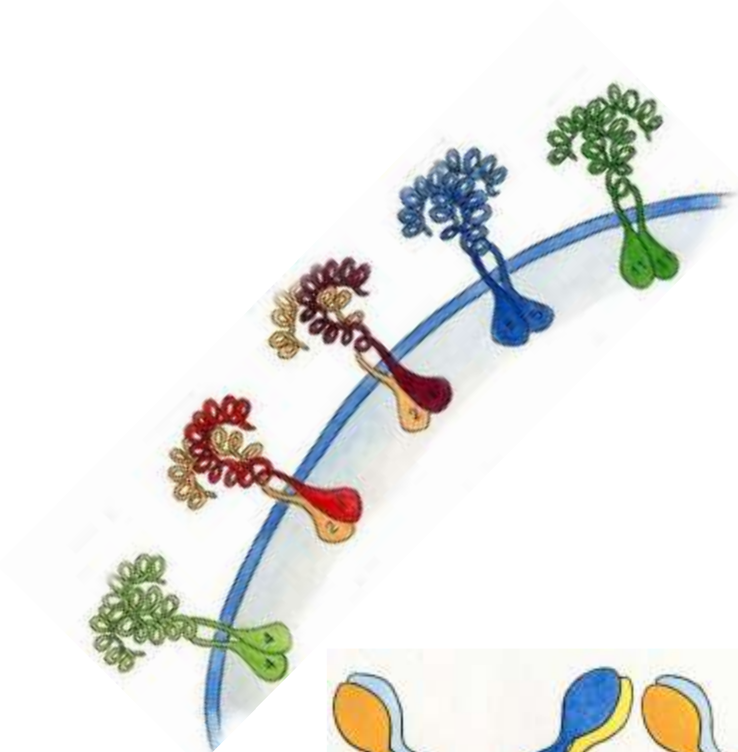
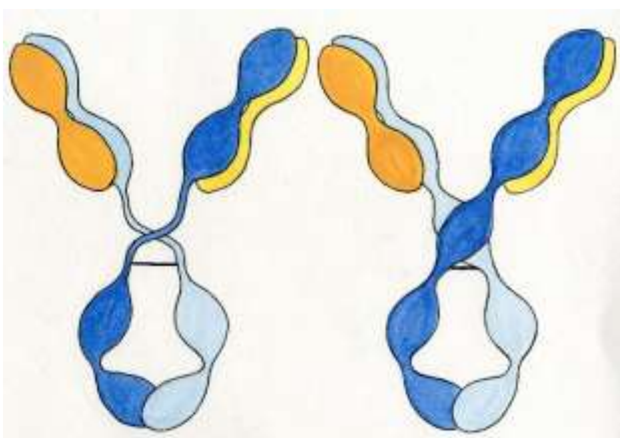
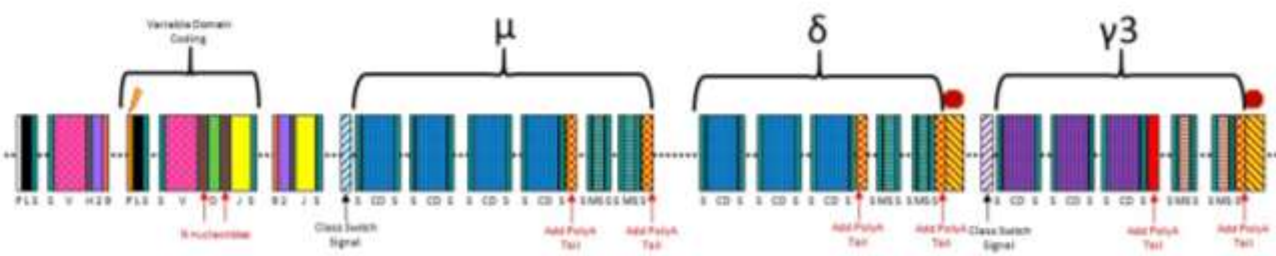
Fundamentals of Immunology:
Innate Immunity and B-cell Function







Alma Moon Novotny

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BIOC 372x Study Guide

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

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

Video clip 1-1

I. Welcome

Video clip 1-2

II. Staying Healthy

A. Context

Figure 1.1a, moss

Figure 1.1b, mushroom

1. All organisms (including plants and fungi, *figures 1.1a and b*) have defense mechanisms. These are clearly derived from common ancestral forms, currently classified as innate.

Figure 1.2, Animal Collage

2. Vertebrates have an additional particularly effective defense - acquired or adaptive immunity involving antibody production, *figure 1.2*.

3. Insects (*figure 1.3*), 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, using multiple splicing of DSCAM messages, figure 1.4

Figure 1.3, Dragonfly

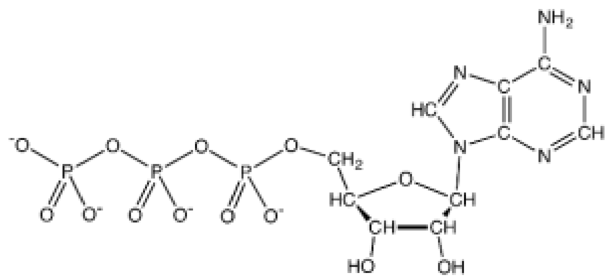


Figure 1.4 ATP

4. The defenses are energetically expensive, *figure 1.4*

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

Figure 1.5a: clean water **Figure 1.5b sewage disposal** **Figure 1.5c mosquito netting**

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 *(figure 1.6).*
5. vaccination (figure 1-7) - OK the engineers didn't give us this one, but without the communications and transportation infrastructure, it’s hard to deliver vaccines

Figure 1.6, infrastructure collage

Figure 1.7, vaccination

Figure 1.8 Hospital in Burundi

C. Disease Burden

1. Economic costs of being sick and having to tend to sick children, *figure 1.8.*

2. Rates of infectious disease and general ill health correlated with lowered IQ.

   a) Increase in height during 20th century parallels increase in overall IQ test scores.

   b) Correlation is about 67%, which suggests that this is not the only factor, but it does provide a possible explanation for the Flynn effect.

   c) Diarrheal diseases rob infant of nutrition at a period of critical brain growth. 87% of the nutritional energy in newborns goes to the brain.

   d) Cerebral malaria can damage the brain directly when infected cells burst and produce clots, leading to strokes.

   e) Measles infections lower overall immune function, compromising resistance to subsequent infections. Measles vaccination in developing countries can lower the subsequent mortality to other childhood disease by as much as 80%.

Figure 1.9, child with measles

Figure 1.10, happy baby

3. Children all deserve a good start in life, *figure 1.10.*

Video clip 1-3

III. Pathogen Varieties

A. Viruses: rhinovirus, flu, small pox, Ebola, polio *(figure 1.11)*

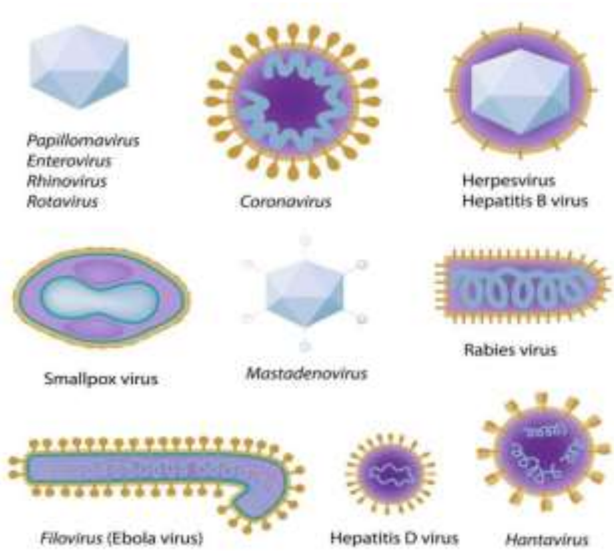


Figure 1.11, a selection of viruses

B. Bacteria: *Mycobacterium tuberculosis* (TB), *E. coli*, anthrax, bubonic plague, strep, cholera, syphilis, *Clostridium difficile (Figures 1.12-1.15)*.

Figure 1.12: Spirochetes

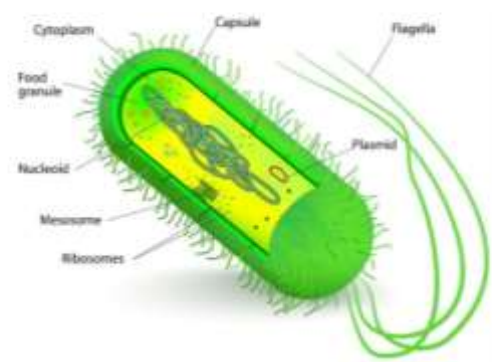
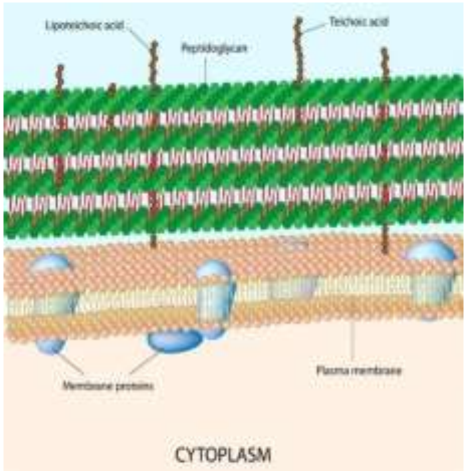


Figure 1.13: Bacterial structure



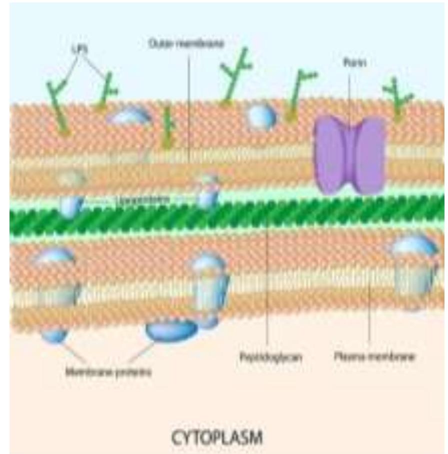
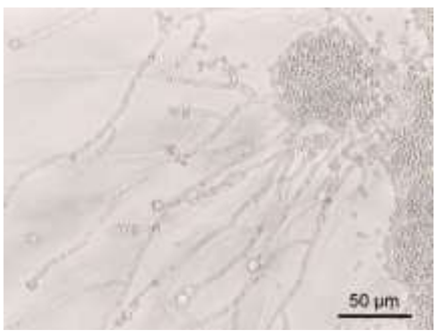


Figure 1.14: Gram positive wall **Figure 1.15: Gram negative wall**

C. Fungi: *Candida albicans* (yeast), athlete's foot, ringworm, *Cryptococcus gattii*



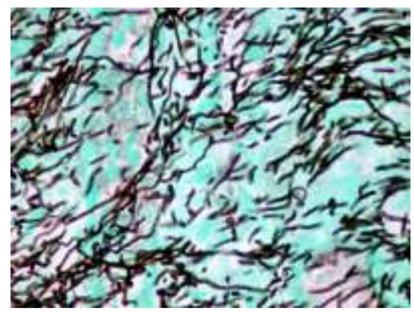
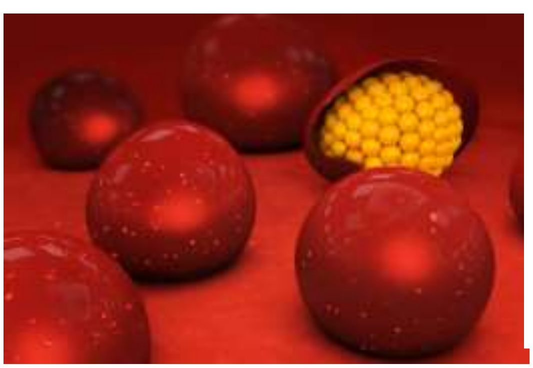


Figure 1.16 Candida **Figure 1.17: Candida** **Figure 1.18: fungi in human tissue**

D. Unicellular Eukaryotes: malaria, trypanosomes, amoebae, Giardia, Chagas *(Figure 1.19-1.20)*



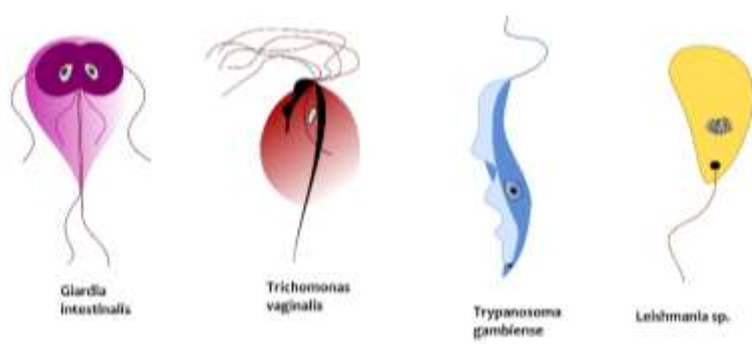


Figure 1.19: Malaria in red blood cell **Figure 1.20: More protozoans**

E. Multicellular Eukaryotes: parasitic worms (Platyhelminthes and Nematoda): flukes, tapeworms, hookworms, heartworms *(Figure 1.21 -1.23)*

Figure 1.21: Schistoma Larvae

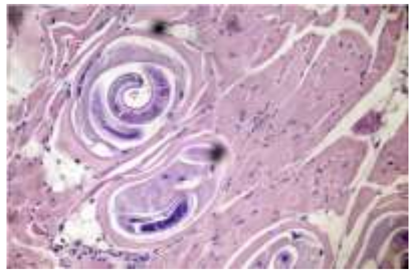


Figure 1.22: Trichinella

Figure 1.23: Hookworm

Video clip 1-4

IV. Pathogen Recognition

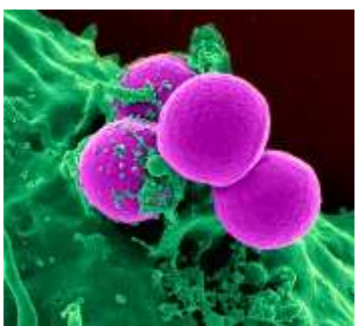


Figure 1.24: neutrophil capturing bacteria

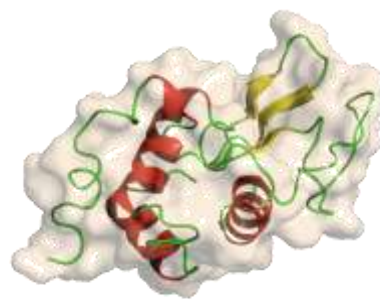


Figure 1.25 lysozyme

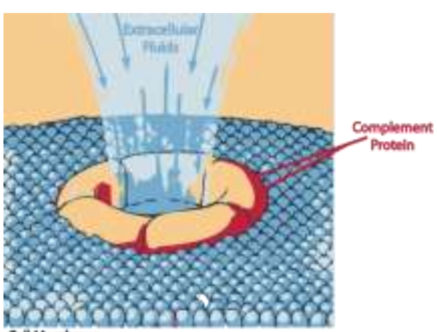


Figure 1.26 complement pore

Figure 1.27 leucine rich repeat

Video clip 1 -5

B. *Pathogen 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.

   a. Neutrophils, *Figure 1.24* – patrol
   b. Lysozyme *Figure 1.25* – mucus
   c. Circulation *Figure 1.26* – complement
   d. Receptors (leucine repeats) *Figure 1.27* - cells

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

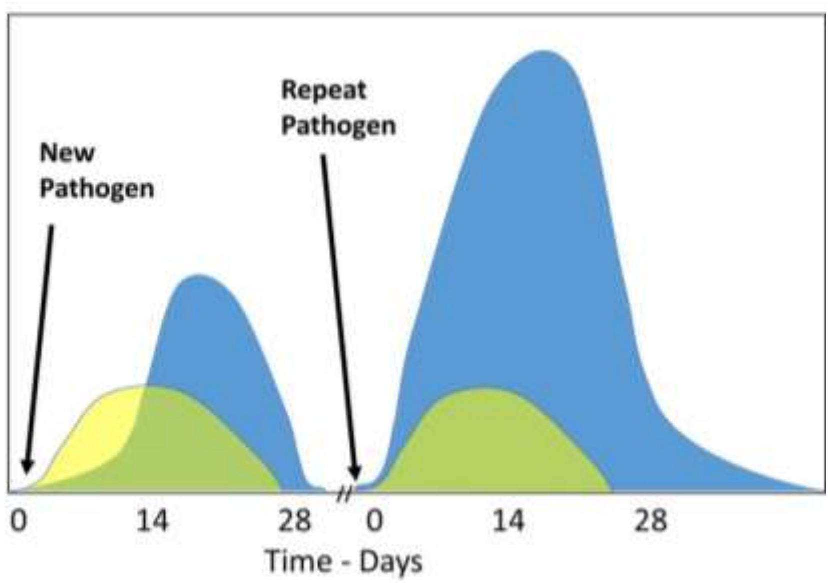


Figure 1.28 Immune responses over time

Properties of Innate Versus Adaptive Defenses

Innate	**Fast:** Minutes after exposure	Always there	**Recognizes** patterns: types of molecules that a pathogen might have and you would not have	Neutrophils, macrophages, NK cells, proteins, barriers
Adaptive	**Slower:** approximately- Two weeks after first exposure Three days after subsequent exposure	Requires gene rearrangement	**Recognizes** specific parts of proteins characteristic of a pathogen	B cells, antibodies, Tc cells, T_H cells

Video clip 1-5

V. 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 pathogens to yourself.
2. You'll encourage the evolution of a more benign strain.

Video clip 1-6

VI. Metaphors of 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

Video clip 1-7

VII. 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

Properties of Innate Versus Adaptive Defenses

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Table 1.1

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 *(You don't get tested on this!)*

A. *Bacterial Adaptive Immunity*

1. Bacterial don't make antibodies, but they can change their DNA to defend against pathogens.
2. They copy foreign DNA into their genomes and then copy it into inhibitory RNAs.
3. The system uses an enzyme called CRISPR, which we have co-opted for use in genetic engineering.

Wikipedia: https://en.wikipedia.org/wiki/CRISPR
Report on therapeutic use in HIV treatment:
http://www.biotechnologyforums.com/thread-6836.html

B. 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 to help you sort out some T cell traits:

Table 1.2 Responding to Foreign Antigen (Inevitably simplified)

Responding Cell	**T_H (Helper)**	**Tc (Cytotoxic)**
Response	Coordinates immune response	Attacks and kills cell
Binds antigen with	αβ T-cell receptor	αβ 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	proteasome
Response	Coordinates immune response	Attacks and kills cell

Lecture L02

Surveying the Cells & Organs of the Immune System

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)

Video clip 2-1

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 draws parallels between their functions and the functions 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.

Video clip 2-2

II. Hematopoiesis - The Source of It All

A. Hematopoietic Stem Cell *(Figure 2.1)*

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. *Lin-* means the cell does not show differentiation surface markers. *Lin*+ cells do: they are lineage positive.

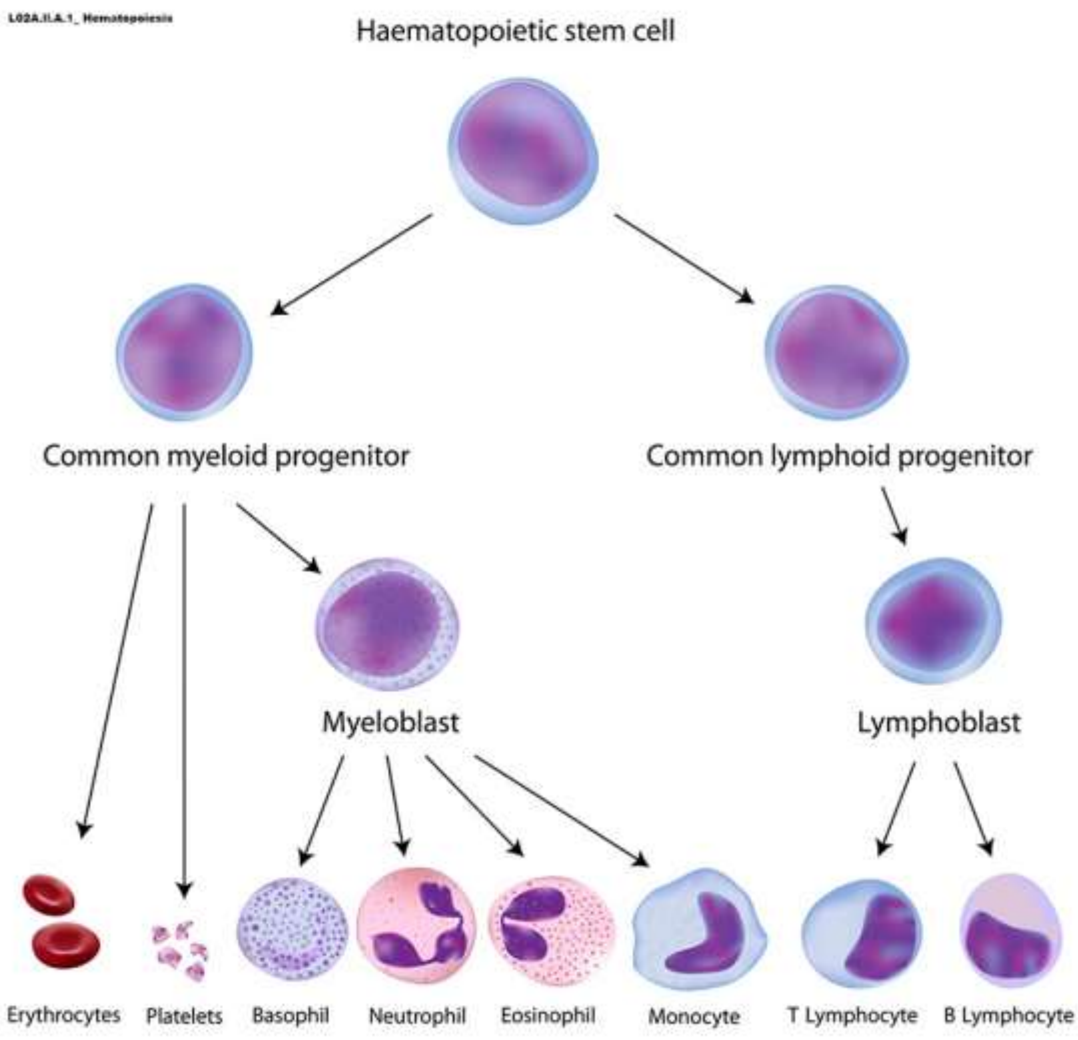


Figure 2.1: Hematopoietic Stem Cell

B. Signaling Differentiation *(Figure 2.2)*

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:

   a. 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.
   b. LMP- If it turns into a common lymphoid progenitor, it expresses the transcription factor Ikaros, and can become an adaptive T and B cell, or an innate NK or dendritic cells. The notch family of transcription factors decides the T- or B- cell choice.
   c. Dendritic cells (these are not nerve cells) – can arise from either myeloid or lymphoid lineages.

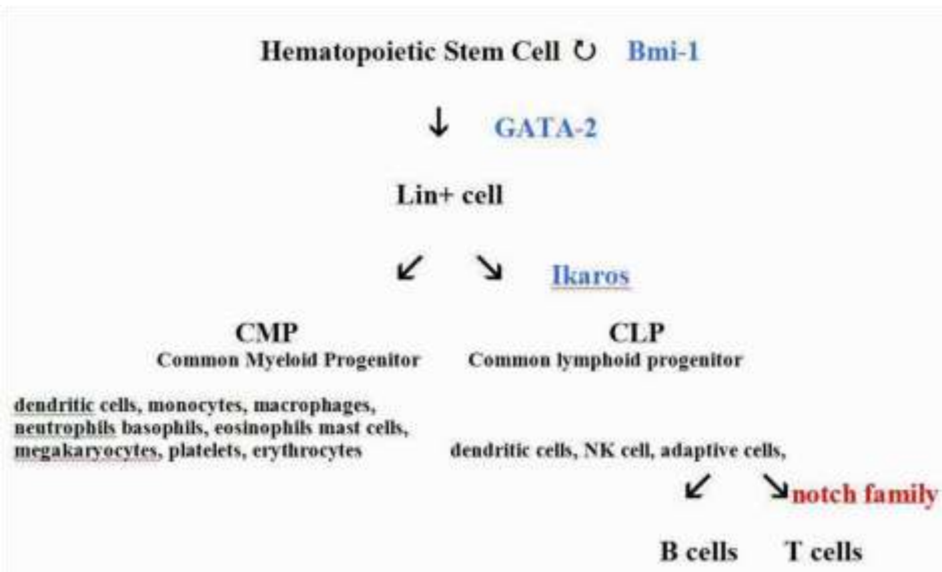


Figure 2.2: Signaling

C. Historical Baggage

1. red blood cells, or erythrocytes. *(Figure 2.3)*

2. platelets, or cell fragments from the megakaryocytes *(Figure 2.4)*

3. white blood cells, or leukocytes, which include cells from both the myeloid and lymphoid lineages

Figure 2.3: Erythrocyte

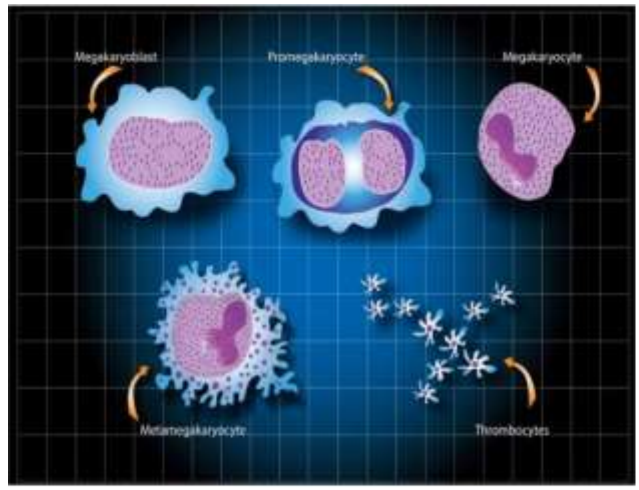


Figure 2.4A: Platelet

Figure 2.5 megakaryocyte

Video clip 2-3

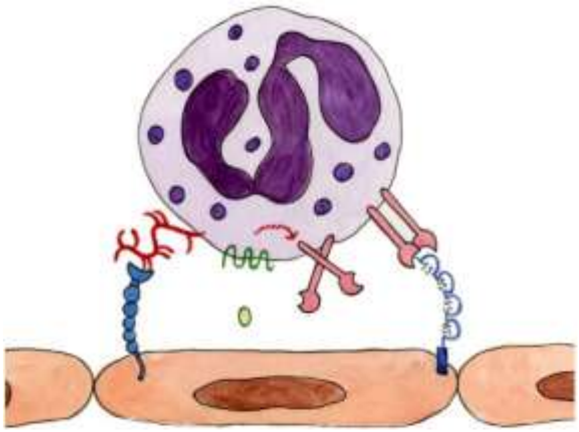
III. Myeloid Granulocytes

A. Myeloid Cells Whose Immune Function is Secondary (for context)

   1. red blood cells – only extrude nuclei in mammals

   2. megakaryocytes – pinch off platelets and cell fragments without nuclei, *Figure 2.5*.

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



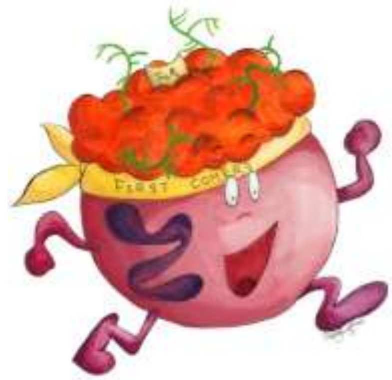
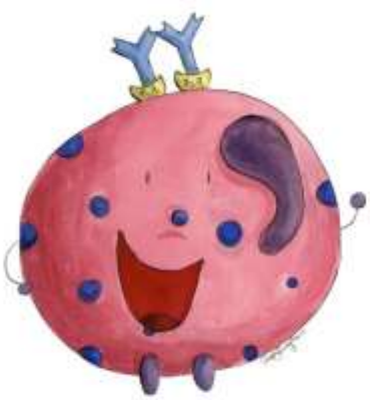


Figure 2.6: Neutrophil **Figure 2.7: Neutrophil**

1. neutrophils *(Figure 2.6 and 2.7)* – the infantry of the system, in the most modern techie sense

   a. strongly phagocytic – first responders to infection and population expands if the infection does not rapidly clear.
   b. typically live for only a day (in some ways, these guys resemble Kamikaze pilots) and remains accumulate in an infected region as pus.
   c. granules stain with both acidic and basic stains (different granules with different functions).
   d. nucleus multilobed (sometimes called a polymorphonuclear leukocytes)



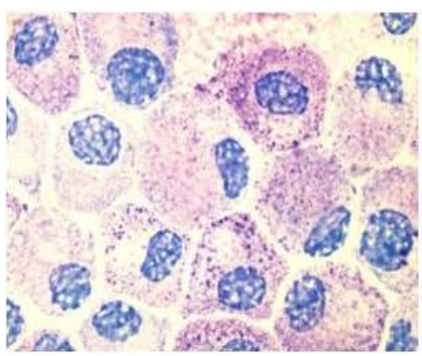


Figure 2.8: Basophil **Figure 2.9: Mast Cells** **Figure 2.10: Mast Cell**

2. basophils *(Figure 2.8)*

   a. granules with histamines stain with methylene blue, a basic stain
   b. lobed nucleus
   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 2.10)*
    b. non-lobed nucleus *(Figure 2.9)*
    c. released as undifferentiated cells, maturing in their tissues.
    d. have other immune regulatory functions

4. eosinophils

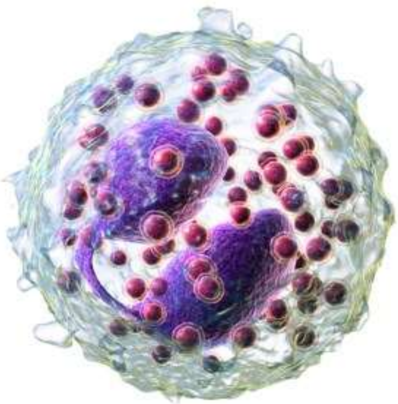


Figure 2.11: Eosinophil

Figure 2.12: Eosinophil

    a. granules stain with eosin red, an acidic stain, have hydrolytic enzymes *(Figure 2.11)*
    b. bilobed nucleus
    c. phagocytic, though less important
    d. target worms *(Figure 2.12)*

Video clip 2-4

IV. Myeloid Antigen Presenting Cells

A. Specific Presenters: 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 2.13)*
    b. enlarge and give rise to - macrophages *(Figure 2.14)*

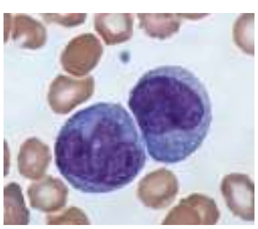


Figure 2.13: Monocyte

Figure 2.14: Monocyte

2. macrophages

    a. migrate into tissues by amoeboid motion, enlarge five to 10 fold *(Figure 2.15)*
    b. phagocytize pathogens and debris from dead cells. Antibodies attached to pathogens make this easier. *(Figure 2.16)*
    c. present antigen derived from phagocytosis to T_H cells.
    d. subtypes guard specific tissues, becoming a fixed part of the structure.

Figure 2.15: Macrophage

2.16: Macrophage

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 2.17, A, B and C)*
    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

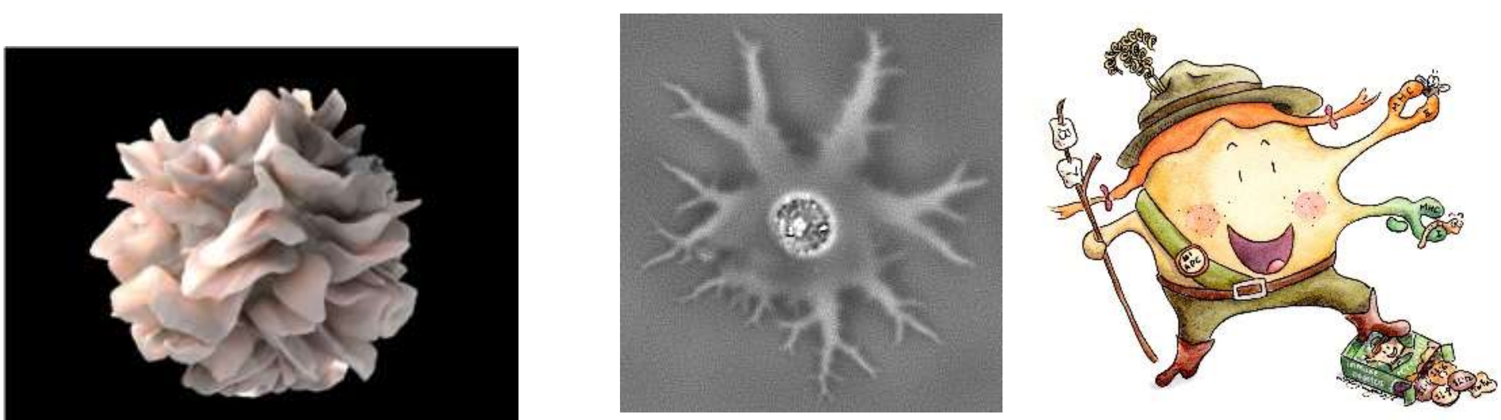


Figure 2.17: Sentinel Dendritic Cells:

A: artist's conception **B: micrograph** **C: cartoon**

B. Follicular Dendritic Cells

2. Do not arise via hematopoiesis

3. Instruct B cells – a little like drill sergeants

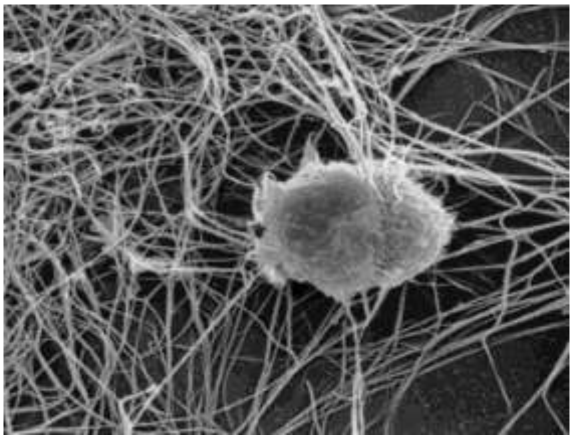


Figure 2.18A - Follicular Dendritic Cell micrograph,

Figure 2.18B, FDC cartoon

4. Help improve antibody production- a little like Q in the James Bond movies.

5. Supply the cells with signals, antibody antigen complexes called iccosomes.

6. B cells with more effective receptors compete successfully for these and survive and multiply

Video clip 2-5

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

A. B Cells: The James Bonds of the Immune system. Adaptable, able to defend and able to summon defense.

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 2.19)*

   b. bone marrow of most mammals

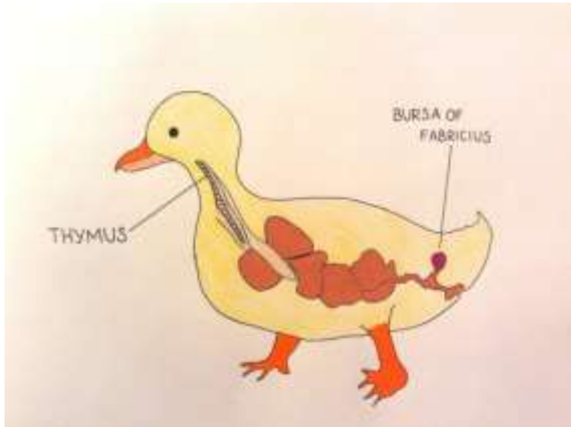


Figure 2.19: Bursa of Fabricius

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 2.20)*

   a. small lymphocytes - naïve, unexposed to antigen. Only 6 μ 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 2.21)*

   c. memory cell - held in reserve if the infectious agent should reappear.

Figure 2.20: B-Cell

Figure 2.21: 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 2.22-2.24)*

   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.

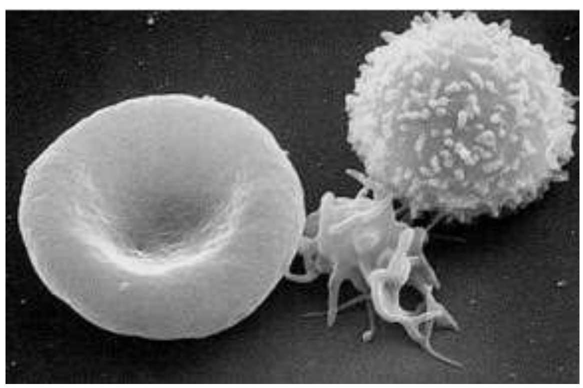
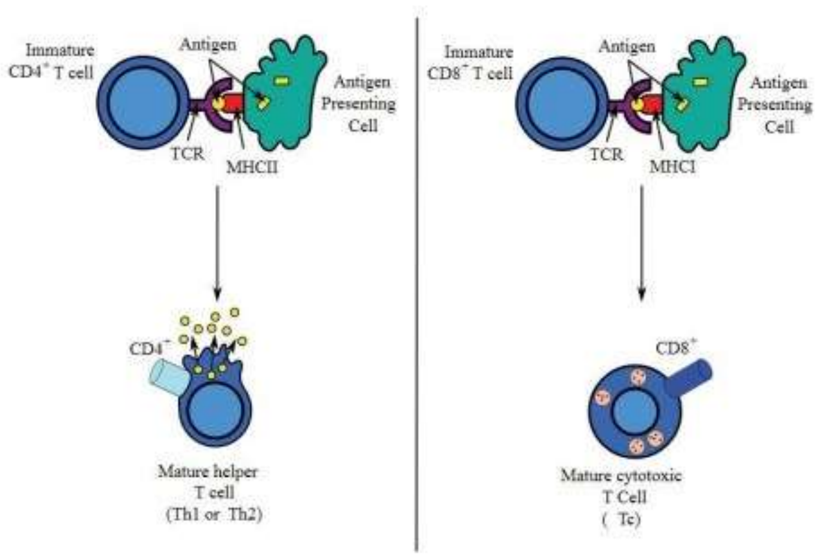


Figure 2.22: T Cell Interaction



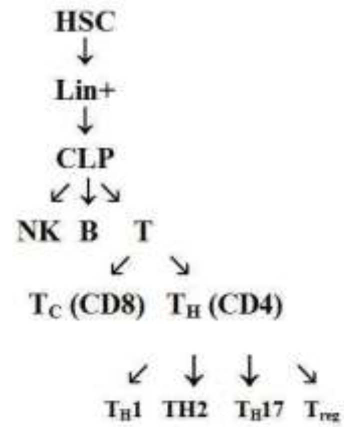


Figure 2.23 Tc and Th cells, compared

Figure 2.24: T Cell Lineage

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.

C. NK *(Figure 2.25-2.26)*, or Natural Killer cells (the Seals or Green Berets of the immune response) – no T-cell receptors, no CD4, no CD8, 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

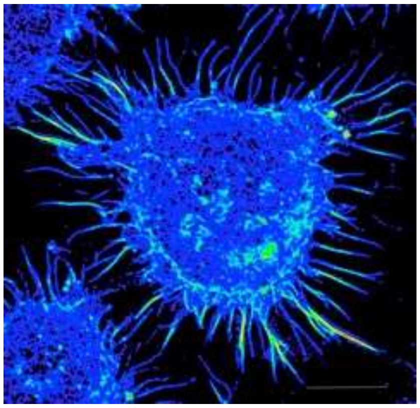


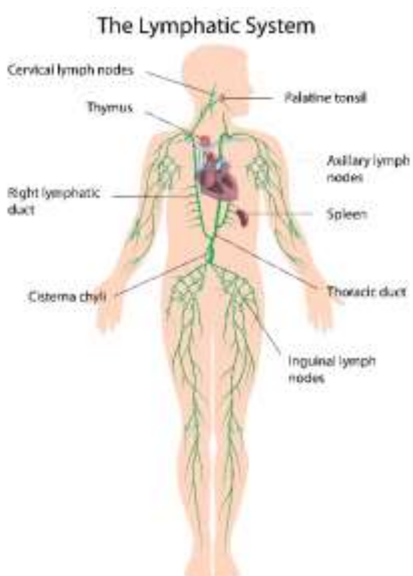
Figure 2.25: NK Cell

Figure 2.26: NK Cell

Video clip 2-6

VI. Primary Organs of the Immune System

A. Bone Marrow *(Figure 2.26)*
   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.



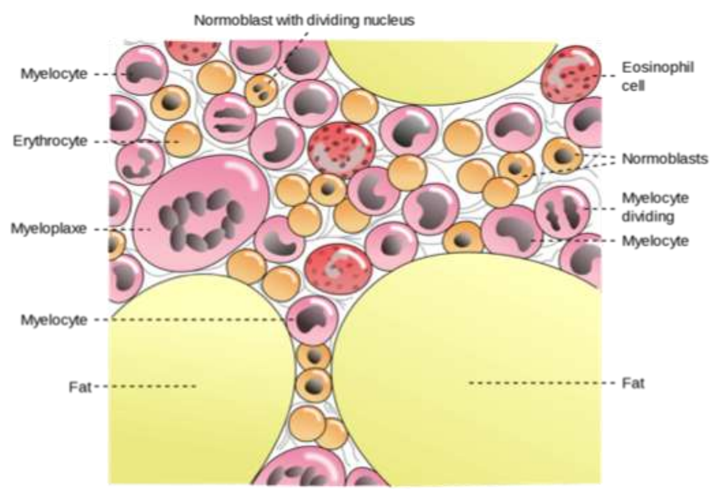


Figure 2.32 Lymph system

Figure 2.27: Bone Marrow

5. Thymus *(Figure 2.32 and 2.28)*

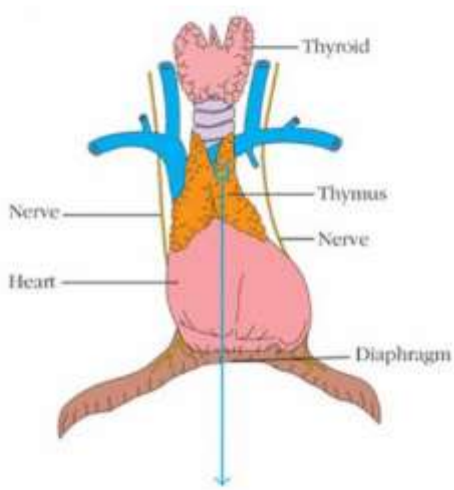


Figure 2.28: Thymus

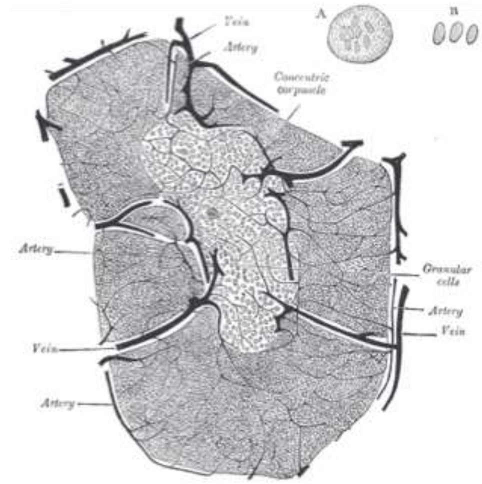


Figure 2.29: Thymus Cross Section

6. 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.

7. Site of T-cell maturation (details of the process later) *(Figure 2.28 and 2.9)*

   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.

Video clip 2-7

VII. Lymph: Fluid, Vessels and Nodes

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 2.30)*

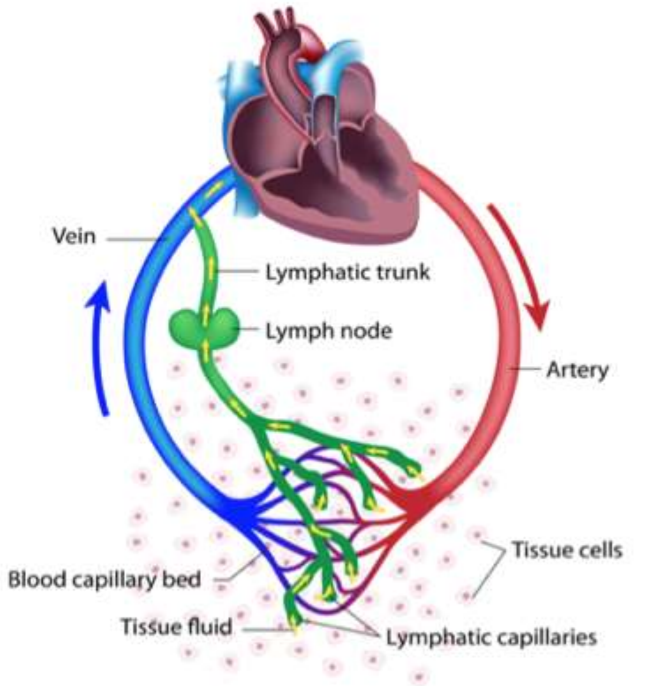


Figure 2.30: Blood Movement

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

b. Drains interstitial fluid from tissues, picking up antigens and white cells *(Figure 2.30)*

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.

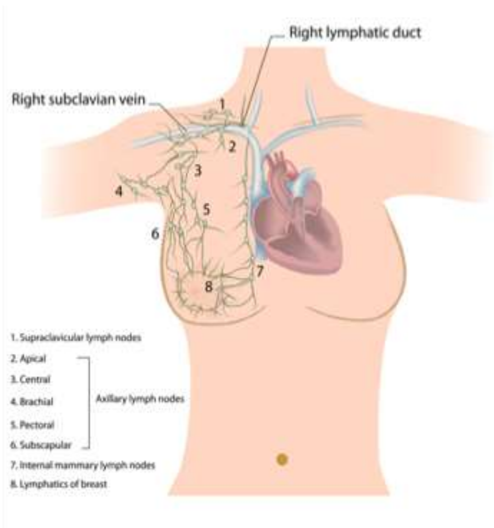


Figure 2.31: Node map

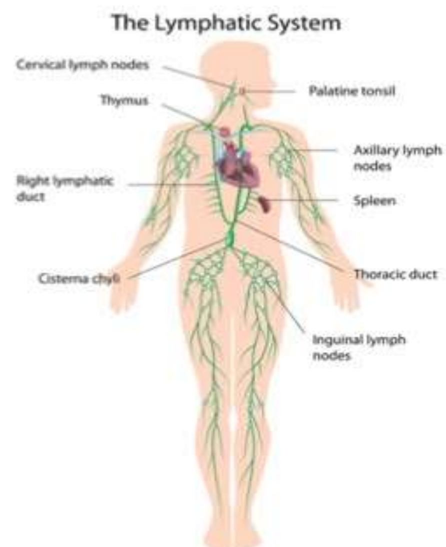


Figure 2.32: Lymphatic System

B. Location of Lymph Vessels

1. Classical *(Figure 2.32)*

2. Meningeal *(Figure 2.33, 34 and 35)*

   a. Collecting vessel runs over the top of the head, connecting with deep cervical (neck) lymph nodes.
   b. Lies between dura mater and meninges (outside the brain) and between meningeal arteries and dural sinuses (veins).
   c. Relative simple, unbranched and only has valves near in region from base of skull.

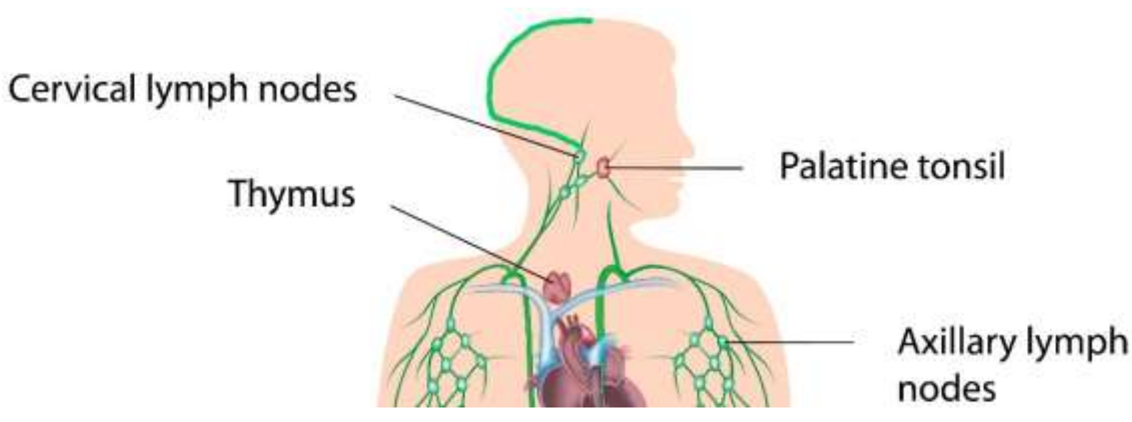


Figure 2.33: Lymphatic System, showing meningeal

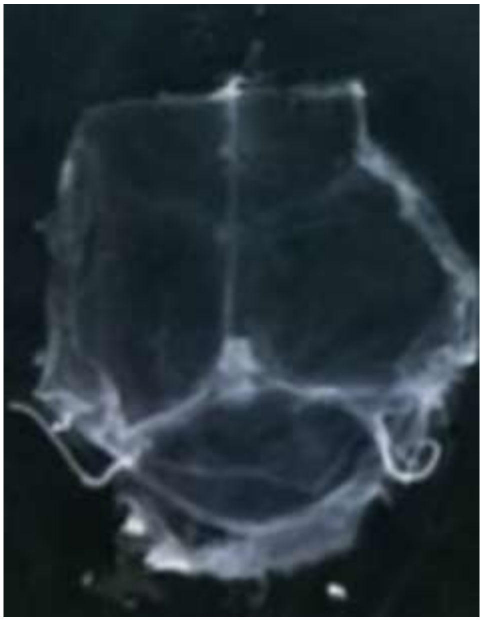


Figure 2.34, mouse dissection

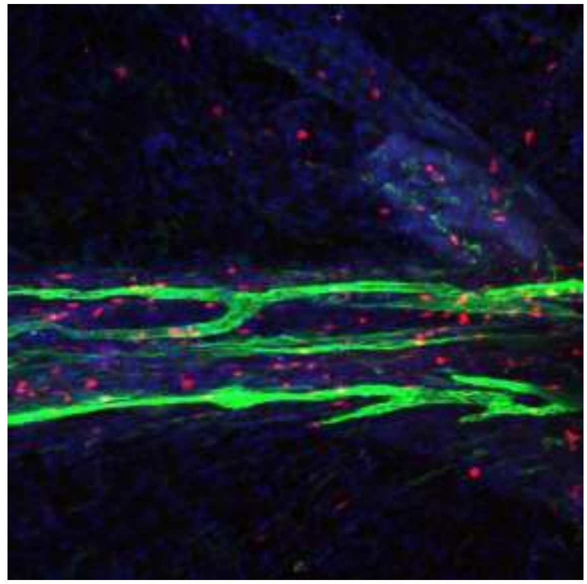


Figure 2.35, cranial lymphatic vessel

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

   1. Basic structure *(Figure 2.32-2.33)*

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

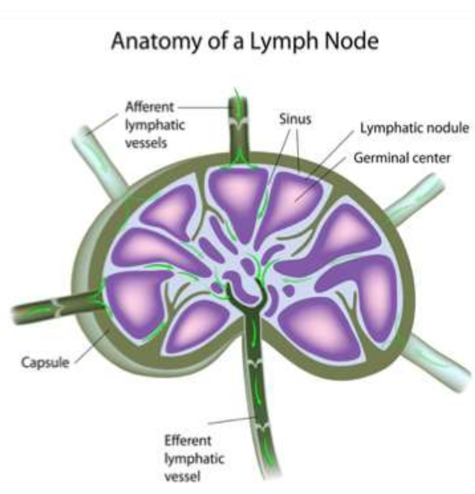


Figure 2.36: Lymph Node

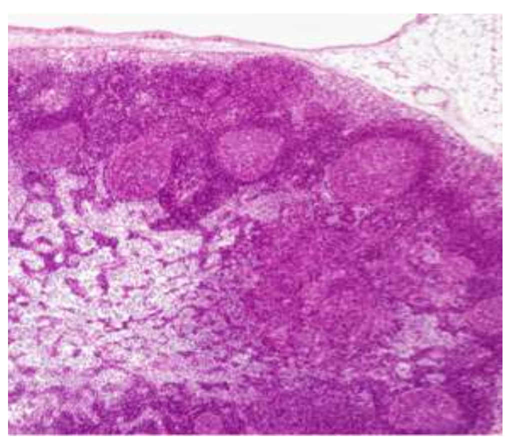


Figure 2.37: Node Cross Section

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.

Video clip 2-8

VIII. Other Secondary and Tertiary Organs

A. Spleen *(Figure 2.38)*

1. in abdomen, next to pancreas
2. filters blood, not lymph *(Figure 2.39)*
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.

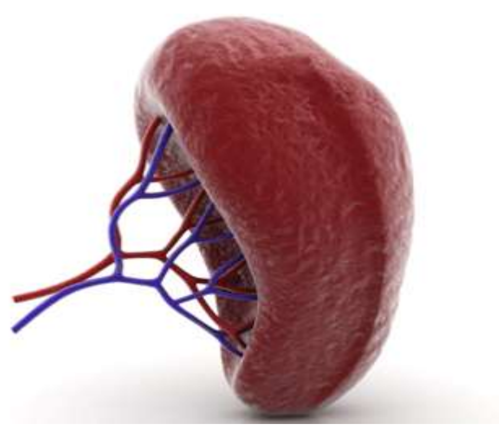


Figure 2.38: spleen

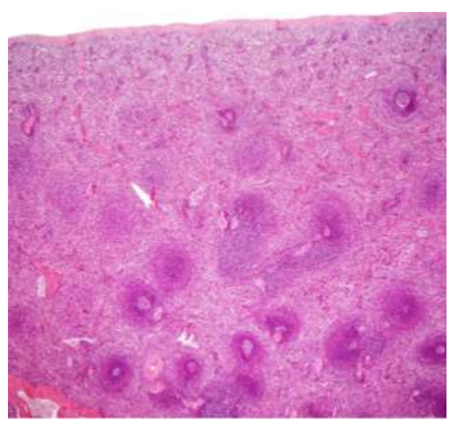


Figure 2.39: Spleen Cross Section

B. Mucosal-Associated Lymphoid Tissue – MALT *(Figure 2.40)*
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 Peyer's patches in the intestine

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.

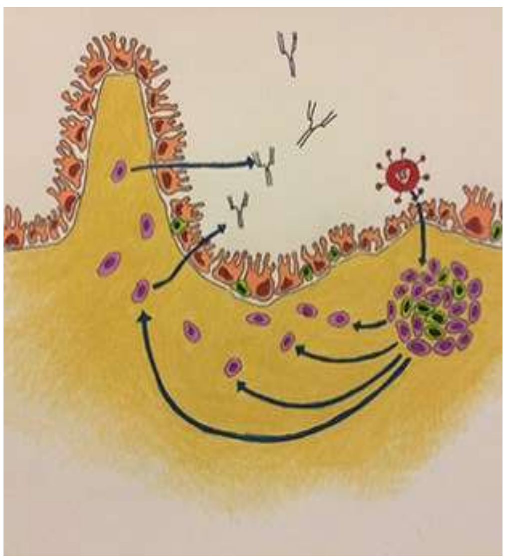


Figure 2.40: GALT

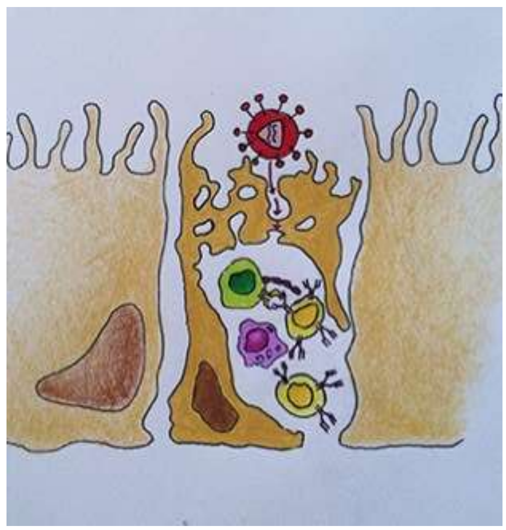


Figure 2.41: M Cell

C. Skin- the largest organ of the body, not technically a Secondary Lymphoid Organ *(Figure 2.42)*

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. Intra-epidermal (a form of intra-epithelial) lymphocyte, or IELs, many with specialized T cell receptors) - activated or memory cells.

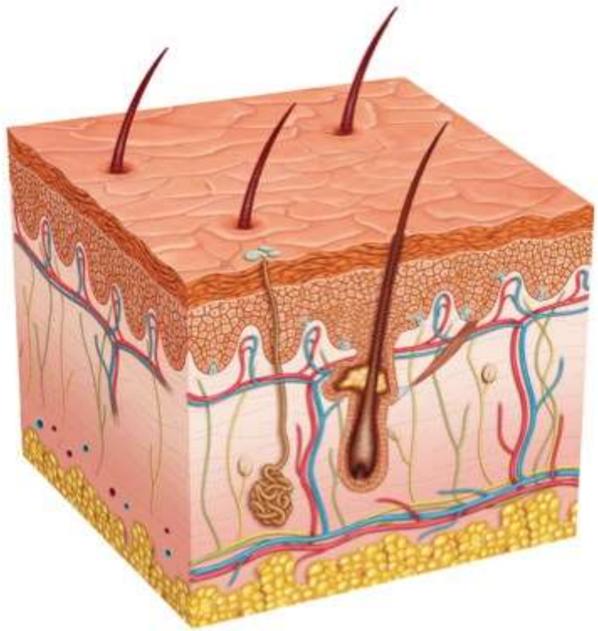


Figure 2.42: Skin

Video clip 2-9

IX. Final Issues:

A. Apoptosis *(Figure 2.43)*

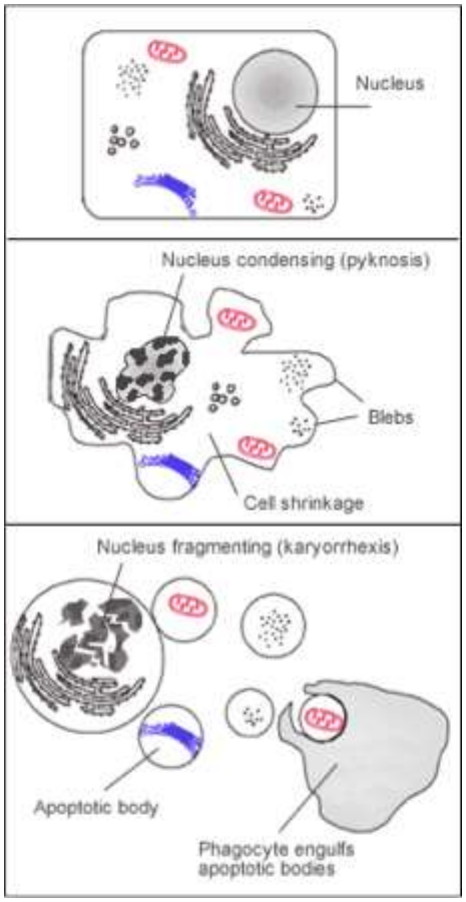
One aspect of the immune system that makes it so energetically expensive is that it produces huge numbers of cells and then gets rid of the great majority of cells before they are even used.

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 2.44)*

   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



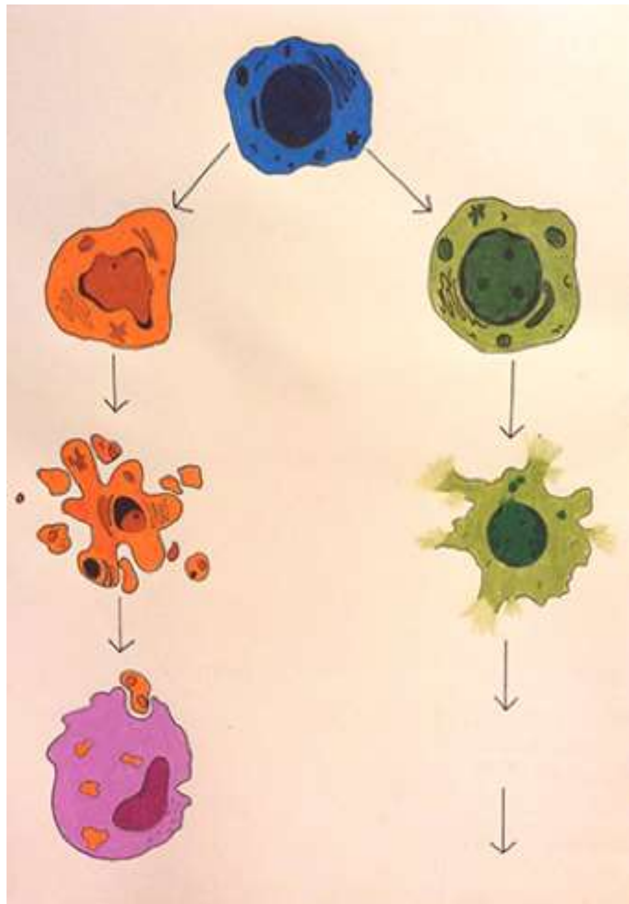


Figure 2.43: Apoptosis

Figure 2.44: Apoptosis and Necrosis

B. Evolution

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

Figure 2.45 tunicate larva

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 2.45)*

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.

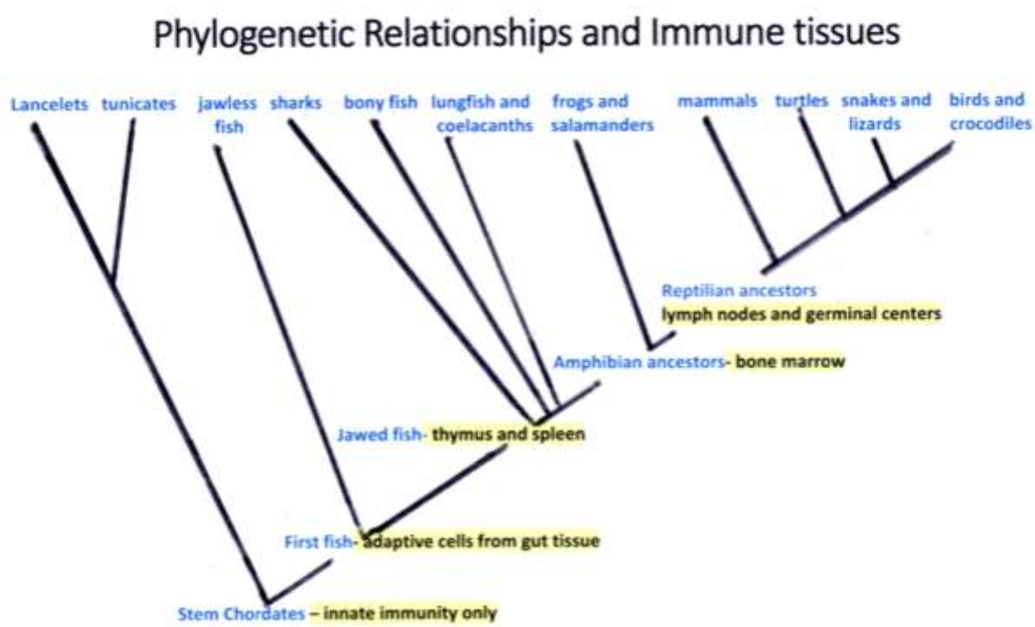


Figure 2.46: Evolutionary Immunology

For fun: 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=mFNxXfwlP3A

Table 2.1 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
lymphoid	adaptive	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
lymphoid	adaptive	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
lymphoid	adaptive	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	
lymphoid	adaptive	Plasma cell	Secretes antibodies	Mature B cell, lives 1 - 2 weeks	No surface antibodies	T_H cytokine signals	antibodies
lymphoid	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	
granulocytic myeloid cells		basophil	Important in allergy	picks up basis dyes, e.g. methylene blue	F_C receptors for IgE	chemokines	prostaglandins and leukotrienes
granulocytic myeloid cells		mast cell	Inflammatory response, especially to allergies	Mature cells in skin, mucosal, digestive tract and other first defense tissues.	F_C receptors for IgE		
granulocytic myeloid cells		eosinophil	defense against parasites	Pick up acidic dyes, e.g. eosin	F_C 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		
antigen-presenting myeloid cells		monocyte	Macrophage precursors	Circulate in blood for 8 hours, then differentiated into macrophage			
antigen-presenting myeloid cells		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 hemtopoiesis		follicular dendritic cells	feed antibody-antigen complexes to B cells	found in secondary lymphoid organs	receptors to hold antibody –antigen complexes		

Table 2.2 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. Alternatively, the conductor of an orchestra
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 Q – the guy who invents and supplies new tech
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	Q's cadre of techies, working to improve defenses. Alternatively teachers and coaches
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 L03

Innate Immunity

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

Video clip 3-1

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 antibacterial 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.

Figure 3.1: Insect

Figure 3.2: Plant

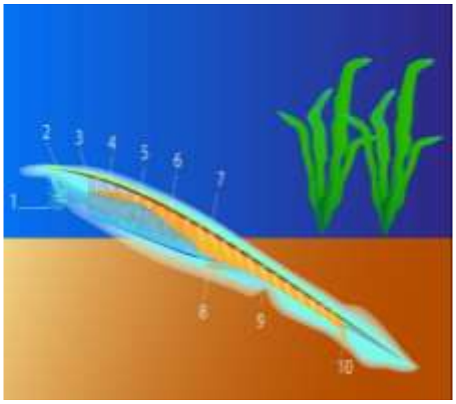


Figure 3.3: Lancelet

Figure 3.4A: Tunicate

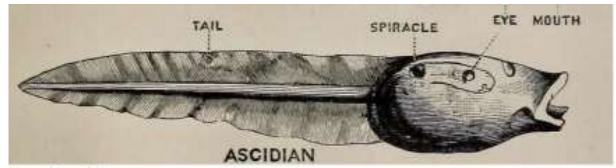


Figure 3.4B Tunicate larva

4. Skin (tertiary immune organ) – Few pathogens can penetrate intact skin, relying instead on bites and abrasions. Water-borne schistoma parasites, causing schistomiasis, 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.

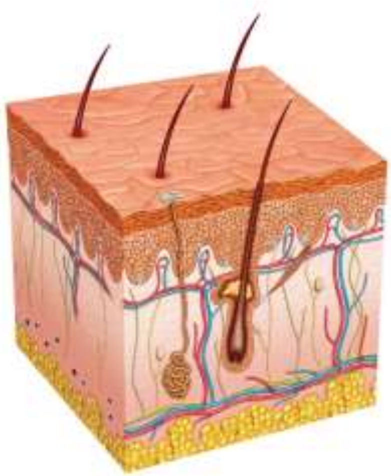


Figure 3.5: Skin

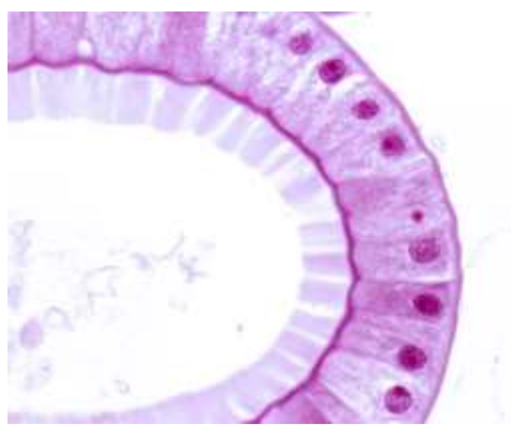


Figure 3.6: Epithelia

5. 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

6. Additional GI defenses:

    a. stomach acid (vultures) *(Figure 3.7)*
    b. enzymes
    c. competing flora (good bacteria)

Figure 3.7: Vulture

Figure 3.8: Inflamed Finger

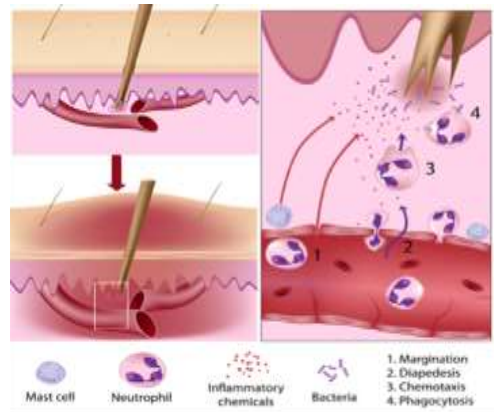


Figure 3.9: Inflammation

Video Clip 3-2

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 temperature 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.

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). It doesn't take an infection to produce inflamed tissue. A 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)*

   That's the good news. The bad news is that healed wounds and resolved or chronic infections may kick off long-term inflammation, with negative consequences.

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.

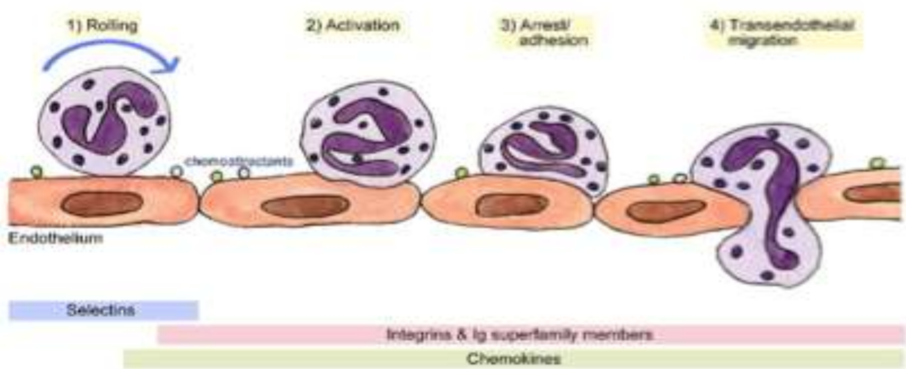
3. Neutrophils leave the blood stream and respond by changing the conformation of their integrins, which makes them stick to the blood vessels near the problem. *(Figure 3.11)*

   a. Rolling - Neutrophils stick briefly and release, which causes them to bounce or roll on the endothelial surface. The mechanical stress leads to internal changes and the next step, activation, to occur.

   b. Activation

   c. Arrest

   d. Transendothelial migration - neutrophils enter the tissue to participate in the inflammatory response. They will respond to local signals and increase their ability to phagocytize and subsequently digest material in the phagolysosome.



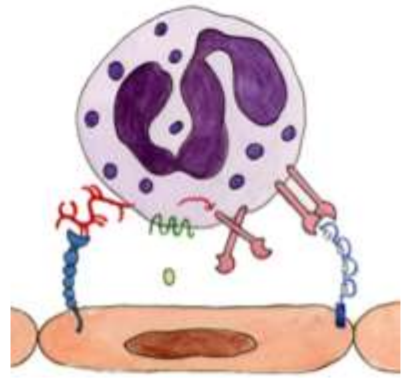


Figure 3:10: Extravasation **Figure 3.11: Neutrophil**

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.

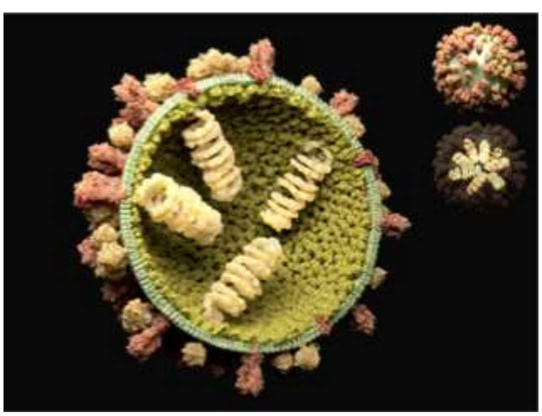
6. The T_H cells then coordinate an adaptive attack on the infections.

Video Clip 3-3

III. Innate Targeting of Pathogens

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

The innate immune system recognizes potentially dangerous pathogens principally characteristic cell surface molecules: lipids, carbohydrates and proteins. Once they phagocytize a pathogen, they can also identify foreign DNA and RNA.



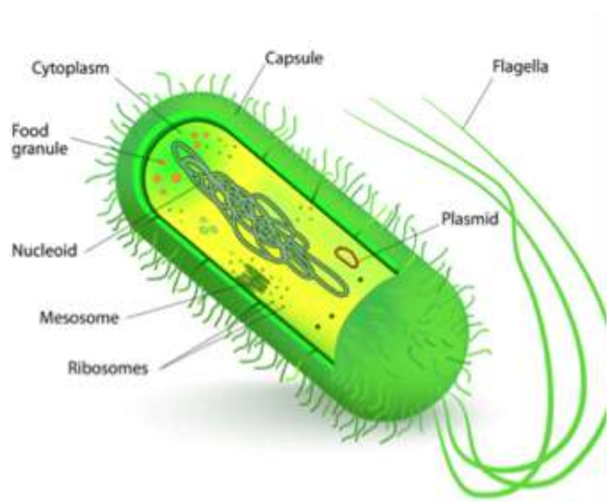


Figure 3.12: Flu Single-stranded RNA **Figure3.13: Bacterial Structures**

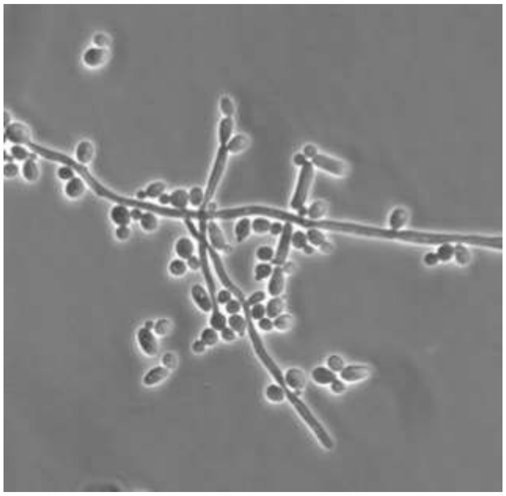


Figure 3.14: Candida (Yeast)

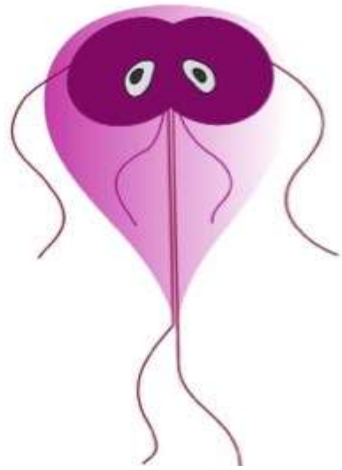


Figure 3.15: Giardia

Figure 3.16: Trypanosome

Figure 3.17: Tapeworm

A. *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:

      a. flagellin – bacteria *(Figure 3.18)*

      b. profilin – surface protein of protozoan (toxoplasmosis) This protein resembles the profilin that performs important control functions in microfilament assembly, but contains an extra peptide loop domain, is secreted to the surface and helps the parasite move toward and enter the host cell.

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. Because the receptors recognize rough overall shape, bacteria can't evolve unrecognizable flagellae and still get them to work.
Fungi	Candida (thrush) athlete's foot, *Cryptococcus*, ringworm	Eukaryotic, unicellular, multicellular or multinucleate	Cell wall: zymosan (ß 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, schistosomia-sis, flukes, tapeworms	Primarily members of Platyhelminthes (flatworms) and Nematoda (roundworms)	Characteristic cell surface proteins

2. carbohydrates and glycopeptides
    a. zymosan – component of fungal cell walls – a vague term because it has not been exactly characterized. No one has identified a PAMP for chitin as yet, but humans are known to produce chitinases in response to fungal infections. These ill also attack the chitins of arthropods and certain worms.

    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

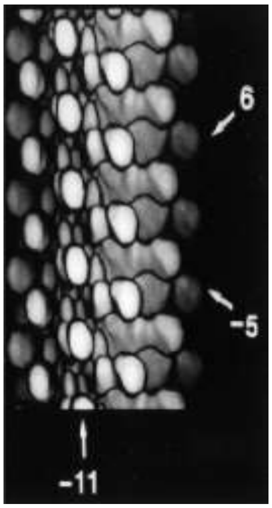


Figure 3.18: Flagellin

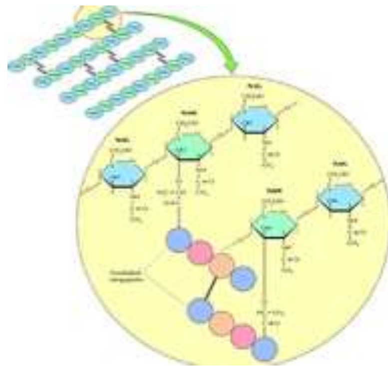


Figure 3.19: Peptidoglycan

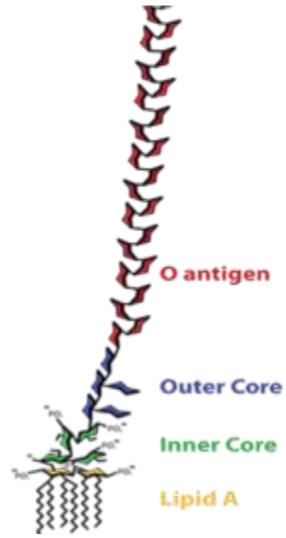


Figure 3.20: Lipids

B. *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 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.
   g. Gram negative bacteria have relatively thin peptidoglycan walls, also have second membrane on outside often covered with lipopolysaccharide -- setting off a HIGHLY inflammatory response. These bacteria are quite lethal and some strains have gained resistance to most antibiotics.

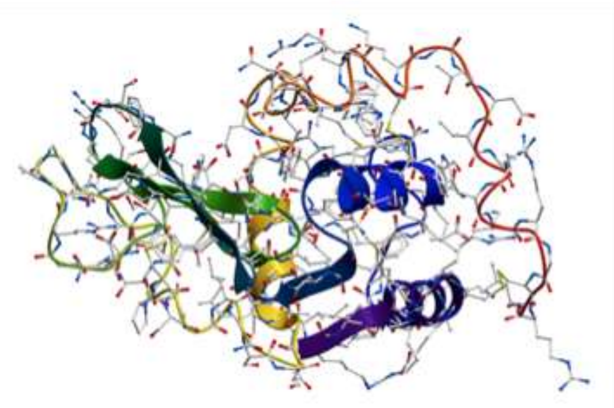


Figure 3.21: Lysozyme **Figure 3.22: CRP**

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-κB, the major internal inflammation regulator.
   d. NF-κB 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

4. TLR – differential function

   a. Internal TLRs (including 3, 7, 8, 9) extend across the RER or phagolysosomal membrane with the recognition region in the lumen. They respond to bacterial/viral DNA and RNA.
   b. External (including 1, 2, 4, 5, 6) extend from the plasma membrane to the exterior of the cell and respond to characteristic pathogenic cell surface components. Usually paired TLRs on outside and unclear if endomembrane TLRs operate singly or paired. However, plasma membrane TLRs may work as either homodimers or hetero dimers, and the same TLR has different recognition properties, depending on its partner.
   c. Negative regulation- you have to be able to turn these off or you get excess inflammation and maybe an autoimmune disease.
   d. Interact with: MD2 at surface for binding LPS (and also certain viral and cancer proteins), MyD88 to initiate internal signal sequence

Table 3.3 TLR

TLR	Recognizes	Pathogen	Membrane
1	Triacyl lipopeptides	mycobacteria	plasma
1 & 2	Peptidoglycan component and triacylated lipopeptides lipotechoic acid zymosan (ß-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	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

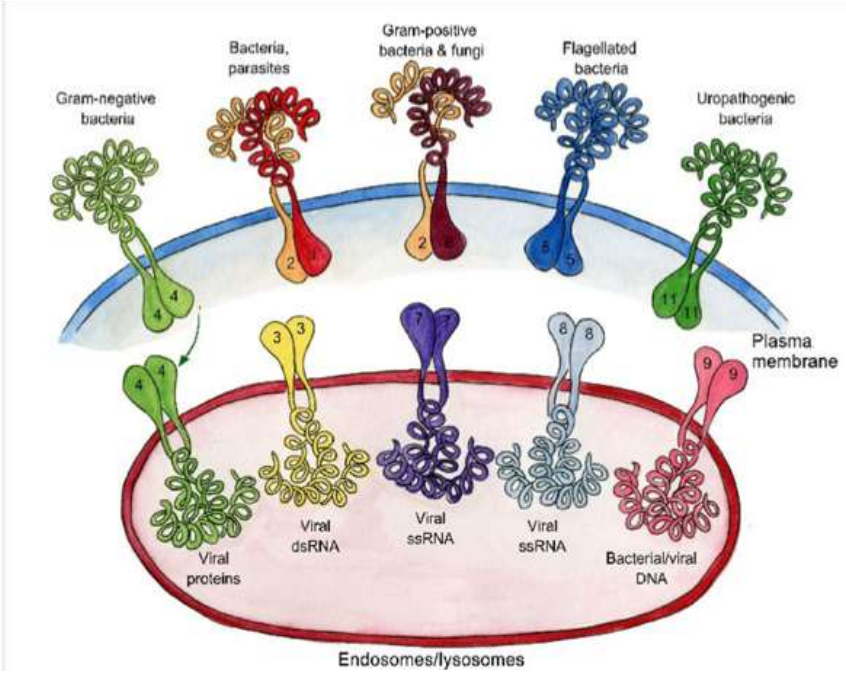


Figure 3:23 TLR Location

Video clip 3-4

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)*

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.

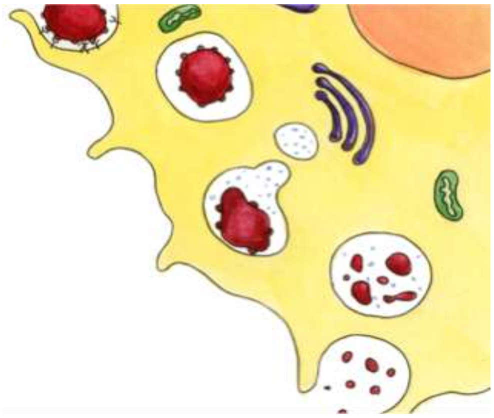


Figure 3.24: Macrophage

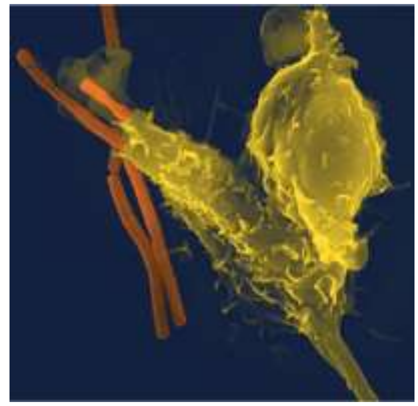


Figure 3.25: Neutrophil attacking Anthrax

3. Superoxide radicals also generate reactive nitrogen species (RNS) including NO, also toxic *(Figure 3.29)*

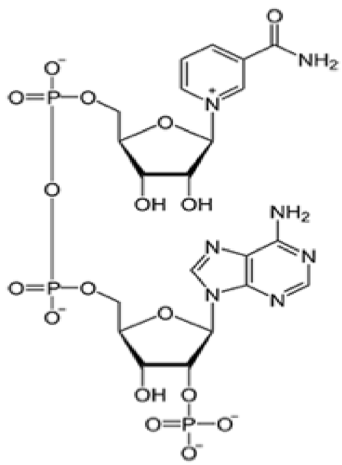
4. Oxidation is coupled to K^+ transport, rendering the interior hypertonic. K^+ enters to compensate for the negative charges, resulting in a rise in pH (8.5). Once the pH reaches this, further neutralization is done by transporting H^+.

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



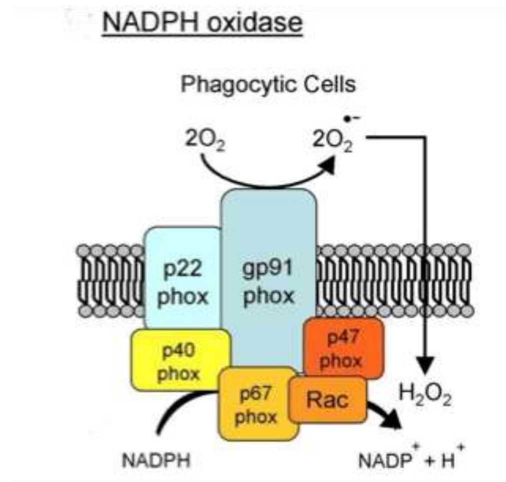
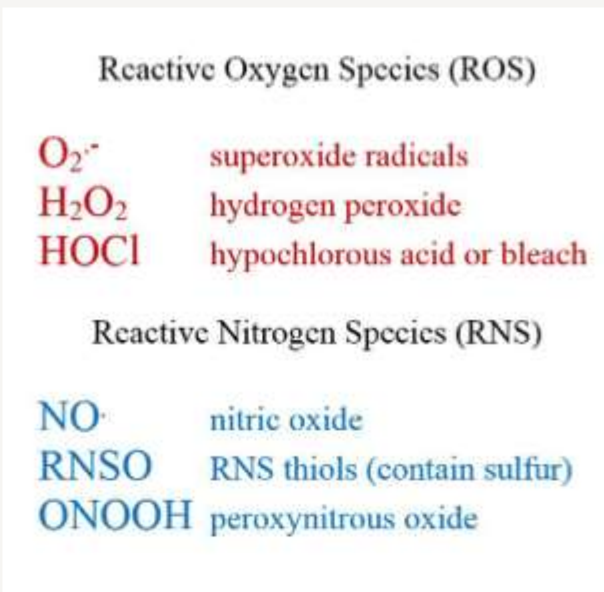


Figure 3.27: NADPH

Figure 3.28: Phox Complex



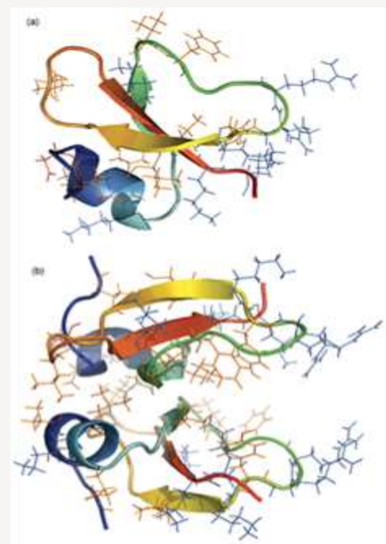


Figure 3.29: RNS, ROS

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 TNFα, all inflammation activators. Always on the lookout for pathogens, and after phagocytizing, they relay info about the pathogen to the T_H cells (unlike neutrophils).

3.

Dendritic cells – the scout and patrols Most important initial trigger of the adaptive response. They phagocytize primarily in order to sample pathogens and activate inflammation. Specifically designed to activate T_H cells and therefore are phagocytes, secrete a lot of cytokines and coordinate with T_H cells. *(Figure 3.31)*

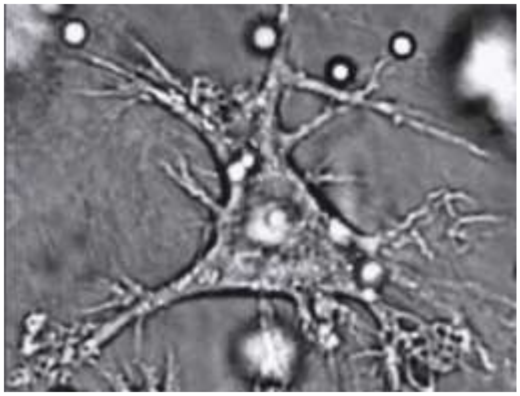


Figure 3.31 Dendritic Cell

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 T_H cells.

Video clip 3-5

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 via pattern recognition**	**(much greater specificity) mostly proteins**
diversity	**hundreds of types, soluble and membrane bound including TLRs**	**VAST number of types, soluble and membrane bound, including every possible antibody.**
gene rearrangement?	**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)		ROS/ RNS

References: More on the vulture stomach defense (spoiler alert- there's more than just acid): http://www.latimes.com/science/sciencenow/la-sci-sn-vulture-gut-microbiome-20141125-story.html

Lecture L04

Antigens & Antibodies

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

Video clip 4-1

I. Context

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

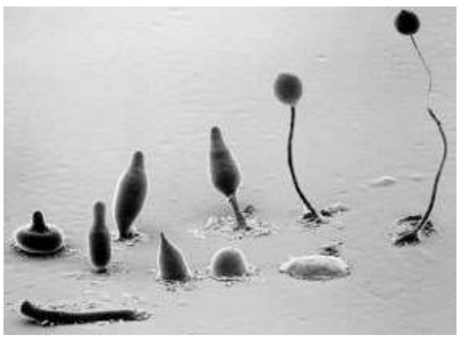


Figure 4.1: *Dictyostelium*

Figure 4.2: Yeast

Figure 4.3: DNA

Figure 4.4: Arabidopsis

Figure 4.5: Drosophila

Figure 4.6: *C. elegans* **Figure 4.7: Mouse** **Figure 4.8: Zebrafish**

2. A Good Model -

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

B. Transferring Information

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

   a. Cell A secretes small protein (**signal**)

   b. 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**).

   c. Binding involves weak interactions

   d. Upon binding, the receptor shifts shape, and transmits the change to its cytosolic region (**transduction**).

   e. The change at the inside sets off a cascade of changes: activates enzymes, brings different molecules together, changes binding properties, etc. 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.

   f. 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.

Video clip 4-2

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)*

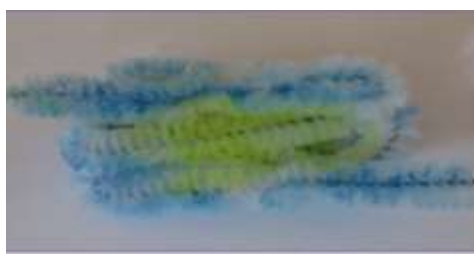


Figure 4.9: Peptide **Figure 4.10: Folding** **Figure 4.11: Disulfide Linkages**

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.

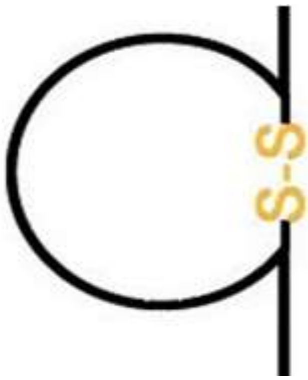


Figure 4.12: Domain Representation

Figure 4.13: Sandwich

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.

Video clip 4-3

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: In the 1960s, chemists classified proteins as fibrous (silk, collagen) versus globular proteins (most proteins, actually). Globular basically meant soluble in plasma.

1. Immunoglobulins: the protein fraction in the plasma involved in fighting disease.

2. Scientist subjected the blood plasma into the gel electrophoresis and the plasma proteins separated into 5 peaks: albumins (small blood proteins to help carry things around and keep osmotic pressure under control- the largest peak), alpha 1, alpha 2, beta, gamma (the heavy one).

3. They isolated the proteins from the gamma peak and found they provided the majority of "immune protection" among the plasma proteins.

4. This "immune effective" region was named the gamma globulins and later found to contain the antibodies.

5. Thus, if you wanted to learn more about antibodies, you started out by isolating the fraction and then experimenting on these 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.

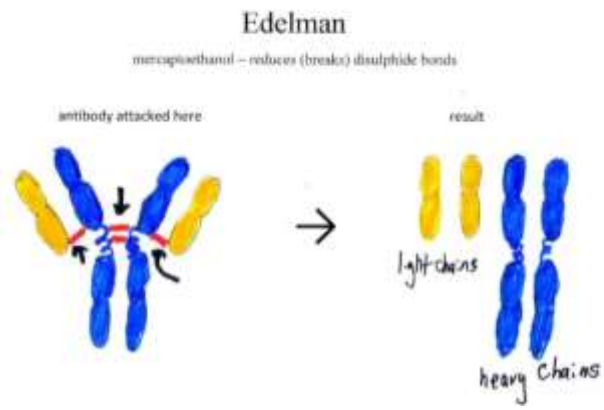


Figure 4.14, Edelman

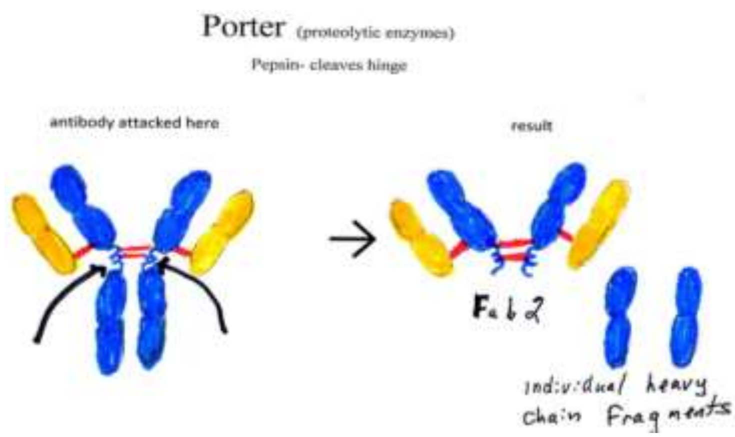


Figure 4.15: Porter - Pepsin

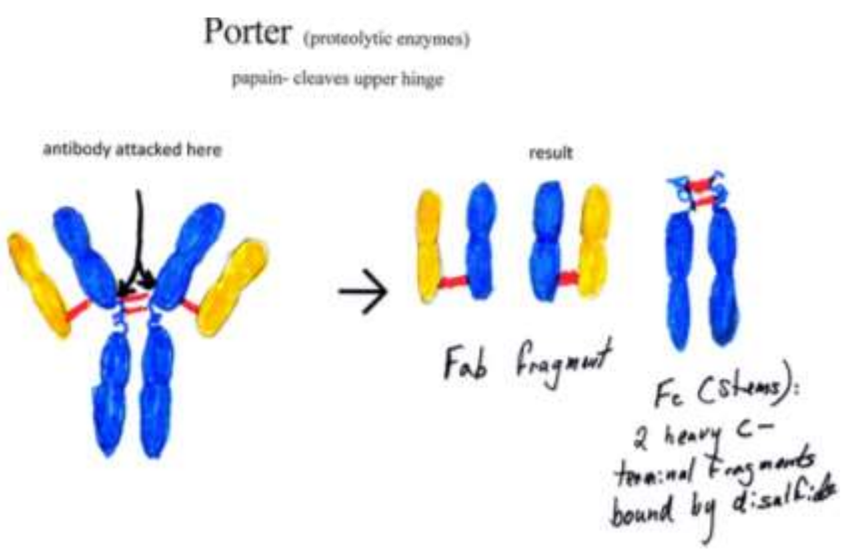


Figure 4.16: Porter – Papain

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 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.

Video clip 4-4

IV. Form and Function (foam board model) (Figure 4.17)

A. Heavy Chains

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.

    a. The constant regions are indicated in light blue at the lower side of each arm.
    b. The variable regions are shown in pink and yellow on the lower side of the model.

2. Two heavy (H) chains (~50,000 MW; MW 100,000 for both), identical to each other, composed of 4 or 5 immunoglobulin domains.

    f. Heavy constant regions are shown in dark blue (3 domains in the foam-board model, 4 in E or M classes.) extending from the arms into the stem.

    g. The Heavy variable region is in pink and yellow at the upper part of the arms and has a green loop.

3. Overall molecular weight: ~150,000

4. The amino (NH_2) 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. Sequins indicate 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. This helps to determine how the antibody interacts with the rest of the immune system.

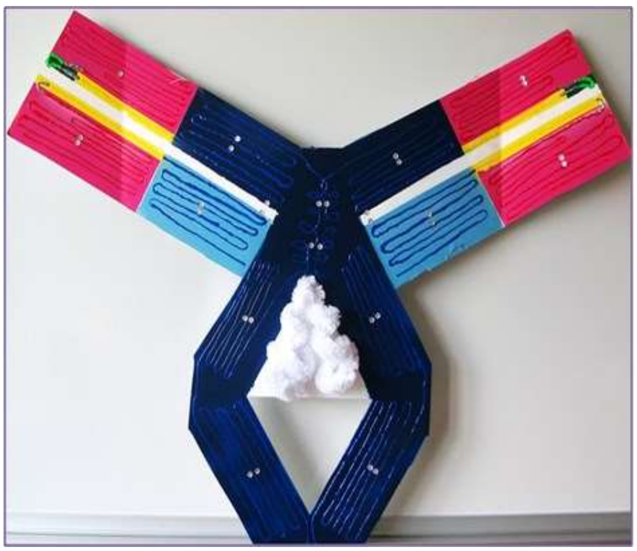


Figure 4.17: Foam Board Model

Figure 4.18: CDR

B. Details of the Structure of the Light Chains

Two basic parts or domains (Figure 4.19)

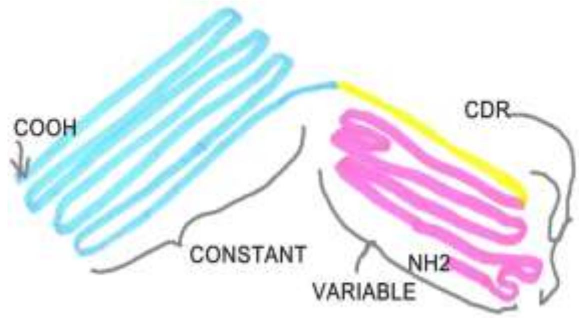


Figure 4.19: Light Chain

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.

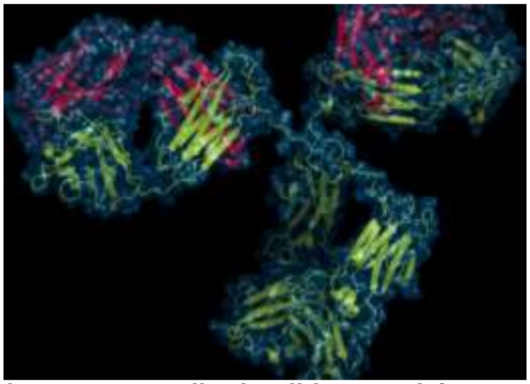
   b. 3 loops that stick out at the end comprise hypervariable region composed of three non-contiguous amino acid sequences (15 to 20% of the domain).

   c. The rest of the domain is the framework region that basically holds the loops in place.

Video clip 4-5

V. Immunoglobulin Classes *(Figure 4.20)*

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.



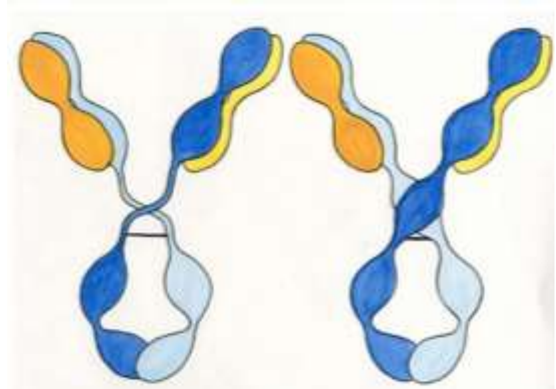


Figure 4.20: Antibody Ribbon Model
Figure 4.21: Comparison

A. Categorization by Heavy Chain Structure

1. flexible hinge or rigid bend – number of constant C Ig domains 3 versus 4 *(Figure 4.21)*
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 com-plexes	J chain	Sub-classes	Timing	Membrane-spanning Ig receptor?	Role
M	rigid	yes	yes	no	first class produced in maturing B cells.	yes, 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_H2 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 μand 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.

Video clip 4-6

VI. Specific Ig Types

A. IgM - µ heavy chain – rigid bend *(Figure 4.22)*

1. rigid bend – 4 constant domains

2. Function- general purpose - First class expressed in plasma.

3. Monomeric form (actually 2H +2L) expressed as a membrane-bound antibody on the naïve B cell.

4. Secreted form occurs as pentamer, looking like 5 IgG's stuck together, stems in, 10 antigen-binding sites out.

5. 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)*

6. Very good at binding large complex structures and activating complement (to kill foreign cells).

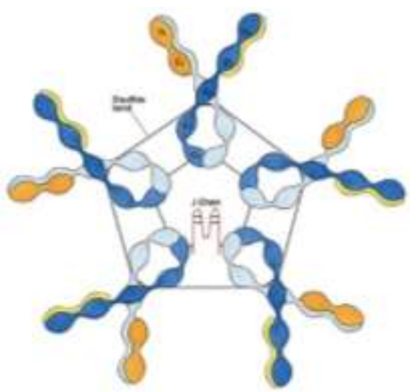


Figure 4.22: IgM Pentamer

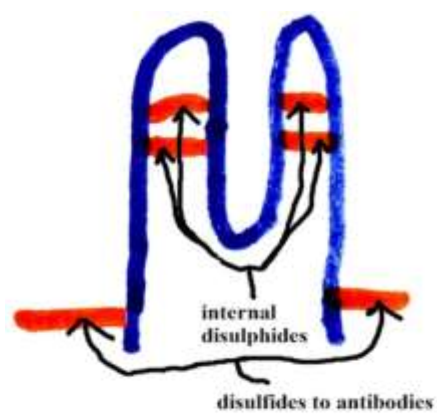


Figure 4.23: J Chain

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

1. Function- aids recognition by naïve B cell.

2. 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.

3. Rarely found in plasma (0.2% of total serum immunoglobulins)

4. Superficially resembles IgG (different amino acid sequence).

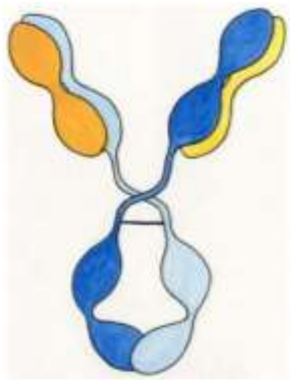


Figure 4.24: IgD

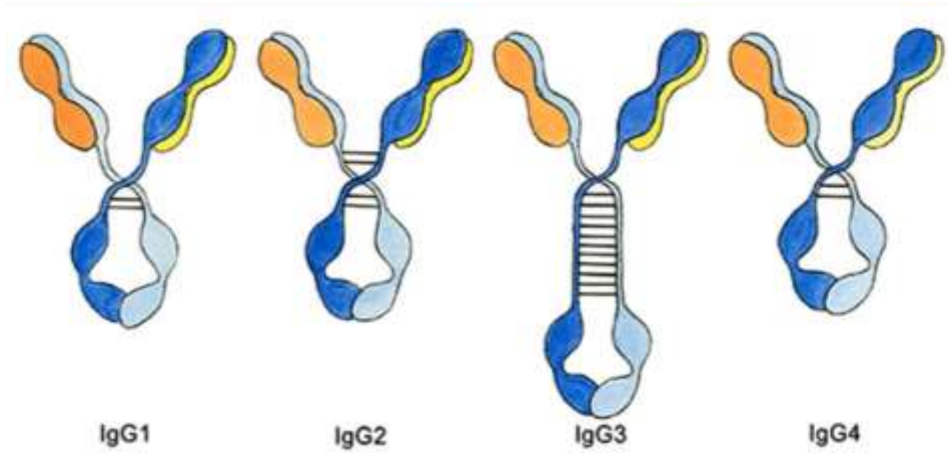


Figure 4.25: IgG Subclasses

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

1. flexible hinge, 3 constant domains
2. standard secreted antibody defending against bacterial and viral pathogens
3. comes in four versions, numbered 1 to 4, varying in biological specificity:
    a. IgG1 – activates complement, Fc receptors bind tightly
    b. IgG2 – weakly activates complement, Fc receptors bind weakly
    c. IgG3- strongly activates complement and binds tightly to Fc (lots disulfides)
    d. 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)

D. IgA - α heavy chain – flexible hinge *(Figure 4.26)*

1. Function- primarily epithelial patrol.
2. Also occurs in serum as a monomer.
3. 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.
4. Two subclasses (1 and 2)
5. Also held together by an additional peptide, the J chain (binds secretory peptide) which is identical to the one in IgM
6. Secreted into mucus, tears, saliva, and breast milk- up to 15 grams per day!
7. Plasma cells that secrete this tend to home in on various epithelial linings.
8. Unfortunately these same pathogens often produce proteases that specifically target the vulnerable hinge regions of this antibody.

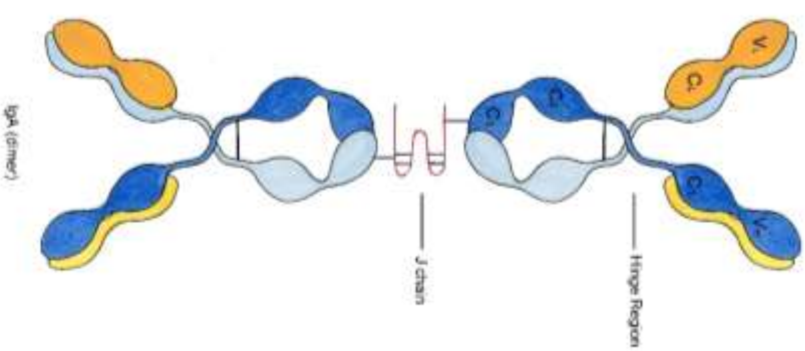


Figure 4.26: IgA

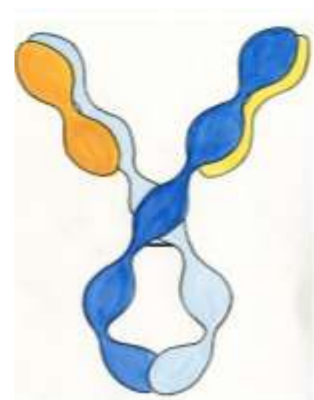


Figure 4.27: IgE

E. IgE - ε heavy chain, – rigid bend *(Figure 4.27)*

1. Function – defense again worm parasites.
2. Monomer superficially resembles IgM (somewhat different amino acid sequence) c. rare, but potent
3. involved in allergic response
4. binds to F_C (stem) receptors on mast cells and basophils, which causes them to trigger the allergic reaction.

Video clip 4-7

VII. 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 anybody 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*: Papier maché* virus 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.

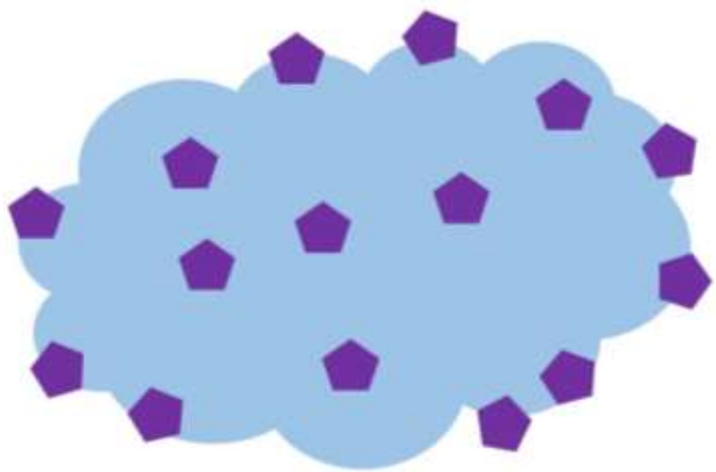


Figure 4.29, hapten (too small to be immunogenic) **Figure 4.30, hapten stuck to BSA**

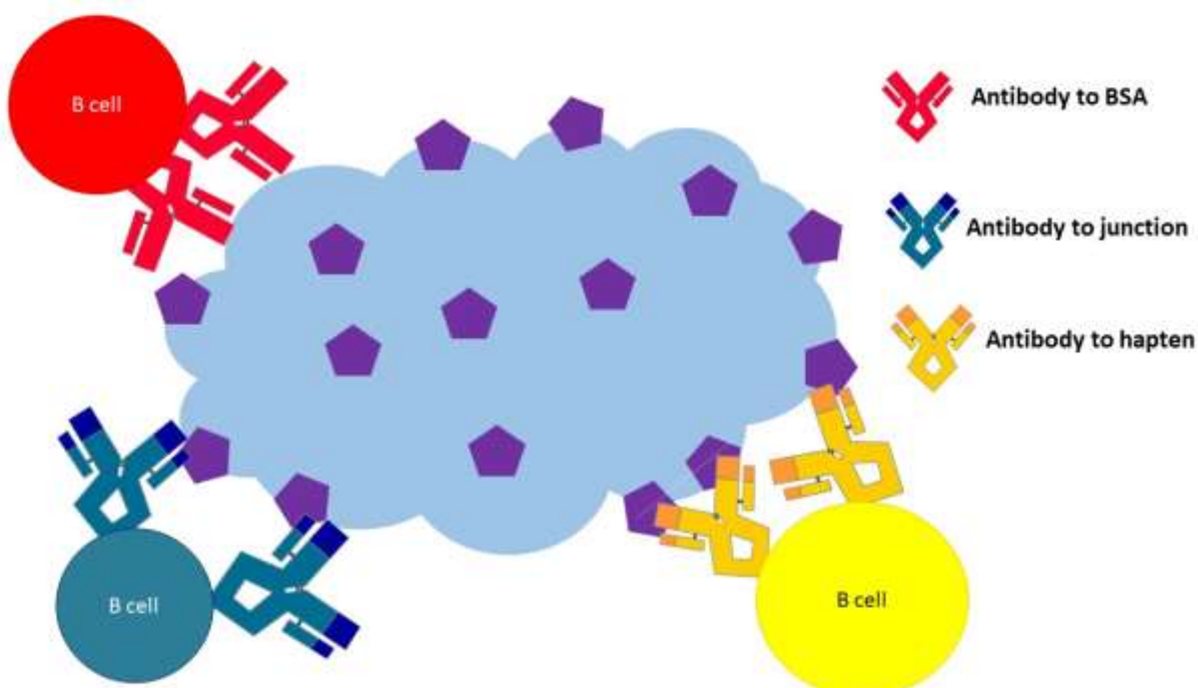


Figure 4.31 – antibodies, including those to the hapten

Video clip 4.8

VIII. Fc Biological Activity

A. Antibody Signaling – property of the Fc, or stem, region of the antibody.

1. Allergic responses – eosinophils, basophils and mast cells have receptors (F_CRs) 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 (F_CRs) for IgG F_Cs.
   b. A bunch of F_Cs sticking out of a bacterium or cluster of viruses will bind to a number of F_CRs 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 of 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

B. 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.

Video clip 4-9

IX. B-cell Receptor *(Figure 4.32)*

A. Immunoglobulin

1. If the antibody is stuck in the membrane, sticking out, it’s an immunoglobulin receptor.

2. B-cells recognize foreign antigen when two neighboring receptors bind to it and cross-link. *(Figure 4.33)*

3. Naïve B cells have M or D class receptors.

4. Memory B cells can have receptors of any class.

B. Co-Receptors

1. The signal is transduced by the associated heterodimer of Igα/Igβ, both of which have long cytoplasmic tails. *(Figure 4.34)*

2. Igα/Igβ current renamed CD79a and CD79b

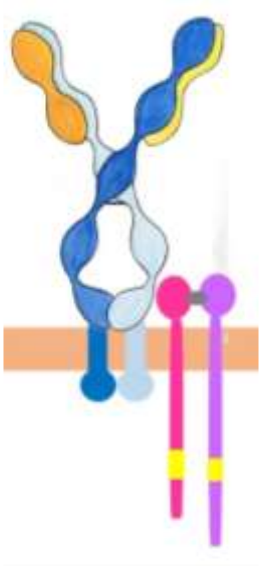


Figure 4.32: Ig Receptor

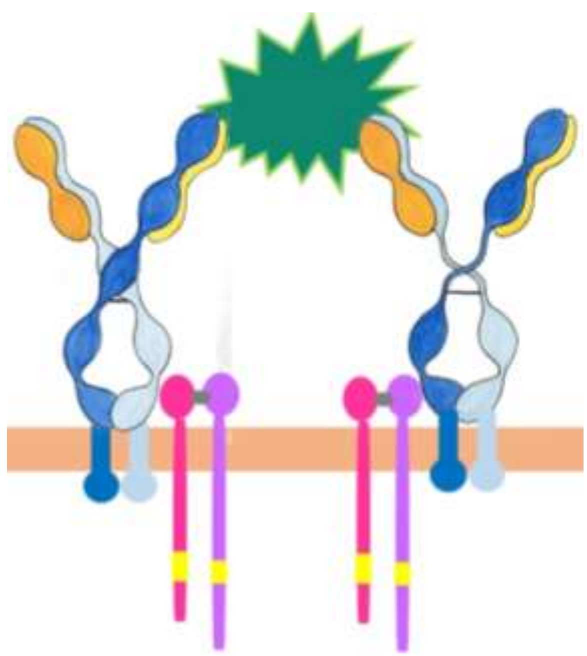


Figure 4.33: Neighboring Receptors

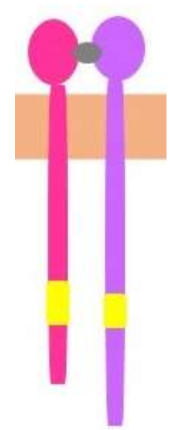


Figure 4.34: Igα/Igβ

Video clip 4-10

X. 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 *de novo* pathway	Can synthesize nucleotides by *de novo* 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.

11. 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 antibody.

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

Video clip 5-1

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.

Video clip 5-2

II. Scale and Models

A. DNA

1. Humans have about 1 meter of DNA per haploid genome- yarn model.
2. DNA is about 2 nm (nanometers or milli-microns or mμ or 10^{-9} m or meters) in diameter.
3. 3D model – about 20 cm (centimeters or 10^{-2} meters) across.
4. Genes comprise 15% of the genome, but only 3% specifies the amino acid sequence of proteins. Huh?
5. And many of the protein coding regions code for proteins that bind to these sequences: hence administratively top-heavy.

B. Implications of the Helical Structure

1. Accessing the information
2. End on view
   a. A one-turn sequence has 12 bases. Counting 12 bases and you make slightly more than full turn around the helix.
   b. A two-turn sequence has 23 bases. Count 23 bases and you make slightly more than 2 full turns around the helix.

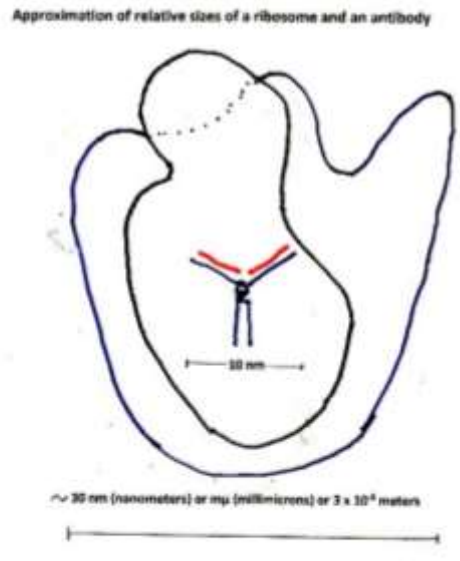


Figure 5.1 end on view of DNA model **Figure 5.2 ribosome and antibody to scale**

C. The Ig genes

1. Genes are on different chromosomes: κ (kappa) light on 2, λ (lambda) light on 22 and heavy on 14.

2. Total base pairs in the three Ig genes is somewhat less than 3 KB

3. Thus we generate all this diversity from less than 1% of our DNA, or less than 1 mm of DNA.

4. Yarn model with rearrangement signals.

D. Scalar Context

1. The *E. coli* genome comparable in size to the total length of DNA used to code for antibodies and T cell receptors.

2. The mitochondrial genome is much smaller- 17,000 base pairs and perhaps several micrometers in length.

3. The antibody molecule is about 10 nm (nanometers, milli-microns or 10^{-9} m) across, and here it's shown up again a ribosome (about 20 or 30 nm across) and if I embedded this in a mitochondrion, that thing would fill up the whiteboard.

Video clip 5-3

III. 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 5.3)*

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."

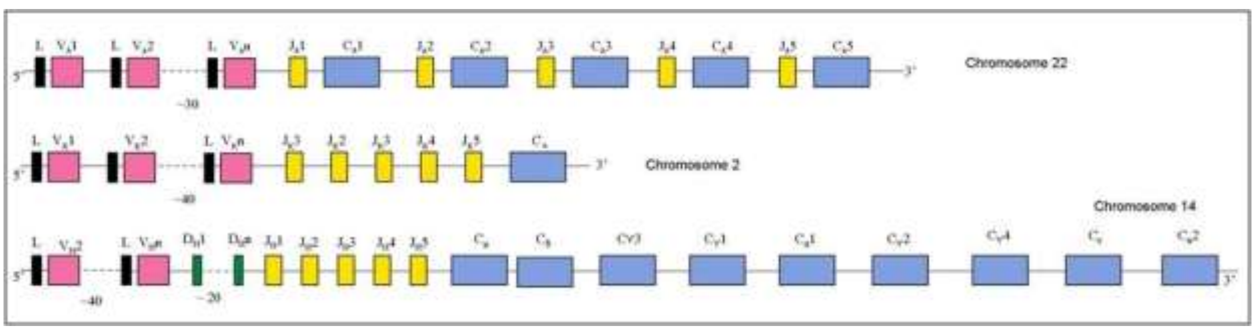


Figure 5.3: Human Version IgG Gene

IV. Light Chain Gene Expression

A. *Lambda (λ) Gene Expression (Figure 5.4)*

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 5.5)*

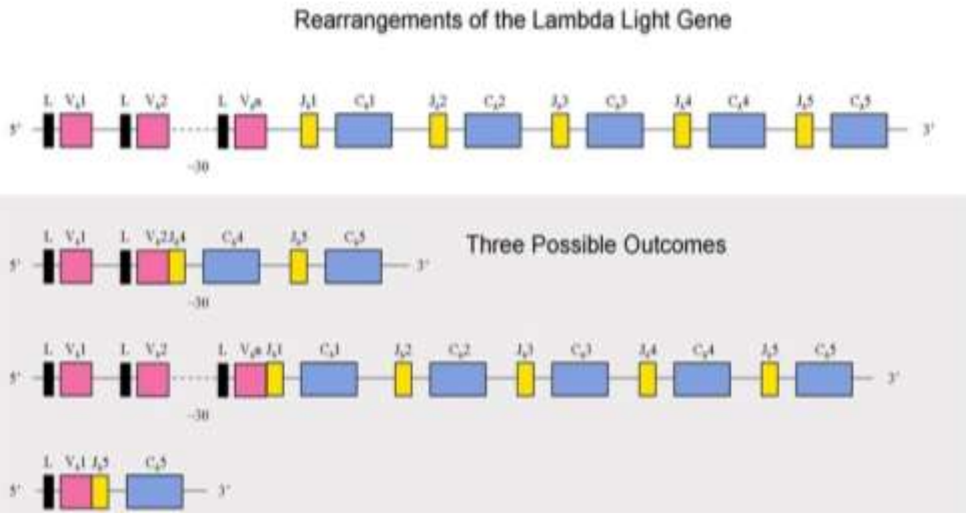


Figure 5-4 – Various outcomes of Lambda (λ) Gene rearrangement

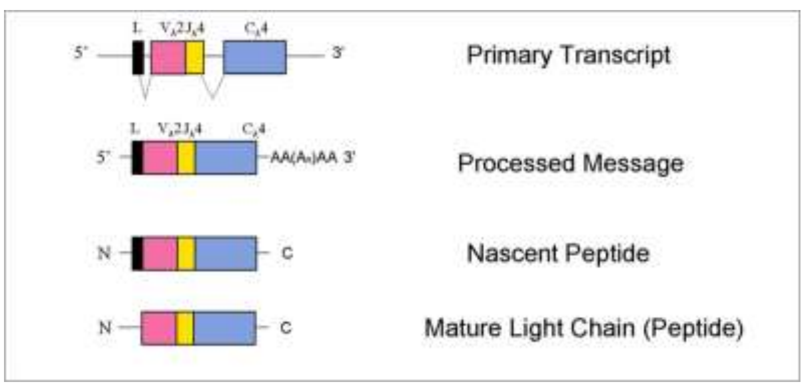


Figure 5.5: Transcription and Translation of the Lambda (λ) Gene

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 massage, 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.

B. *Kappa (κ) Gene Expression (Figure 5.6)*

1. Gene family in humans includes a series of about 40 V_κ (leader–variable) regions, 5 functional J_κ (joining) regions, and 1 C_κ constant region.

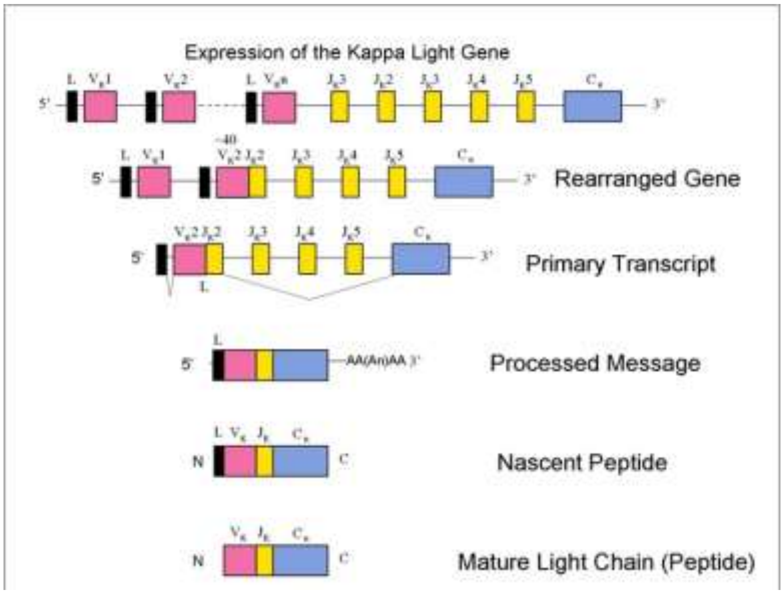


Figure 5.6: Kappa (κ) Gene Expression

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.

Video clip 5

V. Heavy chain Gene Expression

A. Heavy Gene Rearrangement *(Figure 5.7-5.11)*

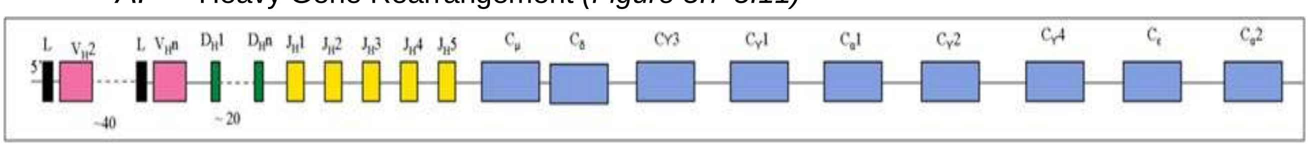


Figure 5.7: Series of Constant Regions is: μ, δ, γ3, γ1, α1, γ2, γ4, ε, and α2

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, 1μ, 1δ, 4 different γs, 1 *ε,* and 2 αs. The order is μ, δ, γ3, γ1, α1, γ2, γ4, *ε*, and α2.

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.

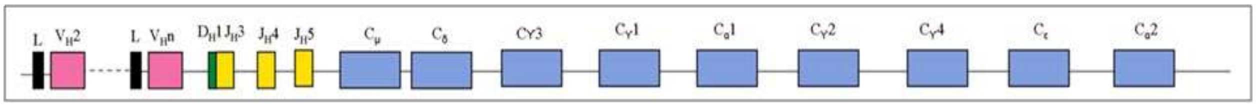


Figure 5.8: D Regions Join with a J

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

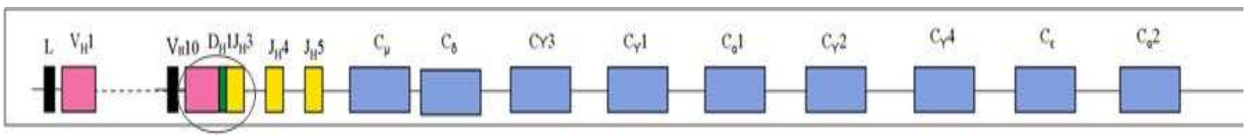


Figure 5.9: VL Joins with D

B. Transcription and Translation

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

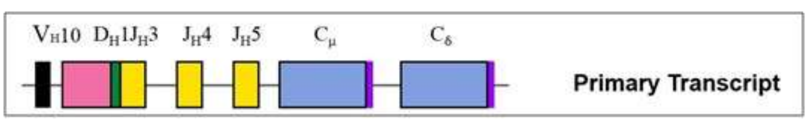


Figure 5.10: Initial Heavy Primary Transcript

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

3. Translation proceeds as with other messages.

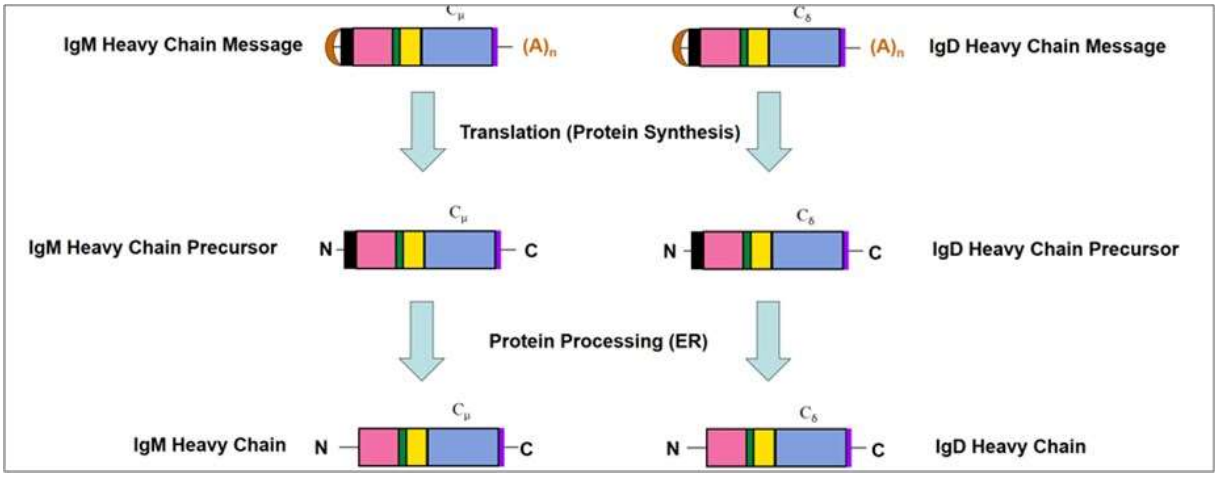
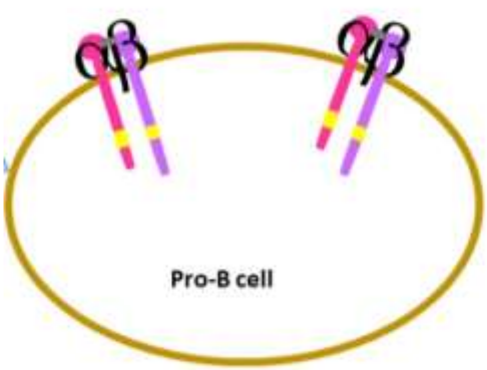


Figure 5.11: Results of Alternative Message Splicing

Video clip 5-6

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

A. Rearrangements (*Figure 5.12*)



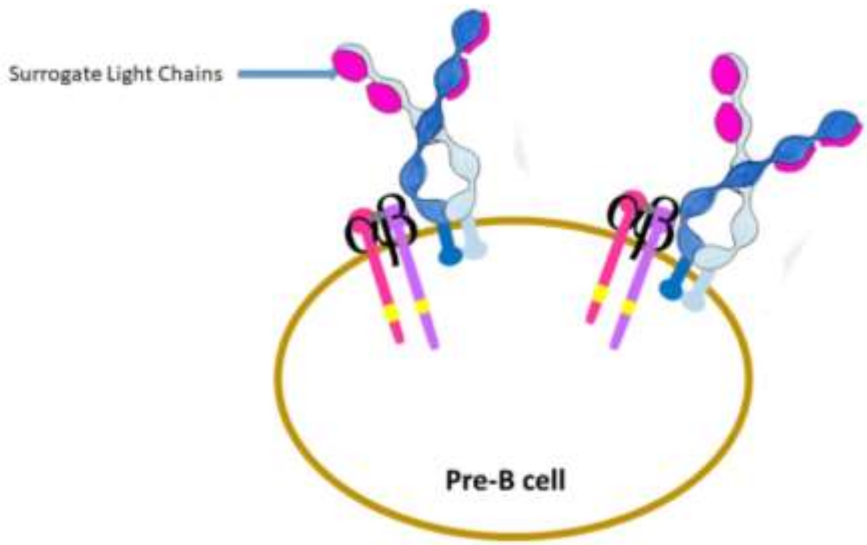


Figure 5.12A **Figure 5.12B**

1. First, the developing B cell synthesizes the co-receptors (Figure 5.12A).

2. Then 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.
3. 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.

4. If you get a successful rearrangement, the heavy chain gene picks up the surrogate light chains and signal to begin rearranging the light chain genes (*Figure 5.12B*).

5. First you rearrange one kappa gene and then, if that does work, the other, again turning off the unused genes.

6. If neither works, you proceed to the lambda, first one then the other.

7. Only if all four genes prove to be duds does the cell apoptose.

8. If there is a productive rearrangement, the receptors then display an M class receptor with the light chain.

9. This is an immature B cell and will undergo negative selection *(Figure 5.12C).*

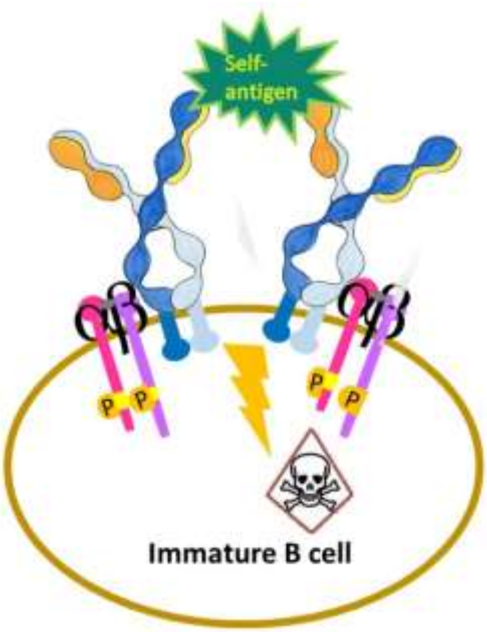


Figure 5.12 C (immature B cell)

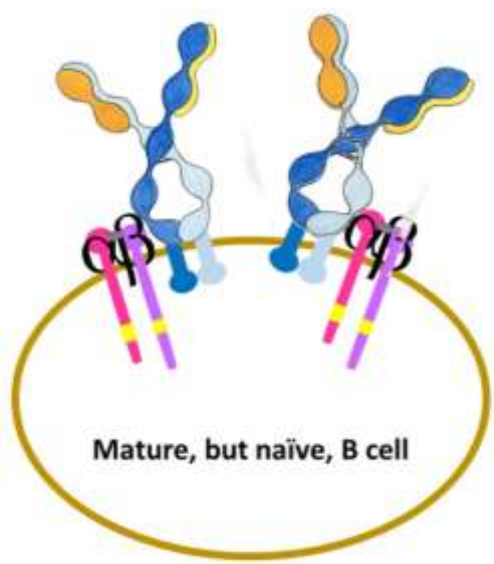


Figure 5.12 D mature B cell (naïve so far)

10. If it passes this test, the cell begins to splice the message so that it can synthesize the D chain.

11. It is now mature and can leave the bone marrow *(Figure 5.12D)*

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.

C. Translation and Processing

1. L and H transcripts exit the nucleus separately, attach to ribosomes, and begin peptide synthesis.

2. The initial signal sequence (leader) binds factors that cause the ribosome to attach to the RER.
3. The ribosome orients so that as the L and H peptide elongate they (separately) enter into the RER lumen.

4. Only if the message for the H chain ends on a membrane-spanning region, the peptide will remain anchored in the membrane. L and antibody H chains float free.

5. In the RER lumen, enzymes clip off the leader and being to add oligosaccharides to the peptides.

6. Assembly and processing occurs in the primarily in the lumen of the RER and the product is then sent to the Golgi.

7. First the heavy chains are put together, then the Ls are added (for the G class first one heavy and one light associate).

8. After assembly, enzymes oxidize the disulfide bonds, nail the structure into position and adjust the oligosaccharide into the version characteristic of the antibody.

Video clip 5-7

VII. The Recombination Signal Sequence *(Figure 5.13)*

A. Location

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

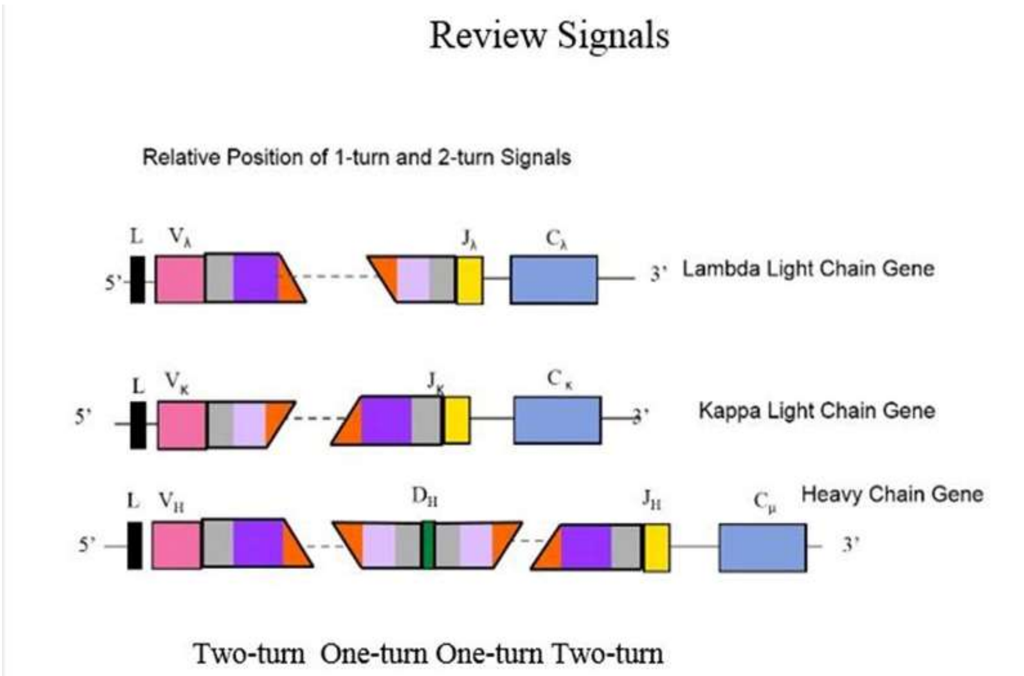


Figure 5.13: Recombination Signal Structure Overview

B. Each RSS has *(Figure 5.14)*

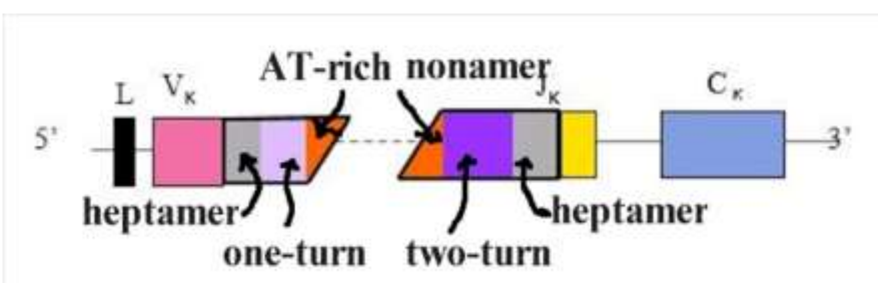


Figure 5.14: Recombination Signal Structure Detail

1. 7 nucleotide palindrome (reads the same forward and back). This region will participate in the ligation of adjacent V(D)J segments.

2. spacer, seems to be important in lining up the adjacent V(D)J regions so that they join properly:

   a. κ 12 base (1 turn of helix - V) or 23 base (2 turns of helix - J)
   b. ii. λ 23 base (2 turns of helix - V) or 12 base (1 turn of helix - J)
   c. iii. heavy chain, V and J, both are two turn, D is one turn

3. 9 nucleotides rich in AT – aid in attachment to the multienzyme complex that performs the whole complex function. *(Figure 5.15)*

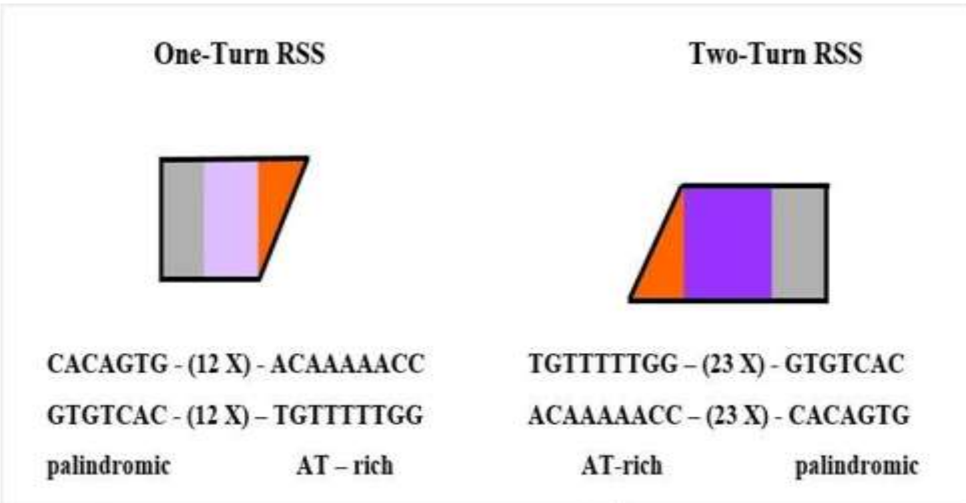


Figure 5.15:

Recombination Signal Structure Nucleotide Sequence

4. Signal sequences with one-turn spacers can only join with those with two-turn spacers, so this protects against mis-joining.

5. The enzyme complex responsible for joining is called the V(D)J recombinase.

Video clip 5-8

VIII. Mechanism of Rearrangement

A. Juxtaposition

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.

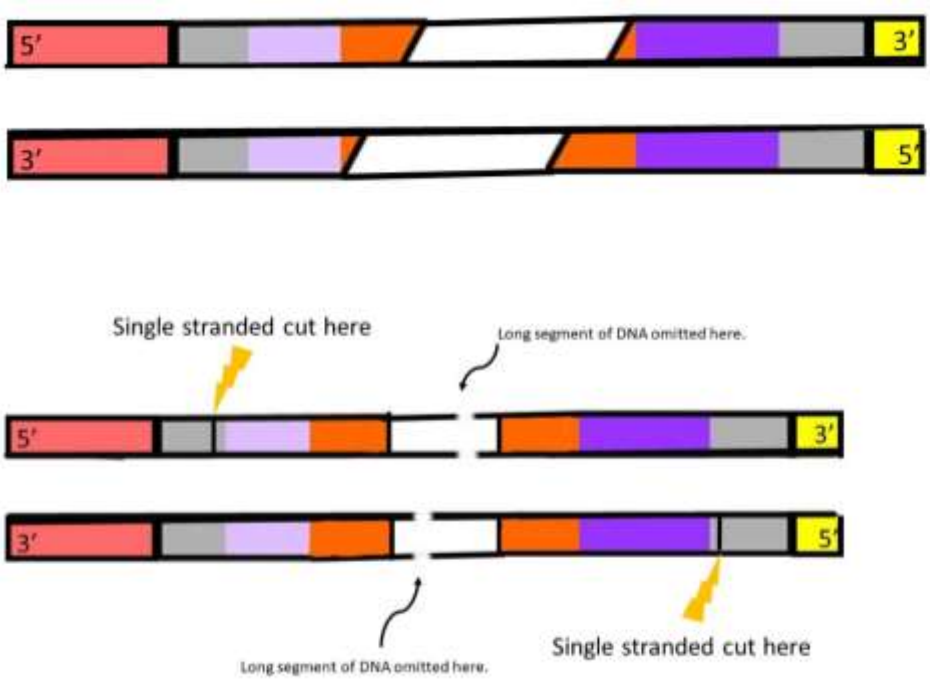
5. The 5'OH end of the cut strand attacks the opposite side of the uncut strand of the same DNA molecule.

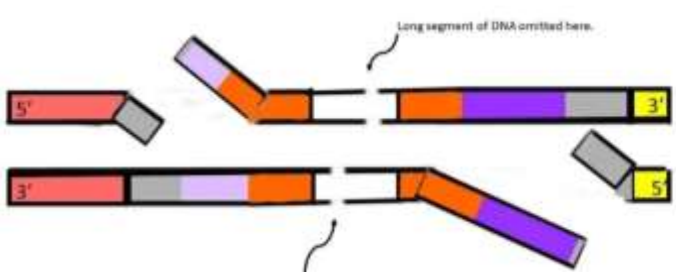
6. This produces a hairpin loop at the downstream end of the V and the upstream end of the J (light chains).

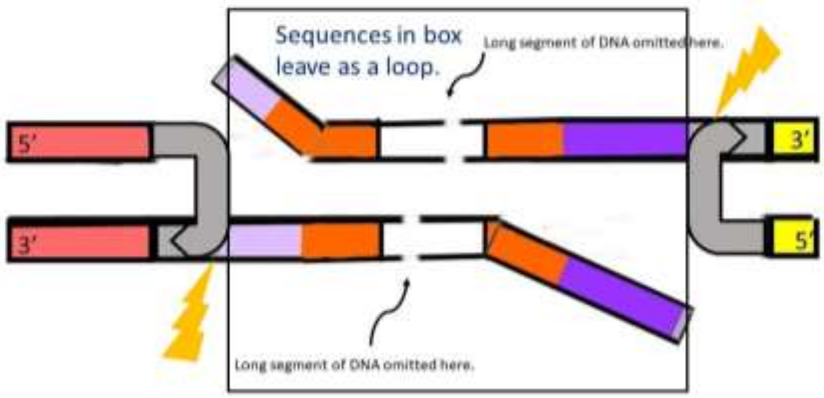
    a. The nucleotides within the loop were originally part of the palindromic sequences.

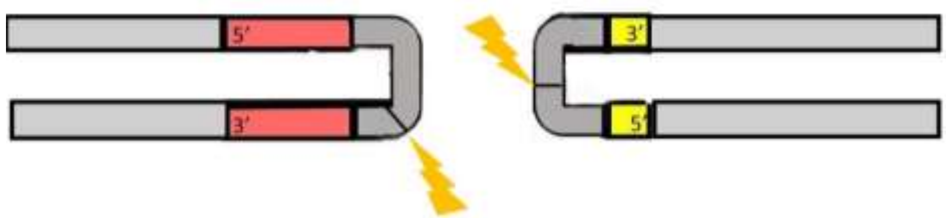
    b. The one- and two-turn sequences, along with the AT region signal end in a flush cut with a 5’ phosphorylated end.

Figure 16, the series showing the whole sequence, continues on the next page and then continues into the one after that.




Long segment of DNA omitted here.
5'
3'
3'
5'


Sequences in box leave as a loop.
Long segment of DNA omitted here.
5'
3'
3'
5'
Long segment of DNA omitted here.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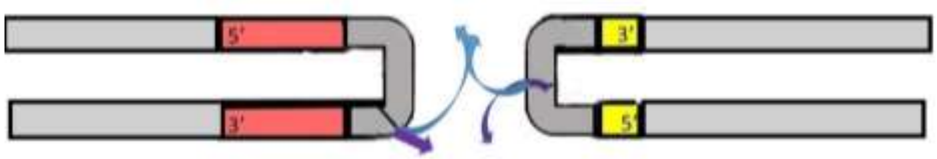
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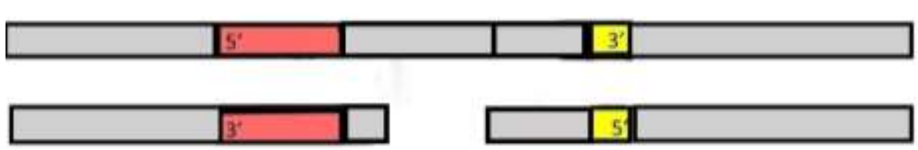

5'
3'
3'
5'

Figure 5-16, Sequence of Events

B. Ligation

7. Enzymes clip the hairpin loop. (*Figure 5.18*) 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 singled stranded regions (**P-nucleotide addition**), matching the ends generated by the cut.

8. **N-nucleotide addition:** For heavy chains only, up to 15 additional nucleotides can be added at random in the junction

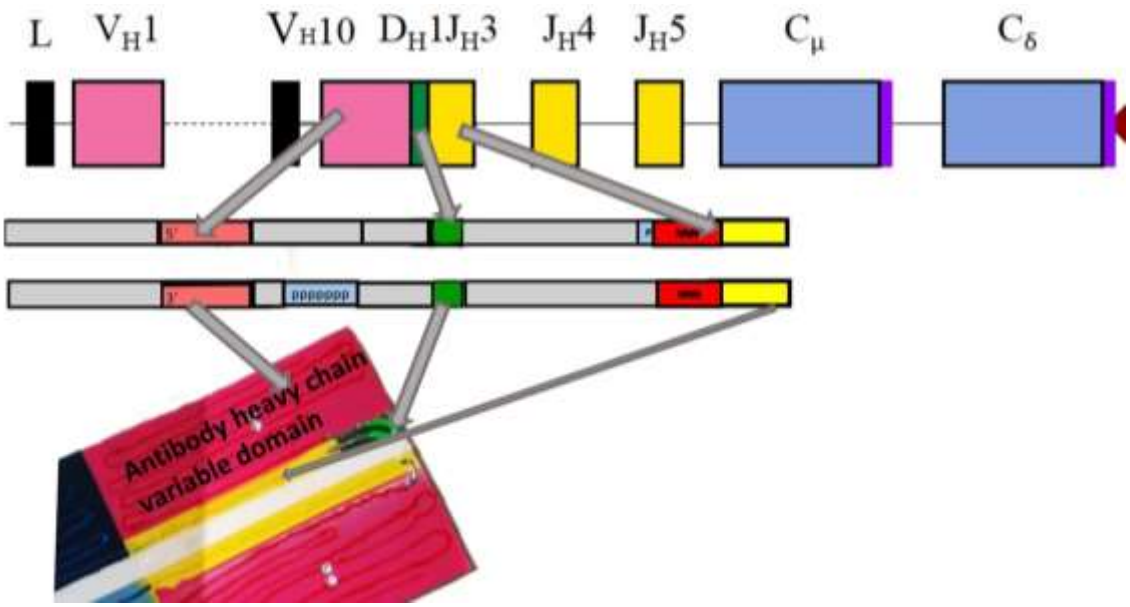


Figure 5-17, N-nucleotide addition at both VD and DJ joints

Figure 5-18, space-filling DNA model showing loop structure

9. Repair and ligation of the coding joint, with release and digestion of the signal sequences (which are retained if there an inversional joining.)

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

Video clip 5-9

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

A. Non- Production Rearrangements

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 if through this.

B. Allelic Exclusion - *only get one good productively rearranged* gene.

1. The heavy rearranges first. Once there's a good heavy chain, this shuts off rearrangement of the other heavy gene.

2. The light rearranges next. First it tries a κ, and if one works, the cell shuts down rearrangement.

3. If the κ doesn't work, it tries a λ. Once one of them works, the cell shuts down rearrangement.

4. If nothing works, the cell apoptoses.

5. Once a cell has a working heavy gene and a working light, then it shuts off its recombination enzyme genes.

C. Dangers and Mechanisms (Figure 5.19)

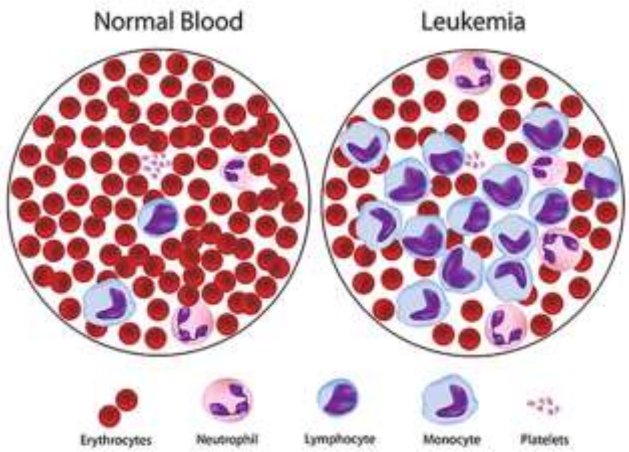


Figure 5.19: Dangers and Mechanisms

Video clip 5-10

X. 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_{κ}, D_{κ}, J_{H}, 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 5.20)

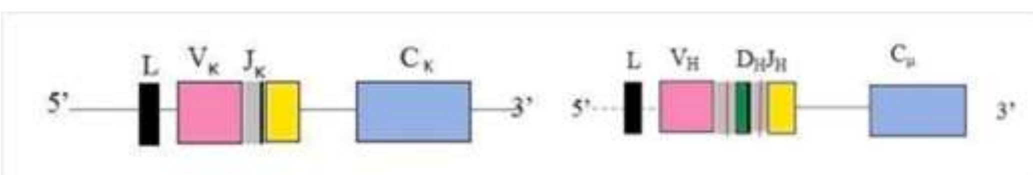


Figure 5.20: Coding Junctions

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 5.21 A and B)*

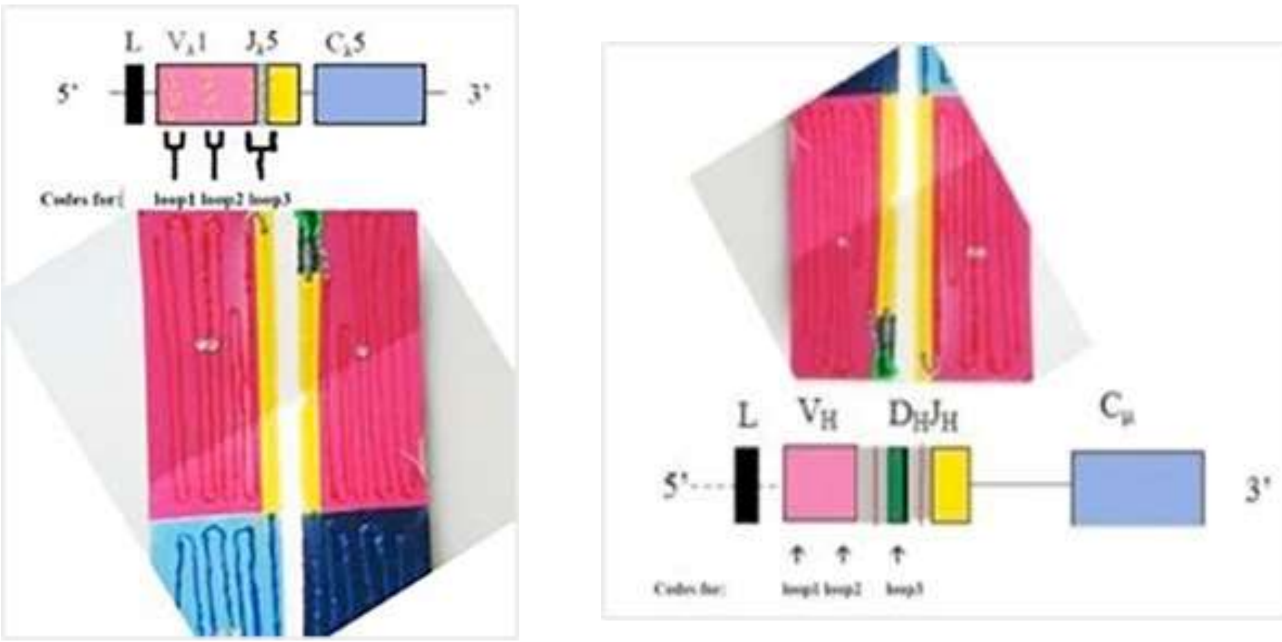


Figure 5.21: Somatic Hypermutation A. Light chain, B. Heavy Chain

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.

Video clip 5-11

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 5.22)*

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.

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

7. Not that there is not a class-switch signal at the end of the last Ca, as removing this will not allow a cell to produce any antibody at all.

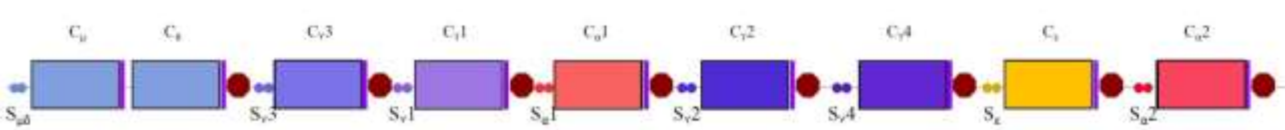


Figure 5.22: Heavy Chain Constant Regions

Lecture 06

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.

Video clip 6-1

I. B-Cell Maturation: Getting Oriented

A. A Closer Look at the Ig Gene Signal Sequences

1. Review Genes, *Figure 6.1*

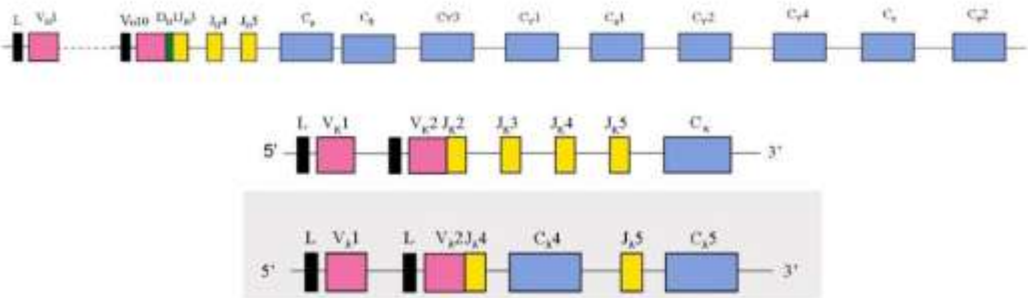


Figure 6.1, Gene Prior to Rearrangement

2. Rearranged heavy gene, with control sequences (*Figure 6.2*) Identify:

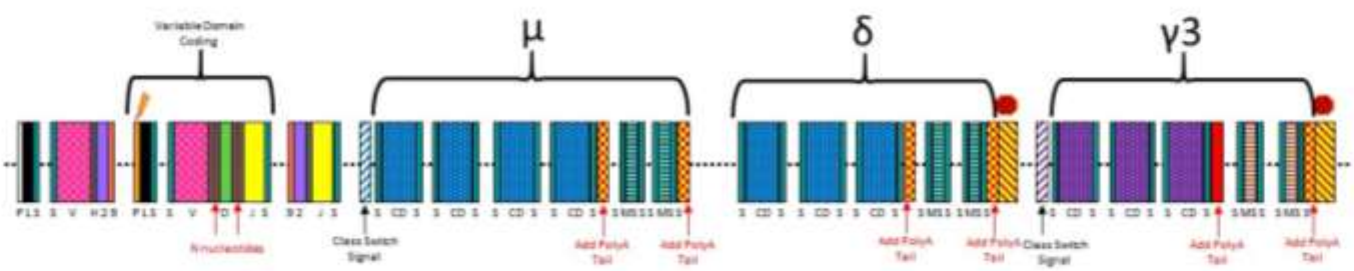


Figure 6.2, Detailed Diagram of Heavy Chain Gene

a. VL with promoters

b. RSS: with palindromic heptamer, one-turn sequence, AT-rich nonamer

c. D and J regions

d. Splicing signals

e. Class switch signals

f. Poly A tail signals and transcription stops signals

g. Constant Ig domains and membrane–span domains

3. Rearranged light genes, with control sequences, *Figure 6.3*

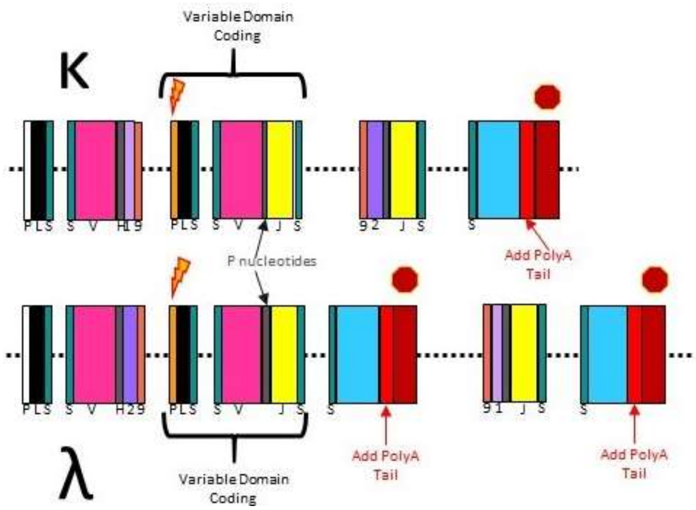


Figure 6.2, Detailed Diagram of Heavy Chain Gene

a. VL with promoters
b. RSS
c. J regions
d. Splicing signals
e. Poly A tail signals
f. transcription stops signals
g. Constant Ig domains

Video clip 6-2

II. Review of Initial Steps in Bone Marrow, *Figure 6.4*

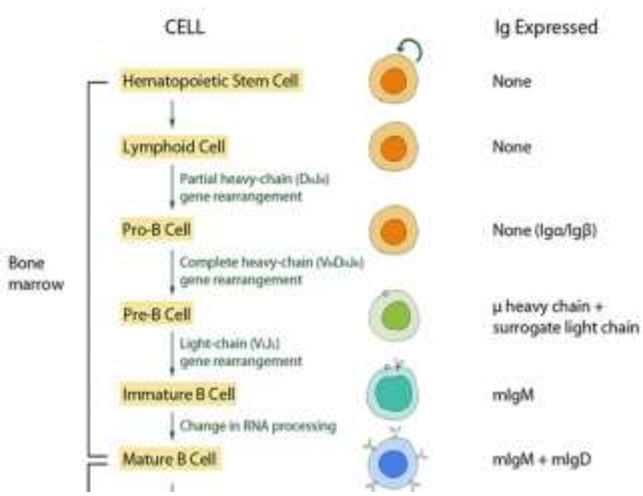


Figure 6.4: B-Cell Maturation

A. Entering Process:

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 6.5)*

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

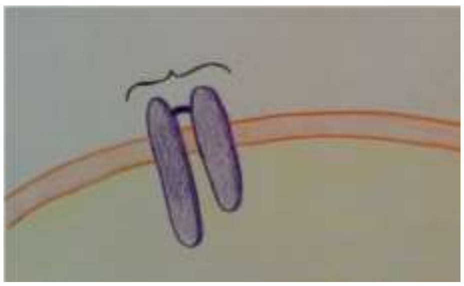


Figure 6.5: Pro-B Cell

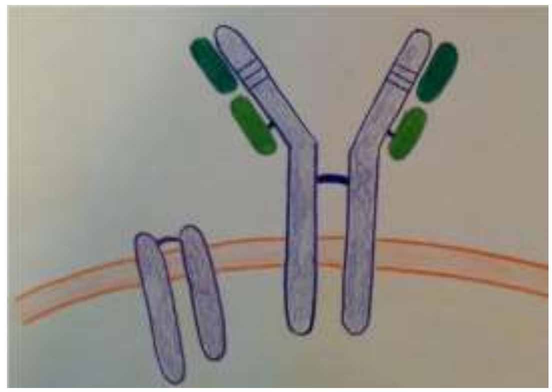


Figure 6.6: Pre-B Cell

B. Differentiation and Gene Rearrangement

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 6.6)*

5. The immature receptor associates with the Igα/Igβ 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 6.7)*

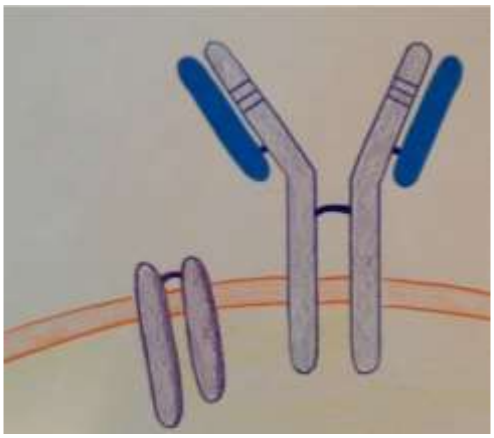


Figure 6.7: Immature B Cell

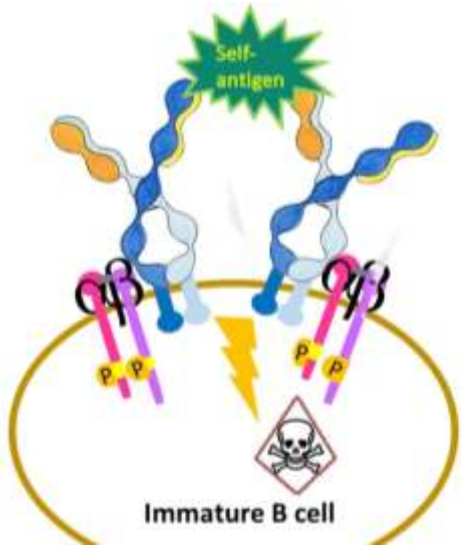


Figure 6.8 B cell undergoing negative selection

9. Undergoes negative selection (section C. 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, (*figure 6.8*).

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.

Video clip 6-3

III. **Thymus Independent B-Cell Activation and Proliferation** - To survive, circulating B cells 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 can also simultaneously activate toll-like receptors.

   a. Type 1 Thymus Independent Antigen - lipopolysaccharide such as those found in the outer bacterial cell walls of gram negative bacteria. These molecules also activate TLR4 to provide the second permissive signal.

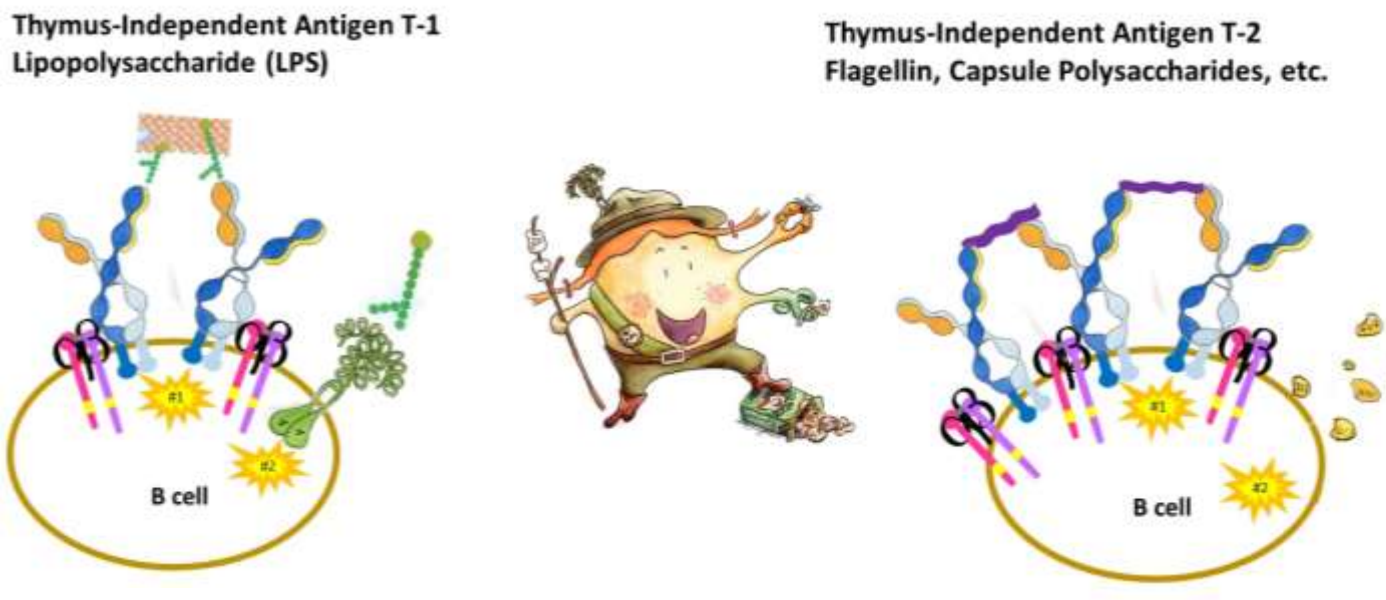


Figure 6.9, TI-1 **Figure 6.10, TI-**2

   b. Type 2 antigen - repetitive polymeric proteins, such as bacterial flagellin and gram negative capsule polysaccharides, can cross link the membrane-bound immunoglobulins, clustering a dozen or more Ig receptor complexes and kick off proliferation. The second signal does not come from a discrete source, but inflammatory cytokines (IL-2, IL-3 and IFNγ) and antigen presenting macrophages and sentinel dendritic cells help with the response

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

B. Activating Signals- Generating signal 1.

1. Review Ig receptor.

    a. mIgM or mIgD molecule
    b. Igα/Igβ heterodimers
    c. **immunoreceptor tyrosine-based activation motif**, or ITAM extends into the cytoplasm

2. When an antigen cross-links one antibody with the next outside the cell, it brings together the cytosolic Igα/Igβ domains, activating the ITAMs. *(Figure 6.11, a and b)*

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.

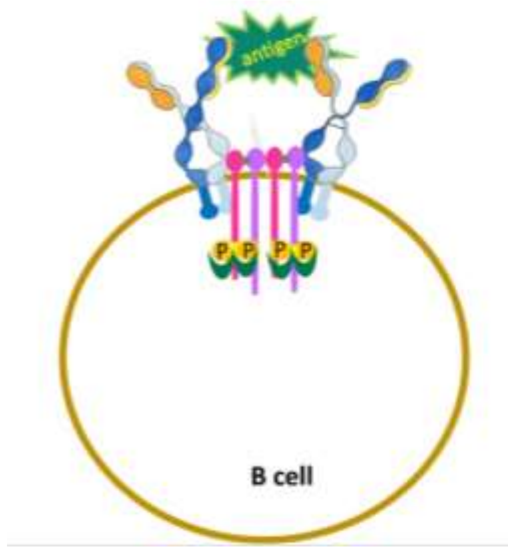


Figure 6-11a, B cell activation

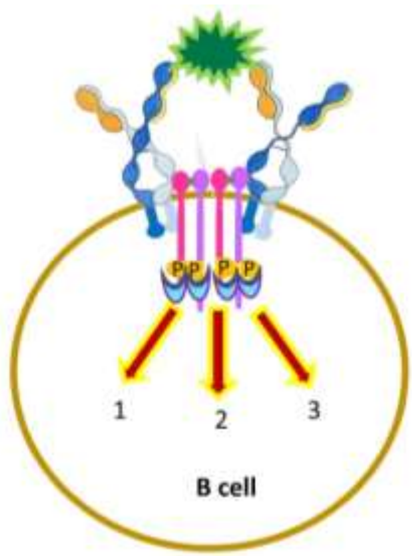


Figure 6-11b B cell activation

4. Once the ITAMs have phosphates, another kinase, syk, docks and triggers several different enzymes cascades.

5. The syk family, in conjunction with other proteins targeted to the ITAM phosphates, sets off a complex interlocking activation cascade. Here's three major elements:

    a. **Triggered by DAG and PIP.** An up-regulated hydrolase cleaves a membrane phospholipid to diacyl glycerol (DAG) and phosphoinositide (PIP). These can trigger a wide variety of responses. One branch leads to release of Ca^{2+} from the endoplasmic reticulum, which activates calmodulin, leading to a removal of phosphate from NFAT. The NFAT now enters the nucleus as an active transcription factor and upregulates genes.

Another branch leads to the removal of iκB (the inhibitor) leading to the up-regulation of the inflammatory transcription factor NF-κB, which also enters the nucleus and up-regulate genes.

b. **The classic phosphorylation cascade of RAS, RAF, MEK and ERK kinases.** Each of these phosphorylates and activates the next member of the sequence, leading to many activated ERK kinases. These phosphorylate a number of different inactive transcription factors, causing them to activate, bind DNA and turn on genes.

c. **Phosphoinositol interconversion**. Cells alter the affinity of various compounds for their plasma and internal membranes by adding and subtracting phosphates to and from membrane -bound inositol. In this case, activation of the receptor causes the phosphoinositol to add a phosphate and block signals leading to apoptosis.

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. This receptor is specific for antibody-antigen complexes. In most systems the Fc receptors are connected to cytoplasmic pathways that up-regulate immune activity. Here, these pathways suppress further antibody synthesis*, Figure 6-12*.

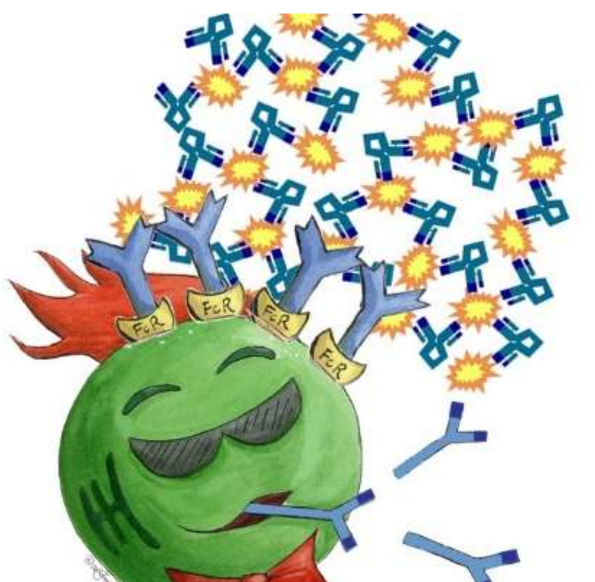


Figure 6-12 – Braking antibody production

Video clip 6-4

IV. Primary and Secondary Response: T cells

A. 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. 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.

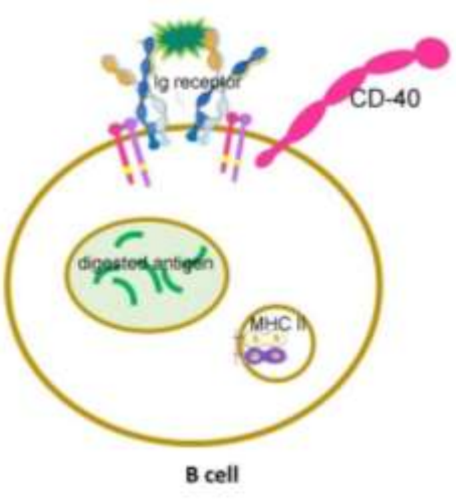
4. Of course the antigen received is different from the antigen presented. The presented antigen is a peptide derived from the overall molecule, figure 6-11a.

5. The cells attach, forming a conjugate or immune synapse.

6. This causes the T_H cell to produce CD40L or CD-154, which is a juxtacrine factor that turns around and signals the B cell through CD40 receptor, Figure 6-11b.

7. This leads to the production of the second signal, activating the interlocking up-regulatory pathways of the B cell and they begin proliferating and differentiating.

8. In the reciprocal part of the conversation between B and T cell, B7 plays a role comparable to CD-154 and CD-28 comparable to CD-40R



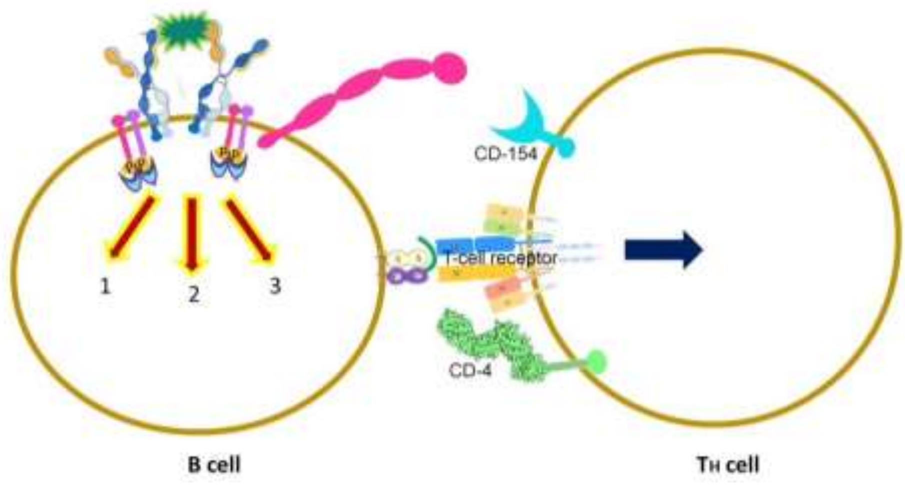


Figure 6-13a – B cell acquires antigen

Figure 6-13b B and T cells synapse

B. Primary Versus Secondary Response

1. The Primary Response

   a. naïve lymphocytes
   b. 4 to 7 day lag time
   c. produces antibody secreting plasma cells
   d. initial antibodies mostly IgM; IgG toward the end
   e. As infection is routed, T cells signal development of memory cells

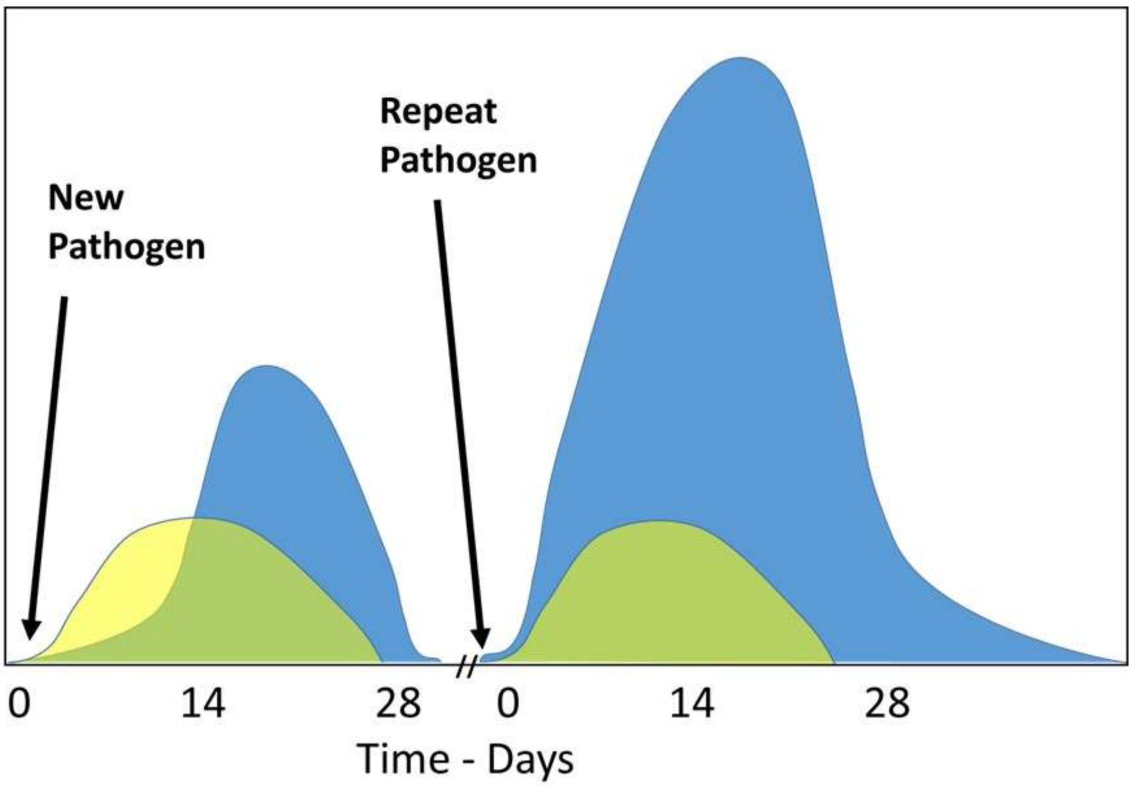


Figure 6-14- Primary and Secondary Responses

1. The Secondary Response: The Immune System is prepared.

   b. One to 3 day lag time
   c. Produced primarily by memory cells
   d. They have already been through affinity maturation, so they're better at binding antigen
   e. much higher proportion of IgG and other isotypes
   f. more antibody secreted, and over a longer time

Video clip 6-5

V. Anatomical and Histological Context

A. Lymph Nodes

1. Lymph drains from tissues and passes through these.

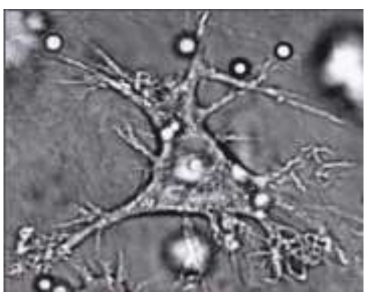
2. Antigen enters. It can be

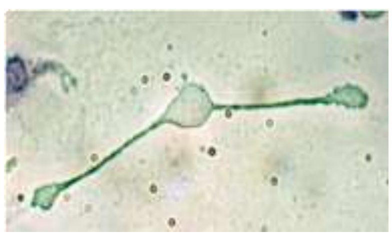
    a. "free" - particle from pathogen, or the whole bacteria or viruses themselves
    b. proteins or other antigens from the pathogens complexed with antibodies
    c. carried in by presenting cell (dendritic or macrophages) that have picked it up elsewhere

B. Cells

1. Free and antibody-bound antigen in the plasma is likely to be picked up by

    a. interdigitating dendritic cells *(Figure 6.15)*
    b. macrophages *(Figure 6.16)*
    c. follicular dendritic cells *(Figure 6.17)*





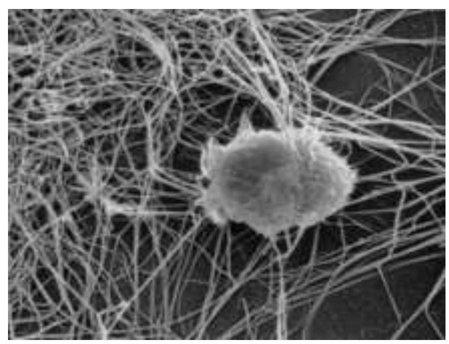


Figure 6.15: Interdigitating Dendritic Cell

Figure 6.16: Macrophage

Figure 6.17: Follicular Dendritic Cell

2. Naïve lymphocytes from the bone marrow enter via the lymph.

3. 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 6.18)*

4. First the macrophages and the dendritic cells activate the T_H cells.

5. 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).

6. The B cell begins to divide, producing a clonal cluster (focus) at the boundary between the paracortex and cortex.

7. A few activated B and T_H cells migrate together from one of these foci to a primary follicle in the cortex.

8. The follicle becomes a secondary follicle, one with a germinal center where B, T_H, and follicular dendritic cells interact.

9. A reminder about follicular dendritic cells: these are **NOT** regular dendritic cells. They capture antigen-antibody complexes in beaded structures (iccosomes) and present them to the B cells.

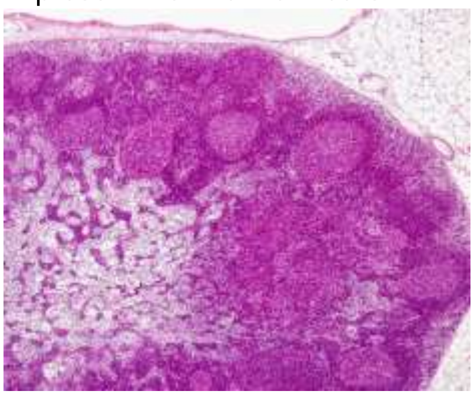


Figure 6.18: Lymph Node

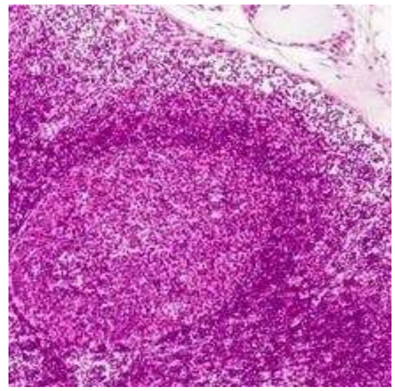


Figure 6.19: Germinal Center

Video clip 6-6

VI. Affinity maturation

A. 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 6.19)*

1. Activated B cells (centroblasts) proliferate and move to one edge or the follicle, forming a dark zone. At this stage the centroblasts:

    a. enlarge and begun to divide rapidly

    b. begin somatic hypermutation- mutating the regions in the heavy and light chain genes that code for the variable loops. *(Figure 6.20)*

    c. stop displaying the membrane Ig (recycles the original via membrane turnover)

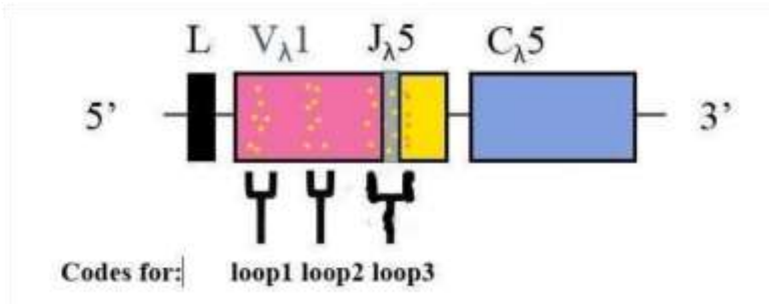


Figure 6.20: Somatic Hypermutation CDR Selection

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.

B. Process and 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.

i. 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.

ii. 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 6.18)*

iii. The mutated centrocyte now displays the new antibody at its surface. *(Figure 6.19)*

iv. As with most random mutations, most of the progeny centroblast cells produced by this will bind antigen less effectively than the original cell.

v. A few however, will bind the antigen more effectively. Cells with improved receptors divide more rapidly than cells with less effective receptors.

Figure 6.21: CDR

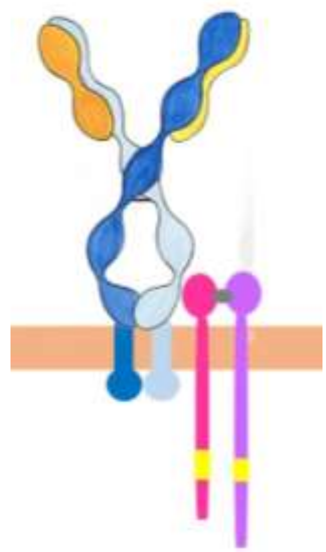


Figure 6.22: displayed Ig receptor

C. Signals

a. 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.

b. However, the beads are essentially a scarce resource, and the centrocytes have to compete for them.

c. 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.

d. 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.

Video clip 6-7

VII. Final Decisions

A. Class Switching – directed by T_H cells

1. 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 6.23)*

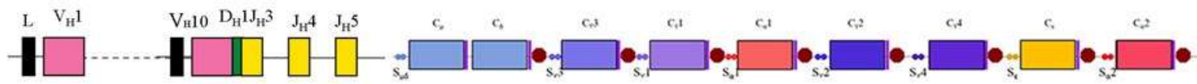


Figure 6.23: Heavy Chain Gene

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

B. 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 6.24)*

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.

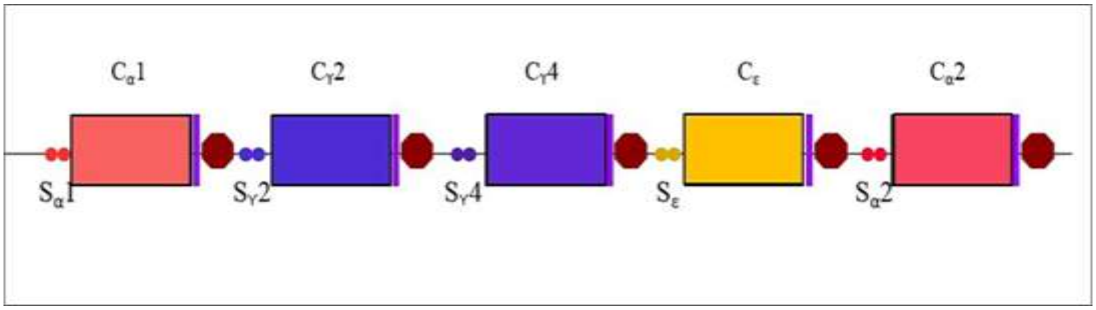


Figure 6.24: Class Switching -- Heavy Chains

Video clip 6-8

VIII. 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:

Table 6.1 B-lineage Development Summary

Name	Location	Surface receptor molecule	Other surface signaling molecules	Activity
Pro B cell	Bone marrow	Igα/Igβ coreceptor	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 coreceptor	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 at paracortex	mIgM and mIgD and co-receptor		Begins clonal expansion
Activated B cell (2)	Cortex, primary follicle, which then becomes 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

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 of 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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