearrangement (*Figure 6.3*)

Figure 6.3: Pre-B Cell



5. The immature receptor associates with the $Ig\alpha/Ig\beta$ transmembrane signal. This signals allelic exclusion and initiates the light chain gene rearrangement.
6. Once there is a productive H chain gene, the cell is a pre-B cell. If there is no productive rearrangement, the cell apoptosis.
7. The pre-B cell then undergoes rearrangements of first one κ , then the other, and one λ , then the other, stopping as soon as there is a productive light chain arrangement and ultimately apoptosing if there is not.
8. Once you have two productive rearrangements, you have an immature B cell, one that has a determined antigenic specificity (CDR) and uses the μ C_H region to produce membrane-bound antibody. (*Figure 6.4*)

Figure 6.4: Immature B Cell



9. Undergoes negative selection (below).
10. Shortly thereafter, the cell also begins to express membrane-bound δ C_H, and become a mature (but naïve) B cell, expressing IgM and IgD on the cell surface.
11. At this point the cells are released into the plasma and head to the peripheral (secondary) lymphoid organs: lymph nodes, spleen and mucosa.

C. Removing Self-Reactive B Cells.

1. 90% of the B cells produced by the above process never make it to the plasma. At least some of the negative selection occurs as cell lines expressing antibodies to self-antigen are eliminated.
2. The elimination is signaled by the crosslinking of IgM.
3. Artificially crosslinking IgM will lead to apoptosis of developing B cells.
4. However, B cells can sometimes get a second chance. If cross-linking occurs, the cells may arrest and reactivate their RAG enzymes, and try rearranging again.
5. If they have a κ chain involved in the CDR for a self-antigen, they may try to replace it with a λ .

II. B-Cell Activation and Proliferation - To survive, circulating B cell must encounter antigen that can bind to its receptors or they will undergo apoptosis within a few weeks.

A. Initiating Antigen Exposure

1. Thymus-dependent activation: In most cases, when antigen cross-links a B cell's Ig receptors, this sets off a signal (signal 1) and the B cell seeks out a T_H cell.

2. Thymus independent activation; there are a few antigen (TI antigens) that can prompt B-cell development independent of T_H cell co-stimulus. These antigen also simultaneously activate toll-like receptors.
- a. Type 1 antigen - lipopolysaccharide such as those found in the outer bacterial cell walls of gram negative bacteria, which also activates TLR4. (*Figure 6.5*)
 - b. Type 2 antigen - repetitive polymeric proteins, such as bacterial flagellin, can cross link the membrane-bound immunoglobulins and kick off proliferation if the simultaneously activate TLR5. (*Figure 6.6*)

Figure 6.5: Type 1 Antigen

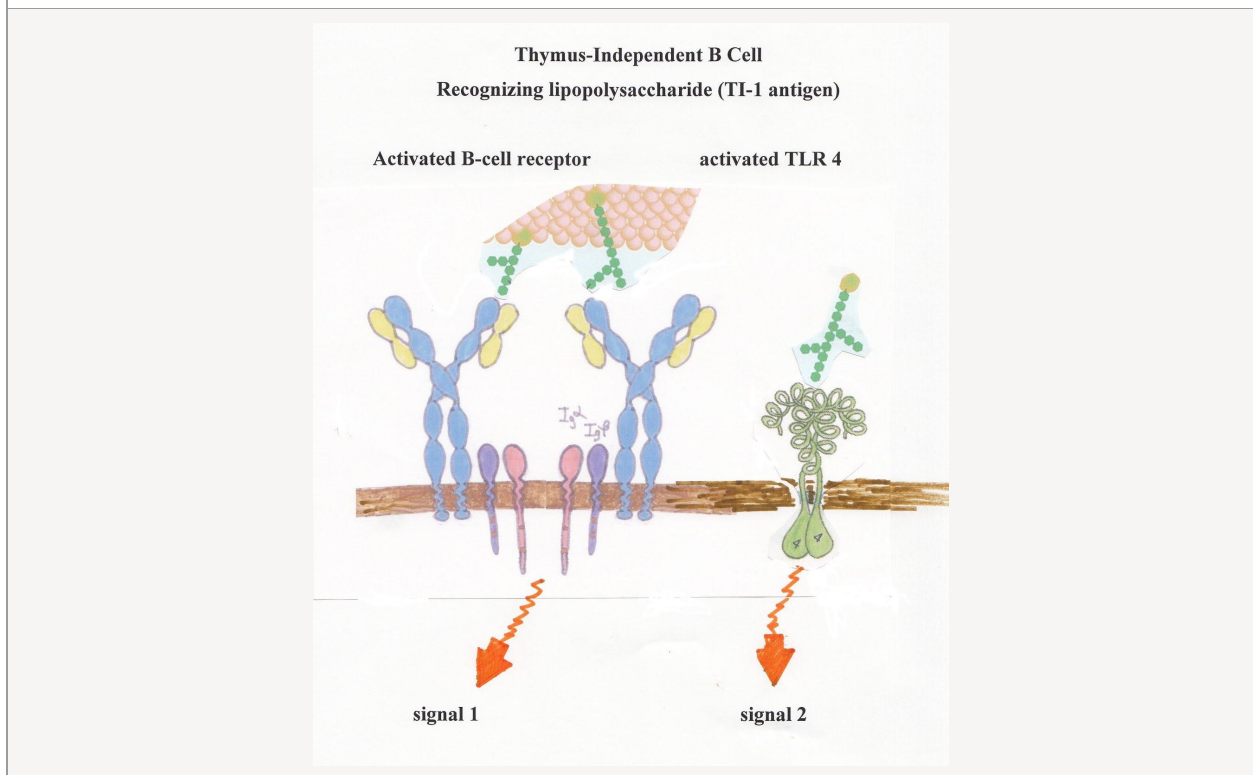
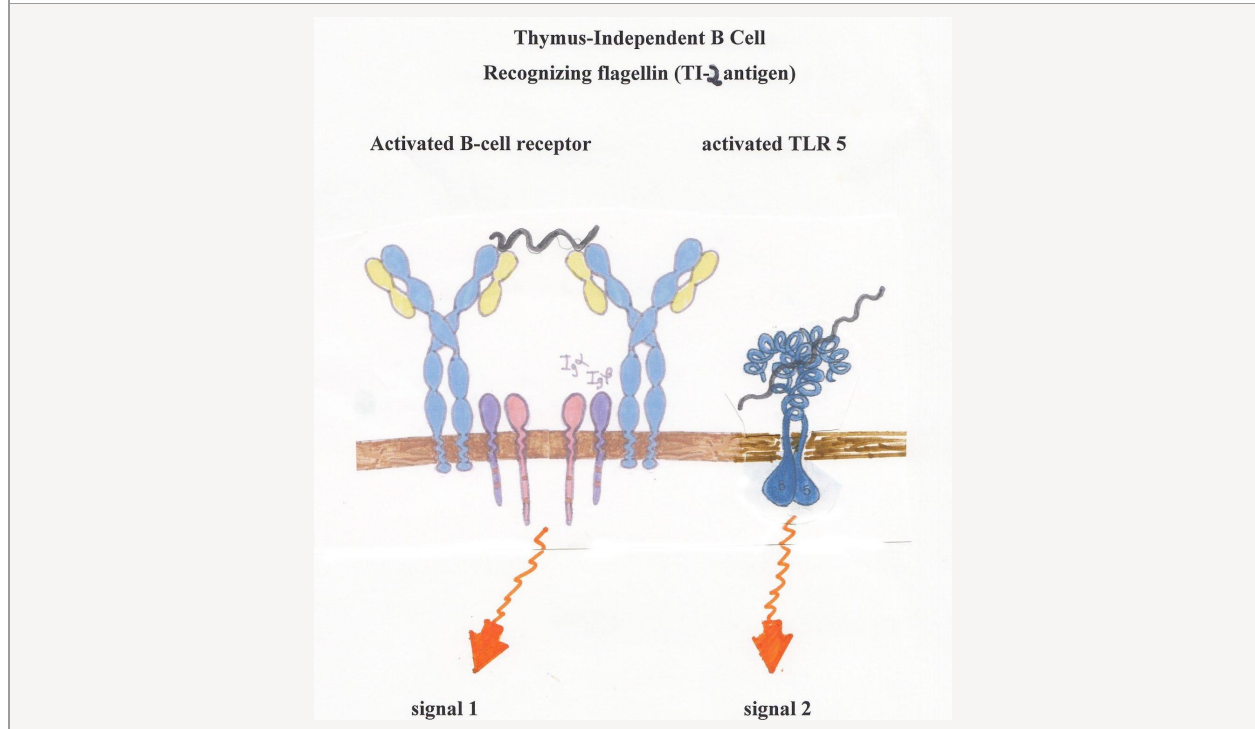


Figure 6.6: Type 2 Antigen

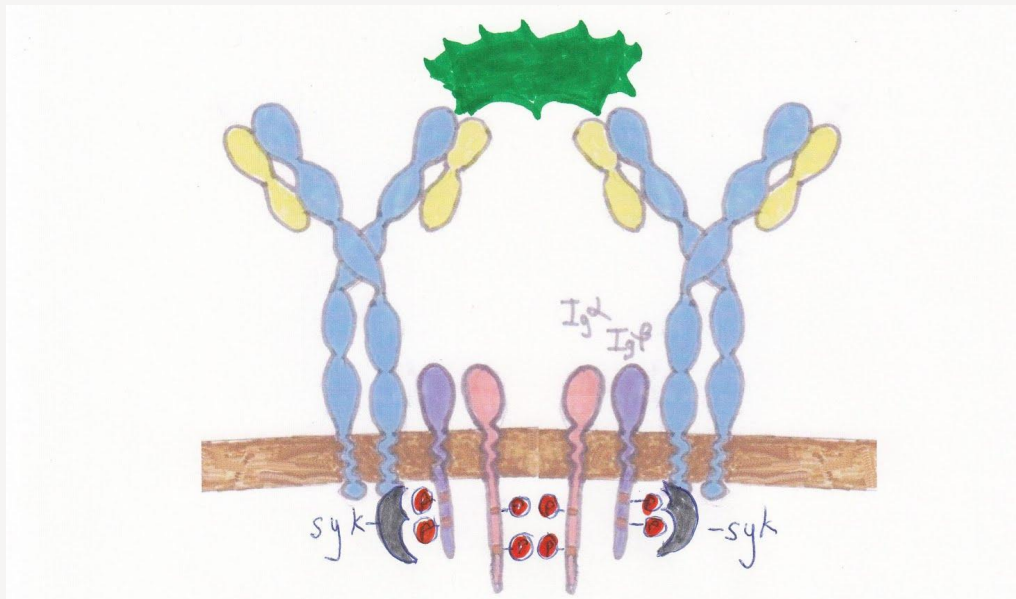


3. However, TI activation does not induce class switching (you mostly just make IgM) and does not produce memory cells. For that you need T_H cells.

B. Activating Signals- Generating signal 1.

1. Review Ig receptor.
 - a. mIgM or mIgD molecule
 - b. $I\gamma^{\alpha}/I\gamma^{\beta}$ heterodimers
 - c. **immunoreceptor tyrosine-based activation motif**, or ITAM extend into the cytoplasm
2. When an antigen cross-links one antibody with the next outside the cell, it brings together the cytosolic $I\gamma^{\alpha}/I\gamma^{\beta}$ domains, activating the ITAMs. (Figure 6.7)
3. This causes the complex to change conformation and activates src-like kinases. These are enzymes that add phosphate to molecules and they add them to the ITAMs.
4. Once the ITAMs have phosphates, another kinase, syk, docks and triggers several different enzymes cascades.

Figure 6.7: Activation



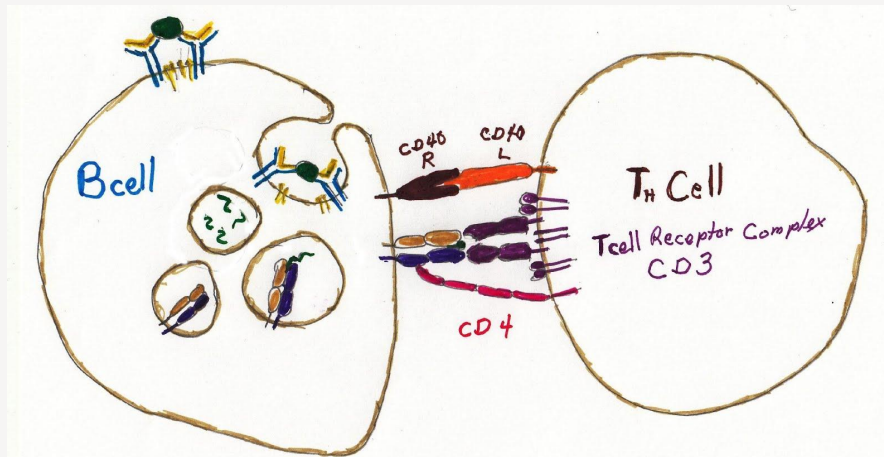
5. These lead to the up-regulation of transcription factors, the inflammatory transcription factor NF- κ B being involved in this.
6. The cells begin to divide and secrete antibodies.
7. As antibodies build up, they bind to CD-22. the Fc or antibody stem receptor, which provides brakes on the system

C. Role of T_H cells

1. However, the BCR does not signal effectively without contact with a T_H cell, nor do the cells divide rapidly without additional stimulus from T_H cell cytokines.
2. When B cells bind antigen, they bring some it inside and hydrolyze it.
3. Some of the hydrolyzed peptide winds up attached to class II MHC molecules, the genes for which are upregulated along with the one for B7.
4. Thus the B cell can present some of the antigen to a T_H cell and also contact the T cell using B7 to CD28.
 - a. Because of its ability to gather up the antigen using the BCR, a B cell is very effective at presenting antigen, and can stimulate a T_H cell at concentrations 100 to 10,000 times lower than those necessary for a macrophage or dendritic cell.
 - b. Of course the antigen received is different from the antigen presented. The presented antigen is a peptide derived from the overall molecule.

5. The cells attach, forming a conjugate or immune synapse.
6. This causes the T_H cell to produce CD40L, which is a juxtacrine factor that turns around and signals the B cell through CD40 receptor. (Figure 6.8)

Figure 6.8: Immune Synapse



7. The contact reorganizes the interior of the T_H cell so that cytokines are released toward the B cell.
8. The B cells begin producing receptors for the cytokines.
9. Cytokine signaling activates the B cells and they begin proliferating and differentiating.

III. Primary Versus Secondary Response

A. The Primary Response

1. naïve lymphocytes
2. 4 to 7 day lag time
3. produces antibody secreting plasma cells and memory cells
4. initial antibodies mostly IgM; IgG toward the end

B. The Secondary Response: The Sadder, but Wiser, Immune System

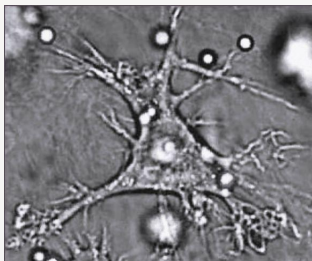

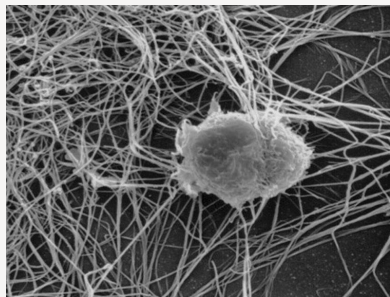
1. Produced primarily by memory cells
2. 1 to 3 day lag time

- a. The number of memory cells specific for the antigen increases.
 - b. These memory cells are more easily activated.
 - c. They have already been through affinity maturation, so they're better at binding antigen
3. more antibody secreted, and over a longer time
4. much higher proportion of IgG and other isotypes

IV. B-Cell Maturation in Anatomical and Histological Context

A. Lymph Nodes

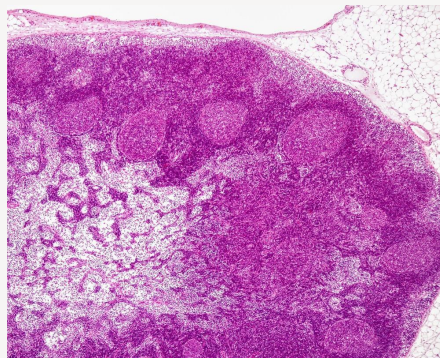
1. Lymph drains from tissues and passes through these.
2. Antigen enters. It can be
 - a. "free" - particle from pathogen, or the whole bacteria or viruses themselves
 - b. proteins or other antigens from the pathogens complexed with antibodies
 - c. carried in by presenting cell (dendritic or macrophages) that have picked it up elsewhere
3. Free and antibody-bound antigen in the plasma is likely to be picked up by
 - a. interdigitating dendritic cells (*Figure 6.9*)
 - b. macrophages (*Figure 6.10*)
 - c. follicular dendritic cells (*Figure 6.11*)

Figure 6.9: Interdigitating Dendritic Cell	Figure 6.10: Macrophage	Figure 6.11: Follicular Dendritic Cell
		

4. Naïve lymphocytes from the bone marrow enter via the lymph.
5. Activation begins in the paracortex, the layer between the outer cortex and the inner medulla, where there is a high concentration of T cells, macrophages, and dendritic

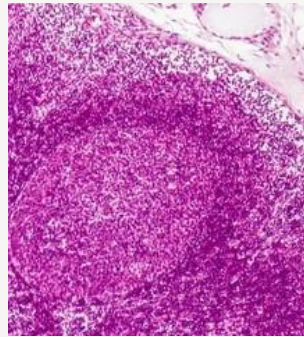
- cells. (Figure 6.12)
6. First the macrophages and the dendritic cells activate the T_H cells.
 7. Naïve B cells contact the T_H cells, presenting any antigen they have internalized via the class II MHC, and forming a conjugate (immune synapse).
 8. The B cell begins to divide, producing a clonal cluster (focus) at the boundary between the paracortex and cortex.
 9. A few activated B and T_H cells migrate together from one of these foci to a primary follicle in the cortex.
 10. The follicle becomes a secondary follicle, one with a germinal center where B, T_H , and follicular dendritic cells interact.

Figure 6.12: Lymph Node



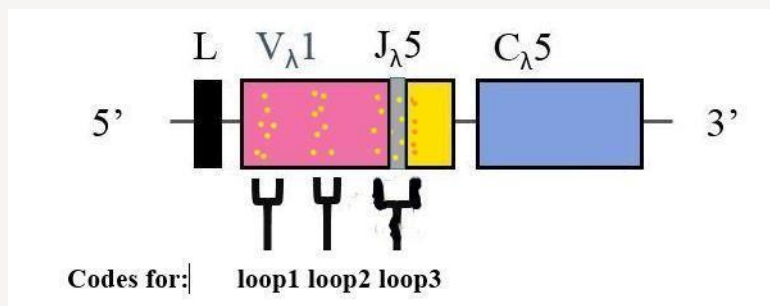
11. A reminder about follicular dendritic cells: these are **NOT** regular dendritic cells. They capture antigen-antibody complexes in beaded structures (icosomes) and present them to the B cells.
- B. Germinal Centers. These are the sites of affinity maturation (somatic hypermutation CDR selection), the processes that refine the ability of a B cell's CDR to bind antigen effectively. (Figure 6.13)

Figure 6.13: Germinal Center



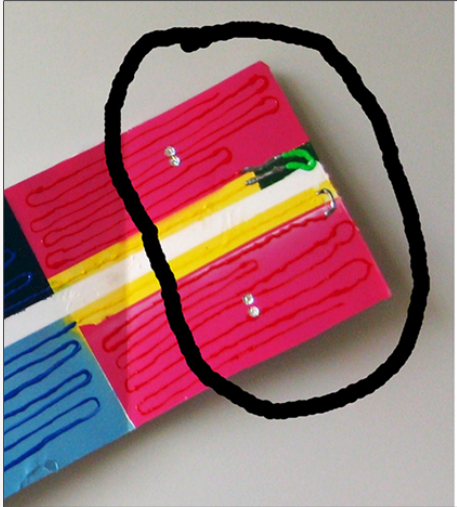
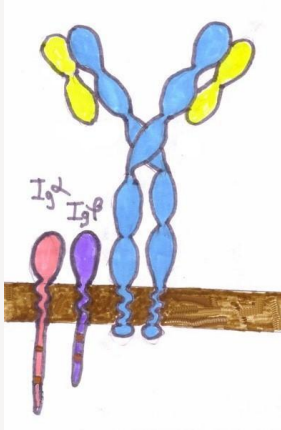
1. Activated B cells (centroblasts) proliferate and move to one edge of the follicle, forming a dark zone. At this stage the centroblasts:
 - a. enlarge and begin to divide rapidly
 - b. begin somatic hypermutation- mutating the regions in the heavy and light chain genes that code for the variable loops. (*Figure 6.14*)
 - c. stop displaying the membrane Ig (recycles the original via membrane turnover)

Figure 6.14: Somatic Hypermutation CDR Selection



2. The centroblasts differentiate into centrocytes which
 - a. stop dividing
 - b. begin expressing membrane Ig
 - c. move into light zone
 - d. contact follicular dendritic cells
 - e. undergo selection B cells during which more effective receptors will survive and multiply at a greater rate.
3. What happens in the follicles is a highly unusual form of accelerated natural selection. It works like evolution in general, except that the time frame is days and not centuries.

- a. random mutation (dark zone)
 - b. excess reproduction (dark zone)
 - c. selection (light zone)
- 4. The centrocytes leave the germinal center as plasma cells, lose their surface antibody again, and begin secreting antibodies.
- 5. Most centrocytes do not contact an antigen that fits with their surface receptors, however, and these die by apoptosis, and are recycled by macrophages.
- 6. Purpose - The environment of the light zone selects for those centrocytes that express the most effective antibodies. Cells with effective antibodies live, those without may return to the dark zone for more mutation, or they may die by apoptosis.
 - a. The maturing B cell that enters the germinal center and begins dividing does so if it can bind (with its surface antibody) to some degree an antigen currently arriving in the lymph node.
 - b. It differentiates into a centroblast that undergoes random mutational events to the very region of the gene responsible for determining the effectiveness of this antigen binding (the CDR). (*Figure 6.15*)
 - c. The mutated centrocyte now displays the new antibody at its surface. (*Figure 6.16*)
 - d. As with most random mutations, most of the progeny centroblast cells produced by this will bind antigen less effectively than the original cell.
 - e. A few however, will bind the antigen more effectively. . Cells with improved receptors divide more rapidly than cells with less effective receptors.

Figure 6.15: CDR	Figure 6.16: Antibody
	

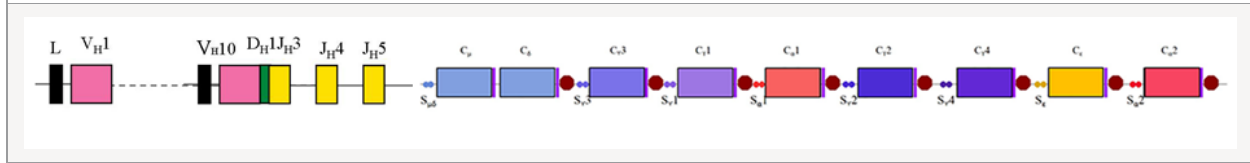
7. Signals

- Follicular dendritic cells play an important role in the selection process. If the centrocyte can bind to one of the little beads with antigen-antibody complex, then it gets a signal necessary (but not sufficient) for its survival.
- However, the beads are essentially a scarce resource, and the centrocytes have to compete for them.
- Thus the more effectively the centrocyte surface antigen binds to the antibody displayed by the follicular dendritic cell, the more likely it is to live.
- In addition, the centrocytes have to receive signals from the T_H cells, especially the contact of CDC40L to the CDC40R. This doesn't work either if the B centrocyte cell does not display processed antigen back to the T_H cell using its class II MHC.

C. Class Switching – directed by T_H cells

- The next decision the future plasma cell must make is exactly what class of antibody to send out with the refined CDR region produced by affinity maturation. (Figure 6.17)

Figure 6.17: Heavy Chain Gene

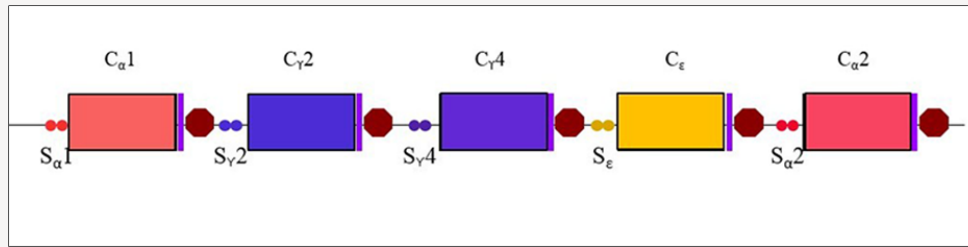


2. Cytokine signals from the T_H cells will determine this (more later).

D. To Remember or to Act: The Final Decision.

1. Centrocyte now decides whether to become a plasmablast and generate a plasma cell or become a memory cell and wait for a subsequent exposure to antigen.
2. Recall that plasma cells do not express membrane-bound antibody. This means that the sequence of differentiation in the lymph node involves:
 - a. dividing mature B cells with surface antigen
 - b. dividing centroblasts with no surface antigen (undergoing hypermutation)
 - c. non-dividing centrocytes expressing surface antibody and undergoing selection
 - d. dividing plasma cells not expressing surface antibody secreting soluble (humoral) antibody
3. The final differentiation to a plasma cell involves the switch that generates the splicing enzymes that do not add the membrane-spanning exons to the μ heavy chain message.
4. Also transcription and translation levels generally rise as the cell begins cranking out antibody, as does the proportion of RER.
5. Memory cells set aside from this process may resemble naïve B cells, but they have undergone class switching and make a variety of heavy chains. (*Figure 6.18*)
6. The receptors of memory cells may therefore also be membrane-bound versions of IgG, IgA, and IgE, and the regions for these genes all also have a region coding for a membrane-spanning portion of the antibody that is not spliced into the message for the secreted form.

Figure 6.18: Class Switching -- Heavy Chains



V. Regulation

A. B-Cell Differentiation

1. B-Cell Specific Activator Protein (BSAP) functions as master regulator.
2. Present **ONLY** in members of the B cell lineage.
3. Present in **ALL** members of the B cell lineage **EXCEPT** mature plasma cells, which are done differentiating.
4. Binds to a variety of B-cell gene promoter regions, including those like the surrogate L chains and class switching regions that are involved in developmental decision making.
5. High levels tend to maintain a cell as a memory cell, low levels tend to promote formation of plasma cells.

B. Overall Immune Effector Response - Tolerance

1. You would like **NOT** to make antibodies against your own proteins, and therefore tolerate them.
2. On the other hand, you do **NOT** want to develop tolerance for foreign antigens, especially those associated with pathogenic infection.
3. Constant monitoring of your antigens by T_{reg} cells suppresses immune responses to your own proteins and to those of benign commensal bacteria and fungi.
4. Moreover, you need to apply brakes once an infection is under control.
5. If you introduce foreign antibodies to an antigen, this will tie up the antigen and prevent it from promoting an immune response on the part of the host:

The More You Know: Optional Resources and Fun Stuff
(You don't get tested in this!)

References and Links:

The Gates Foundation
<http://www.gatesfoundation.org/default.htm>

VIBE
http://bcs.whfreeman.com/immunology6e/content/cat_070/Stanford%20VIBE/index.html

Follicular Dendritic
<http://home.comcast.net/~aszakal/>

Table 6.1 B-lineage Development Summary

Name	Location	Surface receptor molecule	Other surface signaling molecules	Activity
Pro B cell	Bone marrow	Igα/Igβ co-receptor	C-kit, CD45R	Rearranging heavy chain gene
Pre B cell	Bone marrow	Heavy chain (μ) plus surrogate light chain and co-receptor	CD25 (α chain of IL-2 receptor)	Rearranging light chain gene
Immature B cell	Bone marrow	mIgM and co-receptor	No CD 25	Undergoing selection against self-recognition, changing RNA splicing
Mature, naïve B cell	Circulates: plasma, secondary and tertiary lymphoid tissue	mIgM and mIgD and co-receptor		Trolling for antigen: binding and activation necessary for next stage
Activated B cell - 1	Peripheral lymphoid tissue (lymph nodes as example) at paracortex	mIgM and mIgD and co-receptor		Begins clonal expansion

Table 6.1 B-lineage Development Summary Continued

Name	Location	Surface receptor molecule	Other surface signaling molecules	Activity
Activated B cell - 2	Cortex, primary follicle, which then become secondary, with germinal center	mIgM and mIgD and co-receptor	Up-regulate MHCII, CD 40R, various cytokine receptors	Associate with T cells
centroblast	secondary follicle, periphery (dark zone) of the germinal center	No surface Ig receptor		Divide rapidly, mutate Ig V regions in sequences coding for the CDR loops
centrocyte - 1	Cortex, now secondary follicle, periphery, light zone of germinal center	altered mIgM and mIgD and co-receptor	CD40R (signal from CD40L necessary, too)	Stop division, compete for antigen displayed on follicular dendritic cells.
centrocyte - 2	Cortex, now secondary follicle, periphery, light zone	altered mIgM and mIgD and co-receptor	CD40R (signal from CD40L necessary, too)	Decide whether or not to class switch and whether or not to be memory
plasmablast	Lymph node	none		Switch to splicing out membrane-spanning exons, up-regulation of RER and Ig synthesis
plasma cell	Circulation, site of infection	none		Secretes antibody

Lecture 7 (L07)

Complement

Science is a social process. It happens on a time scale longer than a human life. If I die, someone takes my place. You die; someone takes your place. What's important is to get it done. - Alfred Lothar Wegener, shortly before his death at age 50. He died on an expedition to the North Pole to gather information to support his hypothesis of continental drift.



I. Overview and Terminology of the Complement System -

A. What Is It? – the landmines of the circulatory system.

1. A system of over 30 proteins, most soluble in the serum, but some part of the surface of cells.
2. Organized in a cascade system reminiscent of the clotting cascade, only **MUCH** more complicated.
3. Most produced by the liver (along with serum albumins and fibrinogen).
4. Some produced by monocytes, macrophages, and epithelial tissues.
5. Released in an inactive form.
6. Proteolytic cleavage activates them.

B. How Does It Work?

1. Membrane Lysis

- a. Kills cellular invaders and those virus that use components of the plasma membrane.
- b. Produces active MAC, the membrane attack complex that punches holes in the membranes.
- c. Three different pathways can activate the production of active MAC (model)

2. Opsonization

- a. promotes phagocytosis by macrophages and neutrophils
- b. coats foreign cells, free antigen, and antibody-antigen complexes with proteins that promote phagocytosis.

3. Immune Complex Clearing

- a. coats antibody-antigen complexes
- b. attracts phagocytes to them

4. Activating Other Immune Responses

- a. Cleavage fragments produced during activation function as paracrine factors.
- b. These factors activate B cells.

- c. Promote inflammatory response

C. The Name Game

1. Most complement factors are designated by numbers
 - a. examples C1 - C9
 - b. Unfortunately, they are numbered in the order of discovery, not action.
2. Sub factors
 - a. The cleavage fragments are designated with letters. For example, C4 is cleaved into C4a and C4b.
 - b. The peptides that make up the active protein may also be designated with letters.
3. Other names -
 - a. factor D
 - b. homologous restriction factor
4. Active factors - Activation frequently involves this association of more than one factor to form an activated protein, with the complex indicated by overlining.

II. Complement Activation: The Big MAC Attack

A. Convergence of the Pathways

1. You can trigger the pathway with both innate and adaptive recognition.
2. The last stages are the same, so the end results are identical.

B. Sequence of Events

1. The cleavage of C5 to C5a and C5b.
2. C5a serves as an inflammatory signal.
3. C5b attaches to C6, begins formation of the attack complex. If unattached, 5b is unstable.
4. C5b6 complex attaches to C7.
5. C5b67 complex shifts into amphiphilic form and attaches to membrane.
6. Complex attracts C8, and C5b678 inserts into membrane
7. Complex gathers a ring of C9 peptides around it

- a. shallow cylinder with large channel down the middle.
- b. resembles perforins used by T_C cells to kill rogue-self
- c. multiple copies: 10-17 bind to complex, forming the cylindrical ring
- d. produces tube with 70 to 100Å pore - note exact size will depend on the number of C9s in the pore wall.

8. Big MAC Attack: The C9 complex pokes holes in membranes.

III. Three Roads to the Big MAC (*Figure 7.1*)

A. Classical (*Figure 7.2*)

1. C1

- a. complex of proteins that resembles a bouquet of flowers or an amusement park ride frame with struts.
 - i. 6 Cq units with the tails in a bunch and the heads sticking out. Each is made up of three peptides, A, B and C
 - ii. 2 Cr, hooked end to end.
 - iii. 2 Cs, hooked on the ends of these
- b. Cr₂S₂ complex
 - i. When bound to Cq complex, it assumes a figure 8 form in the middle of the spreading stems.
 - ii. Activation of C1 frees of this complex, which now wraps around the outside of the stems, exposing the catalytic activity of the Cr and Cs peptides.
- c. Antigen Binding to C1q (This is what frees the Cr₂S₂ complex)
 - i. Any one of the 6 heads can bind to the F_C (stem) part of an antibody.
 - ii. If any two of the heads bind, then the Cr₂S₂ complex is freed.
 - iii. The oligosaccharide and the domain it is attached to constitute the principal agent of activation.
- d. Binding to IgM, figure 7-4, page 171 and figure 4-17e page 97
 - i. Circulating IgM is pentameric (*Figure 7.3*), so the C1 ought to be able to attach at least two heads to one antibody.

Figure 7.1: Preview of Three Pathways

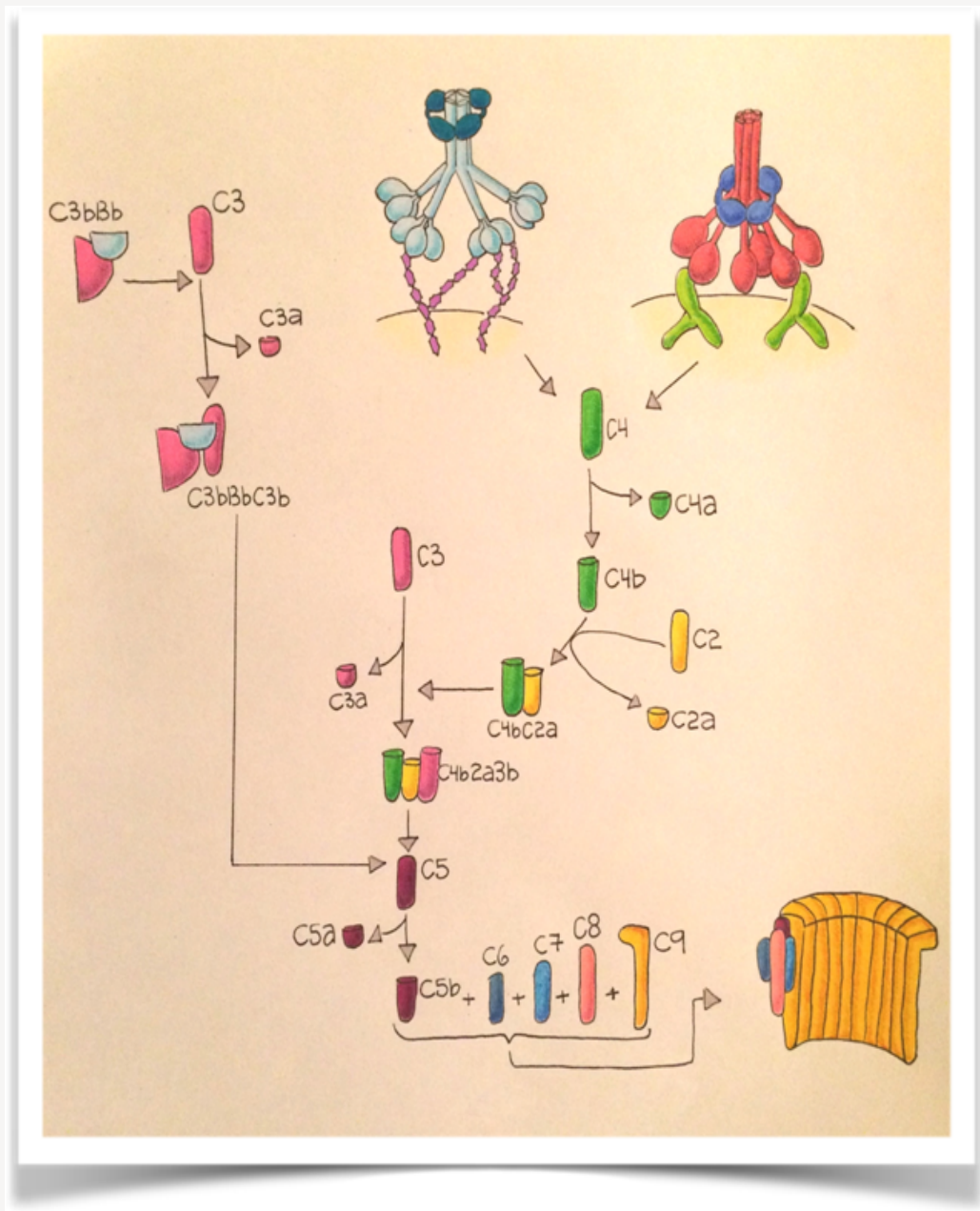


Figure 7.2: Classical Pathway Diagram

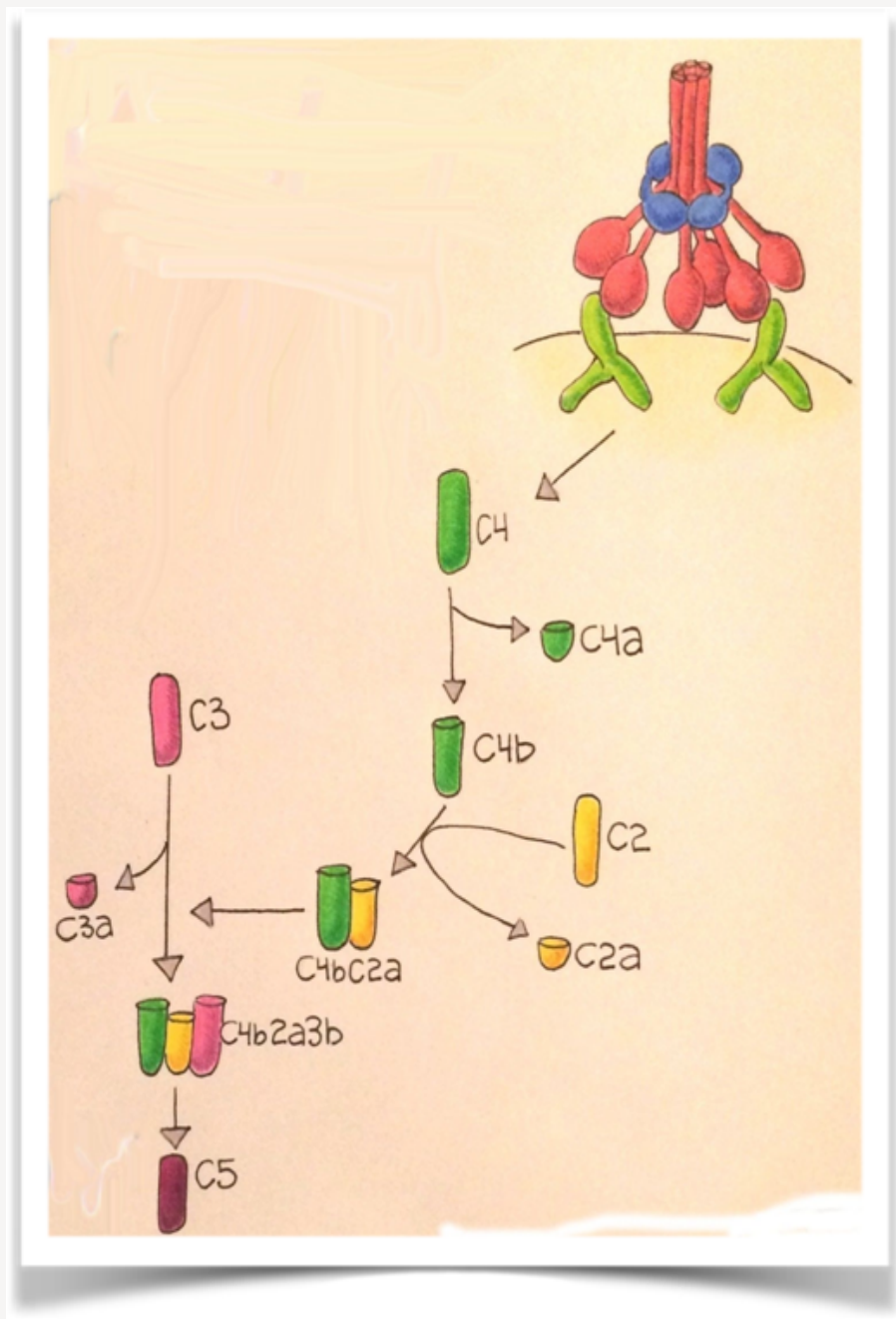
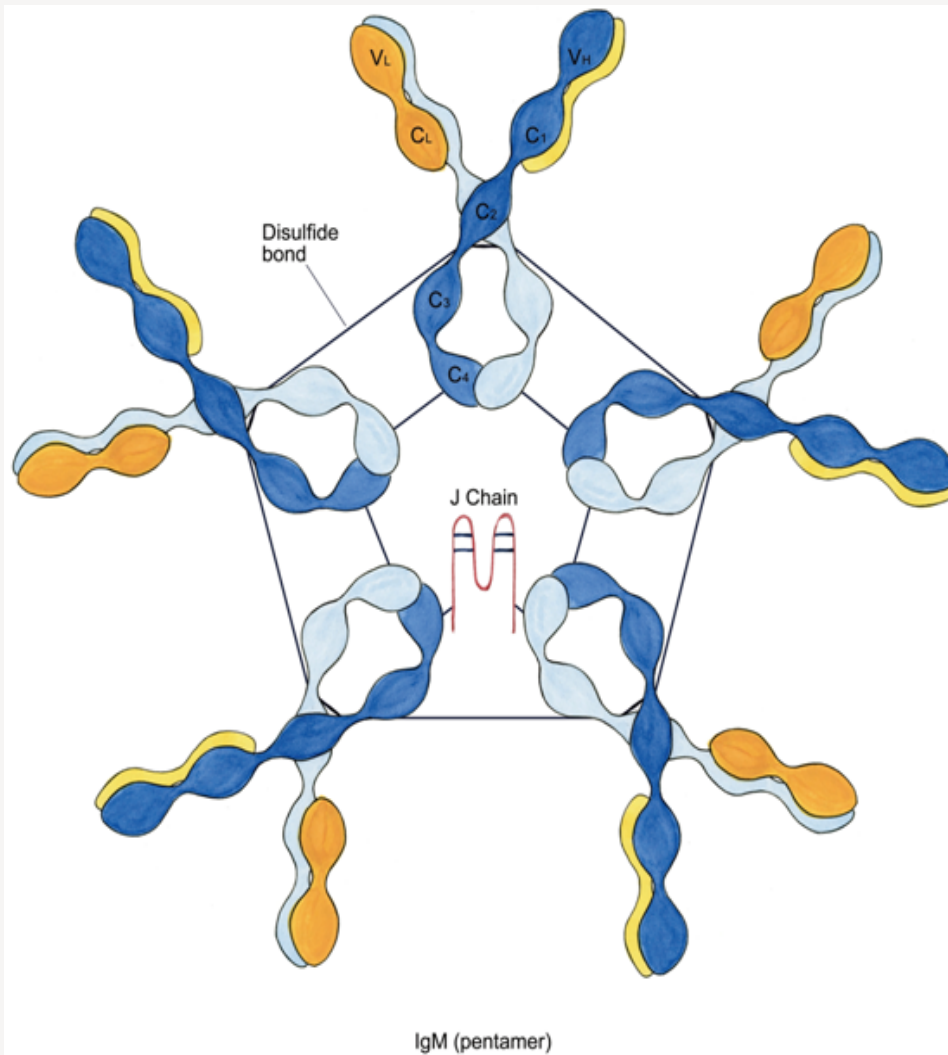
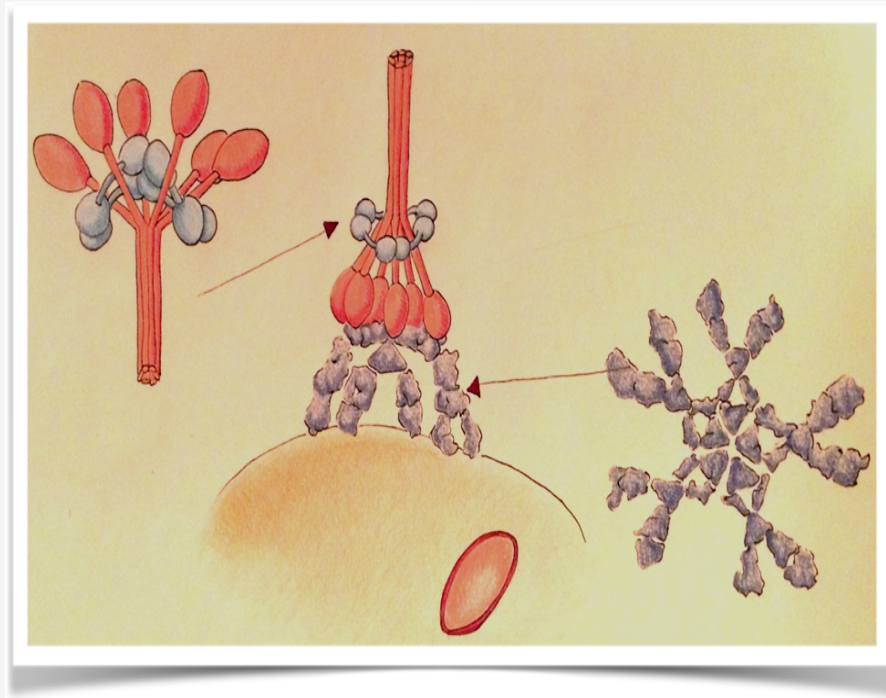


Figure 7.3: Pentameric IgM



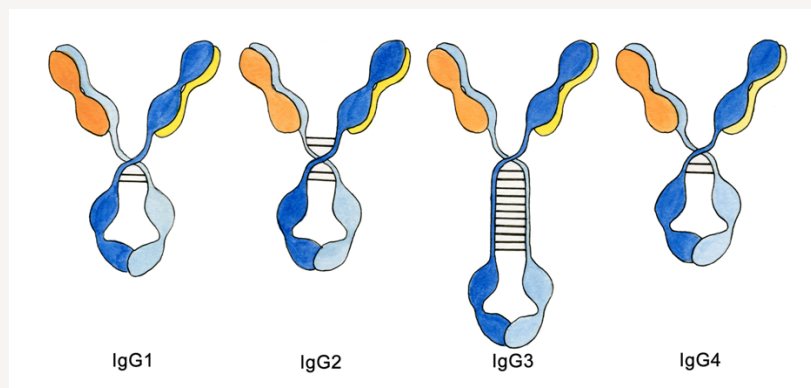
- ii. However, when the IgM is circulating, it's usually in the planar conformation and the heads can't get into the middle well enough to bind.
- iii. When the IgM binds antigen, it flips into the staple conformation, bending the arms away from the F_C region, and making it more accessible. (*Figure 7.4*)
- iv. At that point, even one IgM can activate the complement cascade.
- v. IgM in someone with an infection, even its early stages, will activate the cascade rapidly and effectively.

Figure 7.4: IgM Planar to Staple



e. Binding to IgG (*Figure 7.5*)

Figure 7.5: IgG Subclasses

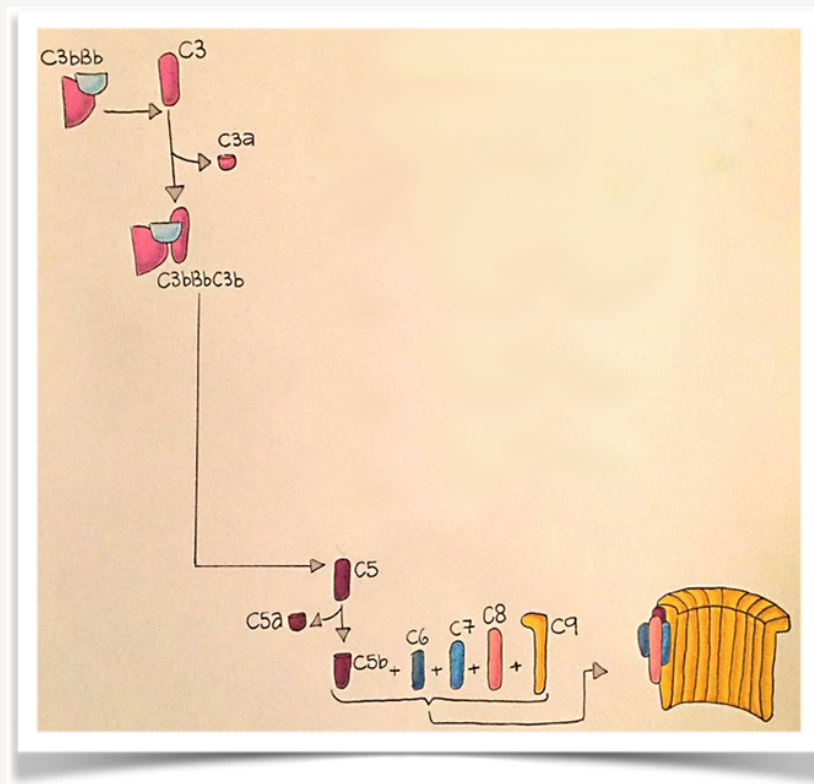


- i. IgG is monomeric, so it's going to take two them to activate the C1.
 - ii. Usually this doesn't happen unless the IgGs are stuck on the surface of a pathogen,
 - iii. This also prevents random activation of C1 by serum IgG.
 - iv. Recall that it is a variant of IgG3 that works best here, followed by IgG1.
 - v. IgG2 barely activates and IgG4 doesn't work at all.
2. Initiating the Cascade
- a. Either IgM or IgG binds to the surface of a pathogen or viral cluster.
 - b. C1 binds to the antibody and changes conformation
 - c. C_{1s} complex activates
 - d. C_{1r} now functions as a serine protease, cleaving $C1s$.
3. $C1s$ triggers production of $C3$ convertase
- a. This is the enzyme that activates $C3$.
 - b. $C3$ convertase is the active complex $C4b2a$, identical to the one used by the classical pathway
 - c. $C1s$ (a serine protease) cleaves both $C2$ and $C4$.
 - d. $C1s$ removes the $C4a$ fragment from the α subunit of $C4$, which diffuses away.
 - e. The remaining large component, $C4b$ can now bind to the target surface near $C1$.
 - f. $C2$ attaches to $C4b$, and is also cleaved by $C1s$, the smaller fragment ($C2b$ - go figure) diffusing away.
 - g. The resulting active complex, $C4b2a$, can now act on $C3$.
4. Activation of $C3$
- a. $C3$ convertase clips a fragment ($C3a$) from the α chain.
 - b. The remaining fragment $C3b$ binds back to the $C4b2a$ convertase complex.
 - c. The total package $C4b2a3b$ is a $C5$ convertase.
 - d. Some activated $C3b$ functions as an opsonin, coating immune complexes.
5. The $C5$ convertase ($C4b2a3b$) activates $C5$

- a. binds to C5 via C3b
 - b. clips off C5a fragment, which diffuses away
6. C5 initiates formation of the MAC

B. Alternative - a different way to generate C5b (Figure 7.6)

Figure 7.6: Alternative Pathway Diagram



1. Component of the innate immune system
2. Initiated by a variety of compounds characteristic of pathogenic surfaces: (Figure 7.7)
 - a. LPS
 - b. Teichoic acid (bacterial)
 - c. Zymosan (fungi)
 - d. Some tumor compounds
 - e. Trypanosome (unicellular eukaryote) markers

Figure 7.7: Surface Triggers

Alternative Pathway Surface Triggers

Lipopolysaccharide

Techoic acid

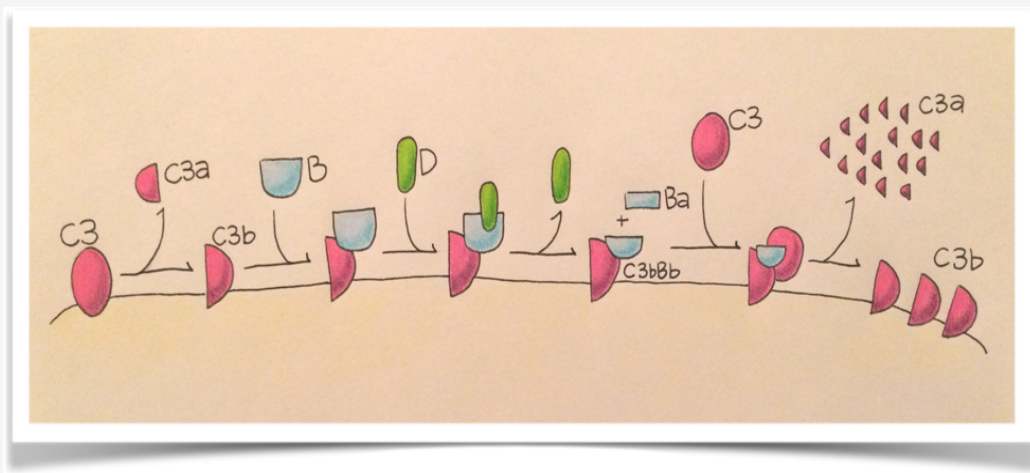
Zymosan

Trypanosome surfaces

3. Activation of C3 (*Figure 7.8*)

- a. C3 spontaneously hydrolyzes at very slow rate to C3a and C3b.
- b. C3b ultimately binds to surface antigens (see above), which stabilizes it.
- c. C3b otherwise decays and is even inactivated on host cells by sialic acid.

Figure 7.8: C3 Activation



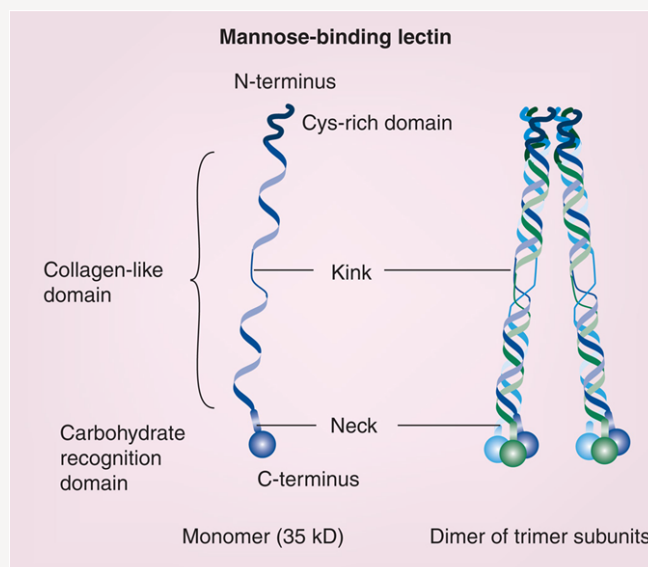
4. B

- a. C3b binds to B, bringing it to the surface.
- b. Binding exposes substrate for D, an active serum proteolytic enzyme.

5. D (sometimes called Fd, for factor D)

- a. D cleaves B to Ba (diffuses away) and Bb, generating the active C3bBb which is also a C3 convertase.
 - b. Properdin (sometimes called Factor F) binds C3bBb (C3 convertase) to the pathogenic surface, stabilizing it.
 7. C3 convertase: C3bBb autocatalyzes more conversion of C3 to C3b in a positive feedback loop. The C3b proteins alone can cover the pathogen.
 8. C5 Convertase: C3b then joins the C3bBb to form the C5 convertase, C3bBbC3b. This complex is analogous to C4b2a3b.
- C. Lectin - also an innate pathway. The Classical Pathway evolved from this.
1. Lectin is a general term for proteins that bind to specific carbohydrates.
 2. MBL - mannose-binding lectin - (*Figure 7.9*) recognizes carbohydrate on surface of microorganisms

Figure 7.9: MBL



3. MBL resembles C1q in that it occurs in clusters of 3, 6 or 18 heads.
4. Bound MBL activates a serum protease, MASP, analogous to C1r and s. (*Figure 7.10*)
5. After that, this resembles the classical pathway, with the cleavage of C4 and C2 to produce a C3 convertase, the cleavage of C3 by this enzyme, and the production of

a C5 convertase made up of C4b2aC3b. (Figure 7.11)

Table 7.1 Pathways

Lectin Pathway	Classical Pathway
Initiated by mannose and other carbohydrates	Initiated by antibodies, using the domain with the oligosaccharide
Changes conformation of MBL	Changes conformation of C1q
Activates MASP	Activates C1rC1s

Figure 7.10: Bound MBL

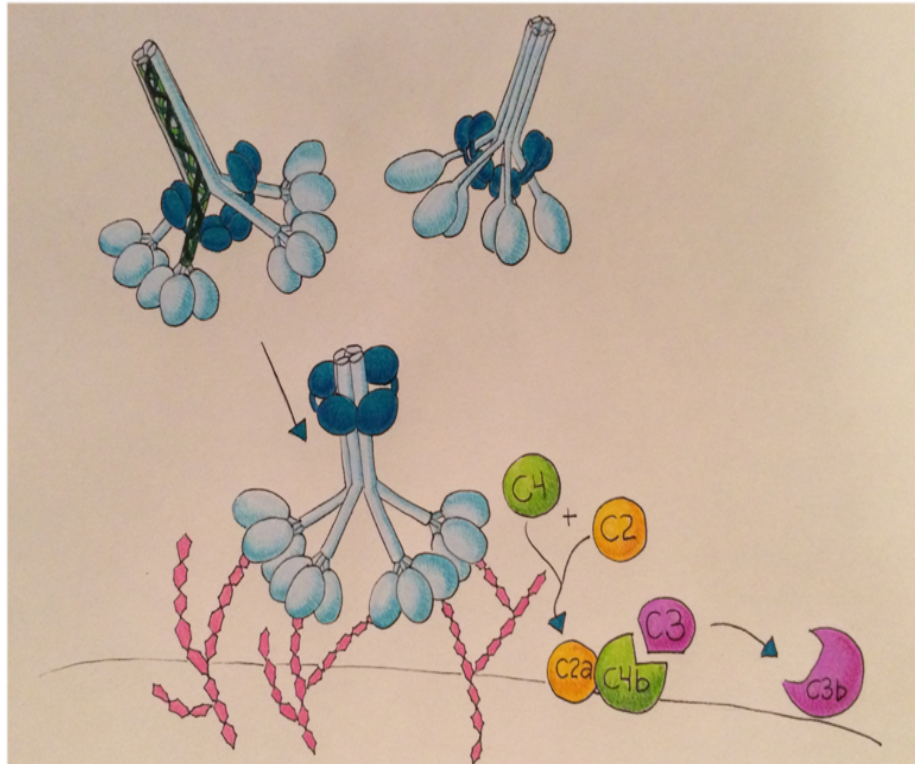
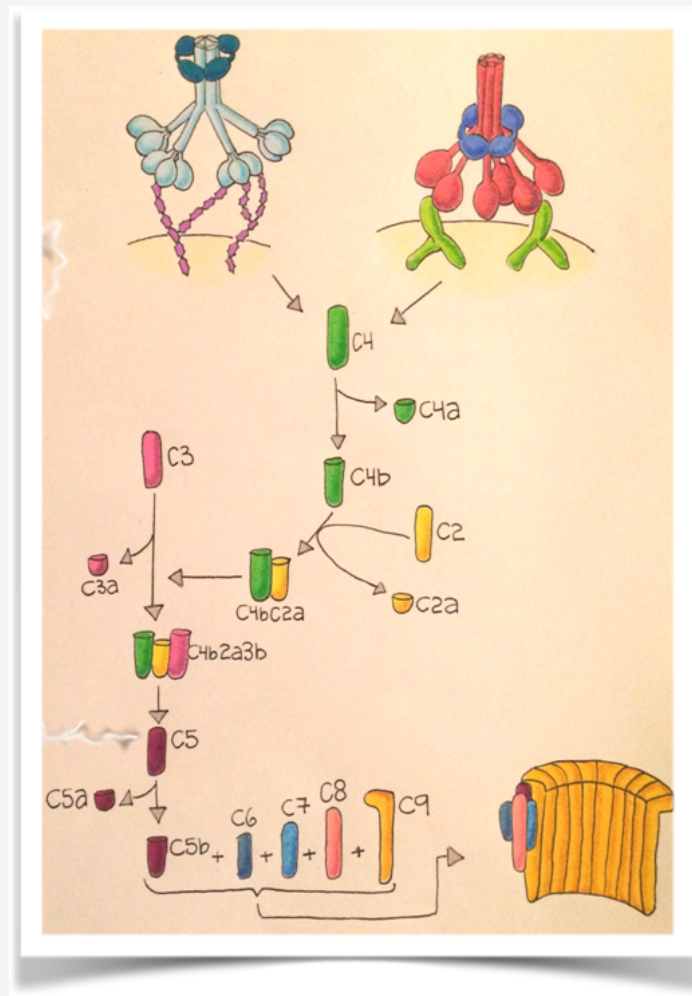


Figure 7.11: Shared Pathway



IV. Endgame and Consequences of Complement Activation - or "What Have You Done for Me Lately?"

A. Cell Lysis

1. membrane-coated viruses:
2. Gram negative (*Figure 7.12*) bacteria (the ones with the thin walls sandwiched between membranes- gram positive, the ones with the thick walls, are hard to puncture: (*Figure 7.13*)
 - a. are usually vulnerable to complement
 - b. "smooth," (with a lipopolysaccharide capsule) are resistant (*Figure 7.14*)

- c. Other gram negatives (a gonorrhea strain) have compounds in the membrane the prevent insertion of the complex.
3. eukaryotic parasites
 4. erythrocytes (red blood cells) are especially vulnerable. (*Figure 7.15*)
 5. cancer cells

Figure 7.12: Gram Negative

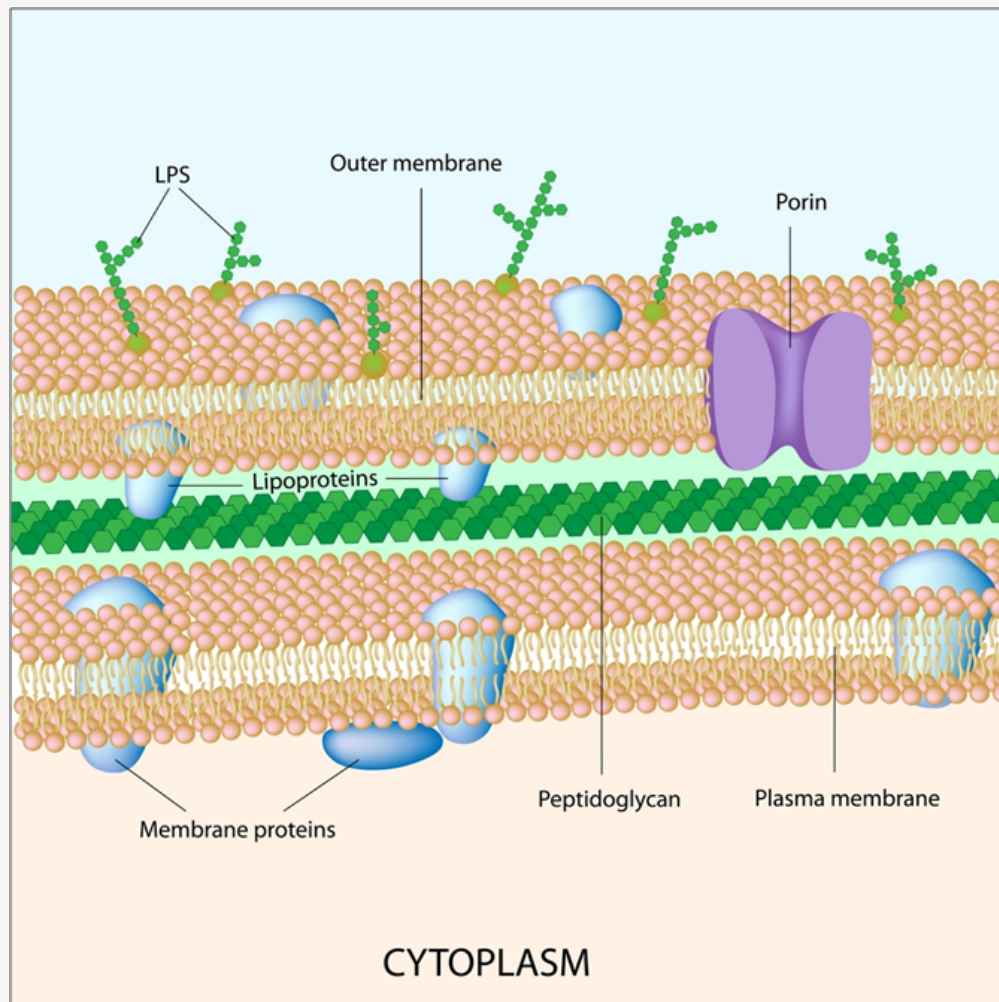


Figure 7.13: Gram Positive

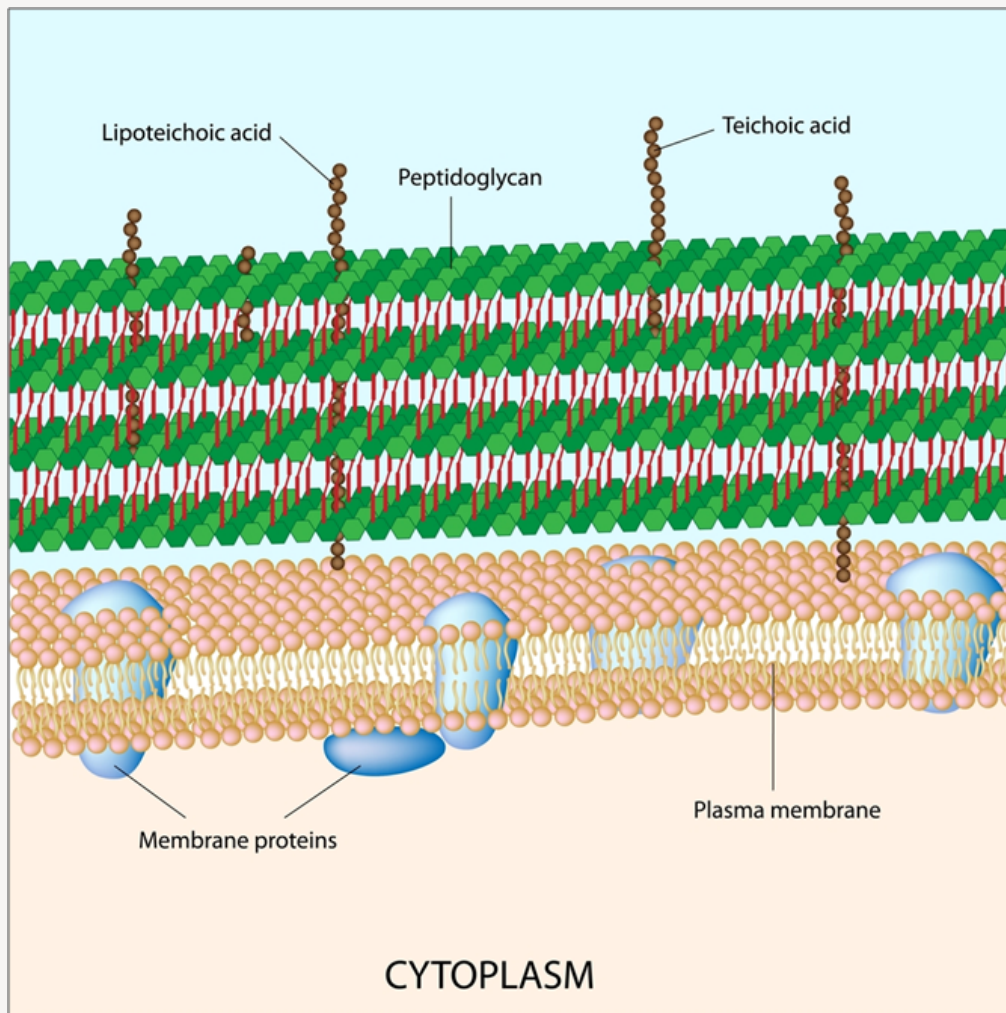


Figure 7.14: LPS

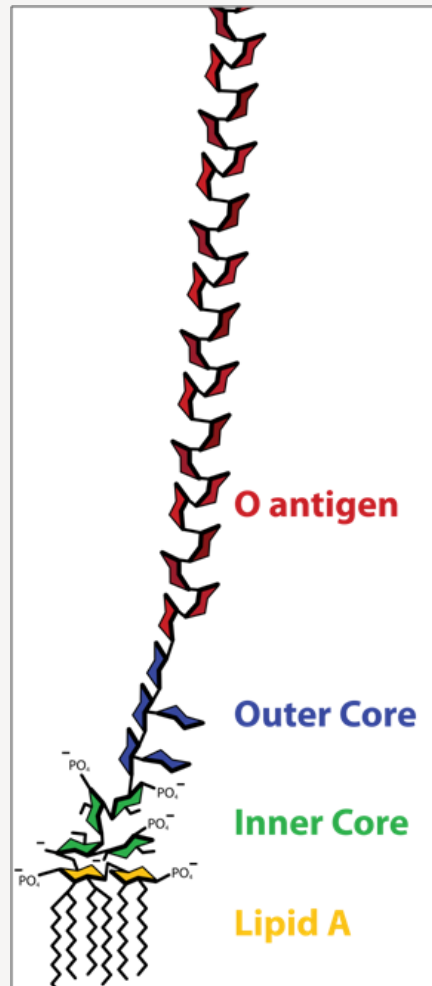


Figure 7.15: Red Blood Cell



B. Inflammation

1. C3a – triggers degranulation of mast and basophil cells, with resulting permeability changes in the endothelium (*Figures 7.16-7.17*)
2. C5a- summons monocytes and neutrophils (*Figures 7.18-7.19*)

C. Coating

1. Opsonization- Neutrophils monocytes and macrophages have receptors for complement proteins (in addition to Fc receptors, TLRs etc.) Binding by these receptors enhances phagocytosis.
2. Viral Neutralization- Even if the complement does not poke holes in a viral envelope, simply being coated and glued to other viral particles makes it much harder for them to attach to and infect a subsequent cell
3. Immune Complex Clearing- works with red blood cells
- 4.

Figure 7.16: Mast Cell

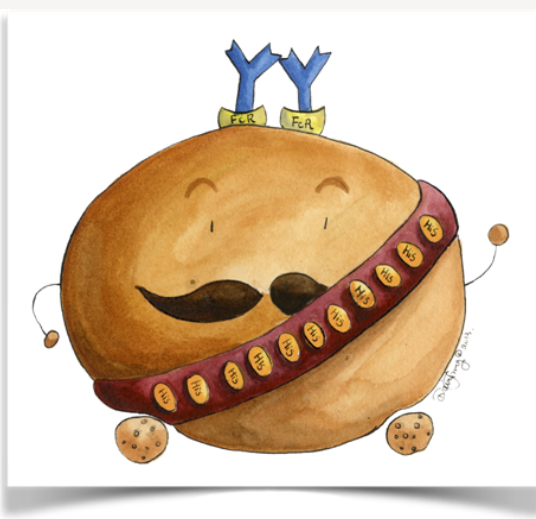


Figure 7.17: Basophil Cell

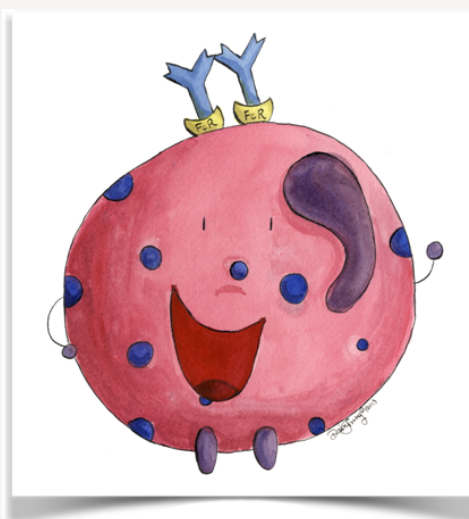


Figure 7.18: Neutrophil

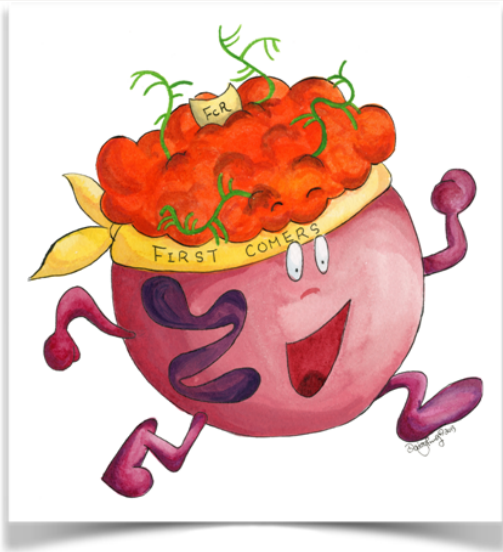


Figure 7.19: Macrophage



The More You Know: Optional Resources and Fun Stuff

(You don't get tested in this!)

My staff and I would like to acknowledge with deepest gratitude the animations produced by Scott Barnum of the University of Alabama at Birmingham (UABRF).

You may order your own copy here:

Complement Activation and Biological Functions by Scott Barnum, ©Scott Barnum and UABRF,
<http://www.microbio.uab.edu/faculty/barnum/complement/index.html>

Unless you have already had some sort of immunology course, the chances are you've never even heard of complement. It's rarely covered in introductory biology texts or even mentioned in sources that non-specialists are likely to read, such as the *New York Times* Science Section or *Scientific American*. If you're teaching a course and looking for more supplements or just want to look at a different versions of the events, you can try the following:

Videos

Complement Proteins: mokhtarr
http://www.youtube.com/watch?v=xcYSGRid50I&feature=youtube_gdata_player

The Classical Pathway of Complement Activation: Carlos Jimenez
<http://www.youtube.com/watch?v=gNvHLStz-VA&feature=related>

Complement Cascade: Doxacurium
<http://www.youtube.com/watch?v=y2ep6j5kHUc&feature=related>

Alternative pathway of Complement Activation: Carlos Jimenez
<http://www.youtube.com/watch?v=qga3Wn76d9w>

Glossary

Adaptive cells – those that rearrange their genes

ADCC- antibody dependent cell-mediated cytotoxicity. A process where white blood cells recognize the stems of antibodies attached to a cell and then attack it.

Allele- A version of the gene. There are two alleles for the enzyme that produces color in four o'clock flowers, one that codes for an enzyme used to make red pigment and a different DNA sequence that does not produce a functional enzyme, leaving the flower white.

ALT – associated lymphoid tissue. MALT (mucosal), GALT (gut), BALT (bronchial), NALT (nasal)

Antibody- a soluble immunoglobulin

Antigen- a molecule that can bind to an antibody, B cell receptor or T cell receptor

APC – antigen presenting cell. Cells that present antigen on MHC II to T_H cells

Apoptosis – programmed cell death

ATP- adenosine triphosphate, directly supplies energy to many biological reactions

BSA – bovine serum albumin. A smallish soluble protein isolated from cow's blood.

CAM – cell adhesion molecule. Any one of a number of different molecules that help stick cells together.

CD – cluster of differentiation. Refers to the isolation of cells by flow cytometry. Depending on exactly what proteins extend from a cell's surface, which in turn influences how the cell moves during the separation process.

CDR- complementarity determining region- the recognition side on the tips of the antibody arms

Chitin – cell wall material of fungi, also an important component of insect exoskeletons.

Chitin – cell wall material of fungi, also an important component of insect exoskeletons.

Chordate – member of the phylum Chordata. Includes vertebrates and invertebrates with a dorsal nerve cord, gill slits, notochord and muscles in blocks.

CLP – common lymphoid progenitor. Gives rise to lymphoid cells, including NK, T cells, B cells, and more.

CMP – common myeloid progenitor. Stem cell that can give rise to any myeloid cell type (including red blood cells and platelets).

Coley toxin – inflammatory material isolated from bacteria used in cancer chemotherapy around 1900.

Complement- a system of proteins that helps identify pathogens and debris for destruction and phagocytosis (the landmines of the plasma.)

CTL – Cytotoxic T cell. Activated T_C cell, ready to kill rogue-self cells

Downstream- the end of the DNA or RNA with the free 3' carbon of the (deoxy) ribose. Nucleic acid synthesis and translation proceeds 5' to 3'.

Epitope – the specific portion of a molecule that binds to an adaptive receptor. For example, a viral protein is an antigen whose different epitopes bind to different antibody idiotypes.

Exon- the part of a gene that codes for a sequence of RNA that will wind up in a message and get translated (expressed.) A gene or gene region may have one to many exons.

Gene region – a sequence of DNA coding for a specific part of the Ig or T-cell receptor.

Granulocytes – Cells with copious granular inclusions and that do not present antigen. Includes neutrophils, basophils, and eosinophils (which have oddly-shaped nuclei) and mast cells (which do not).

Hapten- a molecule that could potentially bind a CDR, but by itself is not large enough to kick off an immune response.

HSC- hematopoietic stem cell. Can divide and regenerate or develop into any type of blood cell. Found in bone marrow.

Humoral response – Immune defense found in the plasma, the word humor derived from the ancient Greek medical theory of body fluids. It really just refers to antibodies. Stupid term. If people stop using it, maybe it will go away.

Hybridoma- a cell or cell line derived for the fusion of a blood cell cancer (myeloma) and a normal, antibody-producing plasma (B lineage) cell.

Idiotype – a category of antibodies that all have the same recognition region

Innate cells – those that do not rearrange genes.

Introns- that DNA sequences of the gene that code for RNA sequences that get clipped out during processing.

Isotype- a category of antibodies of the same class

Lymphoid cells – white blood cell types (innate and adaptive) found in the lymph and (and blood and immune organs as well).

MAC- membrane attack complex- terminal complement pathway produces this, which punches

holes in plasma membranes.

MASP- mannose associated protein

MBL- mannose binding lectin

MHC- Major Histocompatibility Complex. Includes the genes and the proteins they code for. These include the proteins (groups I and II) that hold small peptides so that T cells can recognize them. They also include a variety of other proteins, including enzymes important in immune recognition and promotion. The human versions are names HLA molecules for human lymphocyte antigen.

Monoclonal- refers to a cell line of (theoretically) identical cells derived from the division of a single cell.

Myeloid cells – innate white blood cells rarely found in the lymph.

Necrosis- cell death from disease or injury

NK cell- Natural Killer cell. Kills rogue-self cells, recognizing them by innate mechanisms. Does not require T_H activation.

N-nucleotide addition- During gene rearrangement, when enzymes add nucleotides at random in the palindromic regions of the joint.

NOD – nucleotide oligomer detectors. Soluble pattern recognition receptors found in the cytoplasm of cells. Despite the name, they often recognize cell wall materials.

Nucleic acid – RNA or DNA

Peptidoglycan – mesh-like macromolecules that compose the basic structure of the bacterial cell wall.

Phagocytosis – when a cell engulf large particles

Pinocytosis - when a cell gathers fluid in a vesicle and engulf the vesicle.

P-nucleotide addition- During gene rearrangement, when enzymes fill in the missing nucleotides at the joint by copying the palindromic nucleotides on the other strand.

Receptor-mediated endocytosis – when a cell binds material at its surface using a proteins receptor and then internalized the complex into a vesicle that enters the cell.

RSS – recombination signal sequence. The sequence of 28 or 40 nucleotides that the upstream or downstream end of a gene region providing the signals for gene rearrangement.

Simple sugar – single sugar unit, includes glucose, mannose and galactose. May be modified into sugar units as sialic acid (NANA) or N-acetyl glucosamine.

T_c cell- cells that recognize rogue self-cells by antigen they present on MHC I. They develop

into CTLs after instructions from T_H cells.

TCR – T-cell receptor. Found extending from the surface of both T_C and T_H cells. Recognizes antigen, coded for by rearranged genes.

T_H cell- thyroid helper cell. Coordinates immune responses. T_H 1 cells promote a serious response; T_H 2 promotes a containment response, and Treg tolerance. There are additional types as well.

TLR – Toll-like receptors. Pattern recognition receptors that recognize molecules characteristic of pathogens. Found embedded in plasma membrane and endomembranes of many white blood cells.

Transcription factor – a protein that either up- or down- regulates the copying of RNA (transcription) from DNA. They often have domains that attach to specific sequences of DNA nucleotides. Some attach to other proteins that attach to the DNA. Or both.

Upstream- the end of the DNA or RNA with the free 5' carbon of the (deoxy) ribose. Nucleic acid synthesis and translation proceeds 5' to 3'.

Zymosan – cell wall material of fungi

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