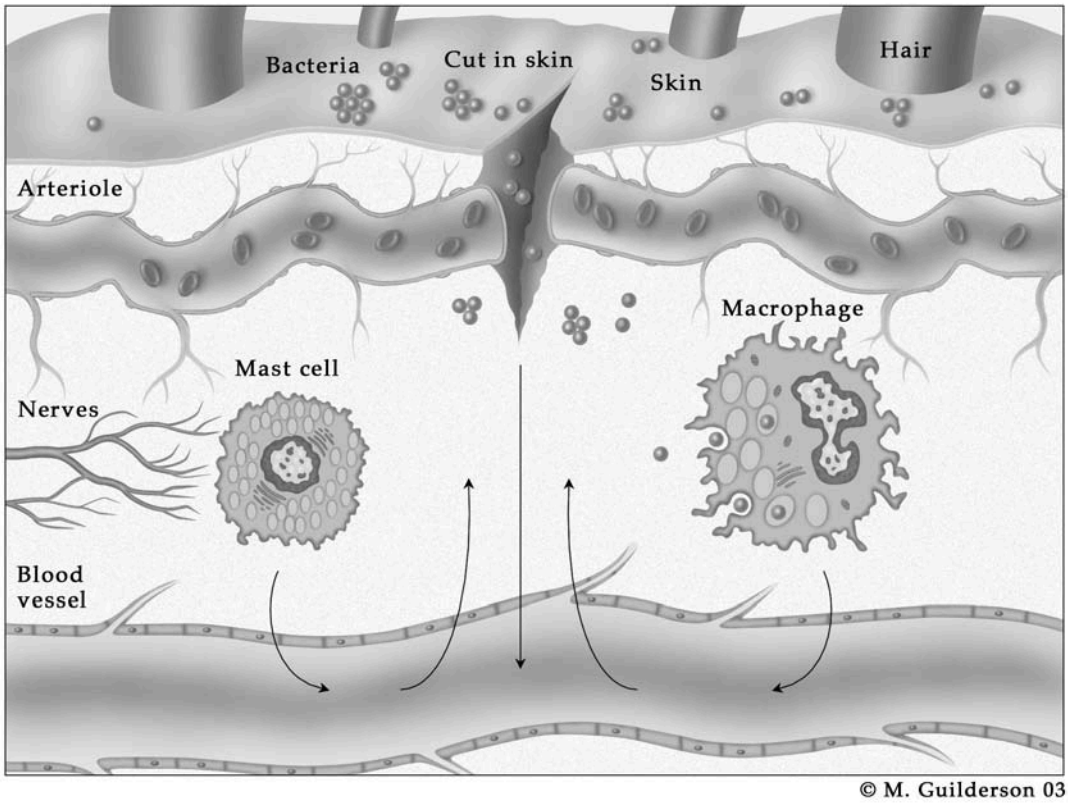
Immunology for 1st Year Medical Students©



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The Two Page Version:

The immune system depends on the complex interaction between many diverse elements. It is important to remember that, although it is involved in a number of disease processes, the primary function of the immune system is to combat infectious agents. Because of this, a good understanding of how the immune system works can be gained by examining how it combats infectious agents such as bacteria, viruses, fungi and parasites. Because these agents cause disease (pathology) they are commonly referred to as "pathogens".

There are four major components of the immune system involved in fighting off pathogens:

1. Antibodies and the cells that make them.
2. Complement.
3. T cells.
4. Non-specific effector cells.

Each of these components will be dealt with in detail in this textbook but their important functions are very briefly summarized below.

Antibodies specifically bind to pathogens to bring them to the attention of other parts of the immune system (Complement and phagocytic cells). **B cells** are the only cells that make antibodies. Antibodies are also referred to as "immunoglobulins".

Complement refers to a cascade of small proteins that bind to pathogens and poke holes in their outer surface causing death (Appendix 2). Complement proteins can bind to some pathogens directly but the activity of Complement is much amplified by the presence of antibody bound to the pathogen. Byproducts of this cascade help initiate inflammation.

T cells are referred to as CD4+ or CD8+ T cells based on their surface protein markers. **CD4+ T cells** are the "orchestra leaders" of the complex immune response. They are generally referred to as "helper" T cells. They help B cells become antibody producing cells and help other cells perform effector functions. They rarely kill pathogens directly. **CD8+ T cells** are involved in direct killing of pathogen infected cells (not pathogens themselves) and are often called "killer T cells" or "cytotoxic T lymphocytes" (CTL).

Non-specific effector cells have a variety of functions. Some, like **macrophages** and **neutrophils,** bind to pathogens then ingest and kill them.

This process works much better if antibody is bound to the pathogen. Other effector cells, like **NK cells,** don't kill pathogens but kill self cells that have been infected with pathogens (such as viruses). Still others, such as **mast cells**, secrete factors that create inflammation in the area of the pathogen to allow rapid access of other immune components to the site.

All of these processes are involved in various aspects of the immune system in which pathogens don't appear, such as transplant rejection, autoimmune disease and asthma. But in these cases the central system is the same as the one involved in the response to pathogens.

The communications network of the immune system is a network of **cytokines** which are soluble protein factors released by one cell that have effects on others cells in the local area or systemically. Some small soluble protein molecules have the effect of attracting cells to a particular site and they are called **chemokines** (because they are **chemo**tactic).

Origin of the Cells of the Immune System

All the cells of the immune system are derived from pluripotent stem cells in the bone marrow by a process called hematopoeisis. This topic will be covered extensively in the second year hematology course.

Under the influence of cytokines, the pluripotent stem cell may become a lymphoid stem cell or a myeloid stem cell. The lymphoid stem cell develops into B lymphocytes (B cells), T lymphocytes (T cells) or natural killer cells (NK cells). B cells mature in the bone marrow (hence the name B cell) while T cells initially develop in the bone marrow but leave the bone marrow as immature cells and mature in the Thymus (hence the name T cell). Natural Killer (NK) cells are lymphocytes that act in a similar manner to cytotoxic (killer) T cells. These cells, however, are not T cells. The lineage of the NK cell is not well understood.

The myeloid stem cell develops into platelets, red blood cells or the granulocyte-monocyte line. Monocytic cells include monocytes and macrophages. The granulocytes include neutrophils, eosinophils, mast cells and basophils. Monocytes circulate in the blood but become macrophages when they enter the tissues.

The development of these cells from a stem cell involves proteins secreted by local cells called “growth factors”. These factors belong to the “cytokine” family (see Chapter 6). Growth factors cause the development of progenitor cells in each line from the pluripotent stem cells. A progenitor cell is a committed cell, meaning that it is committed to that line of cell growth. (An eosinophil progenitor must become an eosinophil; it can't become a neutrophil, for example.) In adults, the hematopoeitic cells grow and mature because of the active support of bone marrow stromal cells (fibroblast-like cells).

Antigens

Antigens are molecules that can be recognized by the immune response as foreign. The term literally means molecules that will **gen**erate an **anti**body response. These molecules are often derived from infectious agents. The pathogen itself is not an antigen; the proteins and carbohydrates that make up the pathogen are antigens. A given pathogen could have thousands of molecules that would be recognized as foreign antigens by the immune system. These antigen molecules are recognized by the immune system by B cells and T cells because they have specific receptors on their surface.

Antigens are not restricted to infectious agents. Tumors often contain modified proteins or proteins not normally expressed which are seen by the immune system as antigens. In autoimmune disease the immune system recognizes normal molecules on the surface of cells as being antigens and these cells are attacked.

OK, that was more than two pages – so sue me!

Chapter 1: Innate Immunity

When I was 8 years old I was playing with a jackknife and I cut myself. As an 8 year old, you can imagine that the knife was far from sterile. The stage was set for a bacterial infection. This would be an infection with extracellular bacteria such as *Staphylococcus* which lives happily on the surface of the skin.

Although the body will develop an excellent immune response directed specifically against the *Staphylococcus*, that will take some time (days). The immune system is no slouch, however, and gets to work immediately, using "innate" responses to hold the fort. These responses don't depend on specific recognition of a particular infectious agent but depend on the recognition of classes of pathogens and situations where infection is likely to occur (such as a cut in the skin). The cells involved respond to general signals elaborated by the trauma of the cut and factors present on the surface, or released by, the extracellular pathogens.

1.1 Nervous System

The skin is well served by nervous tissue. A cut will be rapidly perceived and action will be taken. The most obvious action is verbalization (Ouch!) and removal of the hand from the general area of offense. Not so easily observed are the nervous system triggers of the innate immune system. One of the most obvious is the elaboration of neuropeptides that bind to local cells, particularly mast cells, and cause them to secrete factors (such as histamine) that initiate the innate response by, for example, inducing increased vascular permeability and vasodilation (see below for more on this).

1.2 Clotting Factors

Of course, the cut in the skin will cause bleeding and, under normal conditions, such minor cuts clot very quickly due to the complex clotting cascade. The clotting cascade involving molecules like fibrin and kinins, as well as related molecules such as platelet activating factor, is also able to activate local small blood vessels. Clotting factors also activate the cleavage of Complement molecules into reactive subunits that have effects on local innate cells and on the vasculature. The trauma and the clotting cascade are a finely tuned process such that blood flow will be initially restricted to prevent blood loss and enhance clotting, but then blood flow will be increased to allow elements of the innate response to gain access to the site of bacterial entry.

1.3 Recognition Receptors on Innate Cells

Local mast cells and tissue macrophages were originally thought to be totally blind to the nature of the infectious pathogen. Recognition of pathogens was the hallmark of the more advanced "adaptive" immune response, not the innate response. However, we now know that these cells can recognize certain types of molecules by their molecular "pattern". This is much like being able to recognize a car but not know whether it is a BMW or a Toyota. In some circumstances fine recognition of the make and model of the car is important (such as if you were buying the car) but in others it is not (such as if the car is about to run you over). Innate cells deal with the running over scenario so they need to know it's a car but not what make or model. They do this with pattern recognition receptors (PRR), the most important of which are called Toll-like receptors (TLR). There are a large variety of TLR and they recognize different patterns. Some of these TLR are specific enough to be able to recognize SUVs from sedans, but none can recognize a specific antigen like the T cell receptors or B cell receptors of the adaptive immune system can.

Both mast cells and macrophages have TLR that can recognize bacterial products like bacterial lipopolysaccharide (LPS) and DNA motifs that are relatively specific to certain types of pathogens (CpG motifs). These factors are often referred to a pathogen associated molecular patterns (PAMPS) Some TLR recognize molecules that are released in response to tissues damage. These tissue damage molecules are often referred to as damage associated molecular patterns (DAMPS). Mast cells and macrophages also have receptors for reactive subunits of Complement (C3 receptors) so that they can be activated by the Complement cleavage that occurred due to the clotting process.

1.4 Consequences of Activating Local Innate Cells

The activation of mast cells will cause the secretion of histamine and resulting increased vascular permeability and vasodilatation. This causes more blood to flow to the area of the cut and also causes plasma to flow out of the blood into the tissue. This plasma contains antibodies and Complement which can begin to deal with the bacterial infection (more about these later). The vasodilation (more blood in the area) leads to reddening and heat. The increased vascular permeability causes swelling and pain. These are the four characteristics of acute inflammation (heat, reddening, swelling and pain).

The activation of macrophages (by engagement of TLR, by C receptor binding and by actively phagocytosing the bacteria) leads to the elaboration of cytokines and the attraction of other leukocytes to the site of infection.

1.5 Influx of Plasma

The increased vascular permeability causes an influx of plasma into the site of inflammation. The two primary components of plasma of interest at this point are Complement and antibodies. Complement (see below) is a term that refers to a group of protein molecules that act together to fight infection. Complement can be activated directly by certain pathogens and is capable of killing these pathogens by poking holes in their membranes. Complement is also activated if antibody binds to the pathogen and then to Complement.

Antibody will only bind to the pathogen if you have seen this particular bug before. Unlike TLR, antibody is highly specific and can differentiate between various strains of the same species of bacteria. If you have seen this bug before, antibody will bind by its Fab region, leaving its Fc region open to "fix" (activate) Complement and to attract macrophages to take up the pathogen and kill it (see 4 functions of antibody in Section 2).

1.6 Influx of Cells

When macrophages are activated by Complement, TLR or other factors they become highly activated. They increase their ability to take up and kill pathogens and they secrete a variety of cytokines and chemokines. The most important function of these cytokines and chemokines is to attract more immune cells into the site. In the case of an extracellular bacterial infection the cells most desired are the neutrophils because they are excellent at locating, ingesting and killing bacteria.

The influx of cells is facilitated by the production of chemotactic molecules (chemokines) by the macrophages. These cells also produce cytokines that influence the expression of "adhesion molecules" on the surface of the vascular endothelial cells. The expression of adhesion molecules essentially grabs leukocytes from the circulation and stops them in their tracks. At this point they can sense the chemokines and they migrate through the endothelium and into the site of inflammation. The important cytokines are TNF and IL-1 and the important chemokines are IL-8 and C3a.

Neutrophils, T cells and more macrophages will come flooding into the site of inflammation.

1.7 Link to Adaptive Immunity – The Dendritic Cell

When the innate inflammatory response is in full swing, neutrophils and macrophages are taking up and killing bacteria effectively. When they have killed the bacteria they release the effluent by exocytosis ("putting out the garbage"). These digested bacterial parts can be taken up by local skin dendritic cells that have been stimulated to be phagocytic by the inflammatory response raging all around them. Once they take up material they completely change their outlook on life, leave the skin and travel to the local draining lymph node. There they present antigen to T cells and provide antigen for B cell uptake. The dendritic cell is the link between the innate inflammatory response in the skin and the developing specific, adaptive immune response in the lymph node. The subsequent adaptive response will be antigen specific and thus highly specific for the invading pathogen.

Chapter 2: Antibodies and the Cells That Make Them

Antibodies, also called “immunoglobulins”, are one of two important protein molecules of the immune system that engage in the recognition of pathogens or other foreign material. This process is called “antigen recognition” and is a pivotal process in the immune response (see Appendix 3 – Normal Antibody Levels). The other antigen recognition molecule is found on the T cell and is called the T cell receptor (TcR). Antibodies act as recognition units on the surface of B cells (where they are called the B cell receptor) but usually, when we think of antibodies, we are thinking of the antigen specific soluble proteins secreted into the blood and tissue by antibody producing cells. Soluble or secreted antibody is structurally slightly different from the antibody on the surface of B cells but the antigen recognition sites are the same.

There are five different classes (isotypes) of immunoglobulins: IgD, IgA, IgM, IgE and IgG. There are subclasses of some of the five classes and they vary among species. Each of the antibody classes will be considered separately in this book but let us first consider the basic function of antibodies. Later we will look at the structure and how the genes for these proteins make the amazing diversity of these proteins possible.

Antibodies recognize antigen. If you are unclear what an antigen is, then take a minute and read about it. The ability of antibodies to recognize specific antigen is an important characteristic. Antigen recognition and binding allows antibodies to perform four important effector functions.

1. Opsonization for phagocytosis
2. Activating Complement
3. Neutralizing toxins
4. Blocking attachment of pathogens to cells or tissue

1. Opsonization: This is the process by which things like bacteria, viruses and small parasites are 'tagged' for destruction by macrophages and neutrophils. Antibodies are the 'tags' (or opsonins) because the antigen binding (Fab) area of the antibody binds to an antigen on the surface of the organism. The other end of the antibody (Fc) binds to receptors on phagocytic cells. The antibody signals the phagocytic cell to engulf and destroy the organism. If the organism is too big to engulf (like a parasitic worm for example) the phagocytic cells will release destructive enzymes and other factors onto the surface of the organism (sometimes called “frustrated phagocytosis). IgG is the most important antibody for this because it is very abundant and fits nicely into the Fc receptor

on the surface of the phagocytic cells. IgM and IgA are very poor at this because they are secreted as multimers.

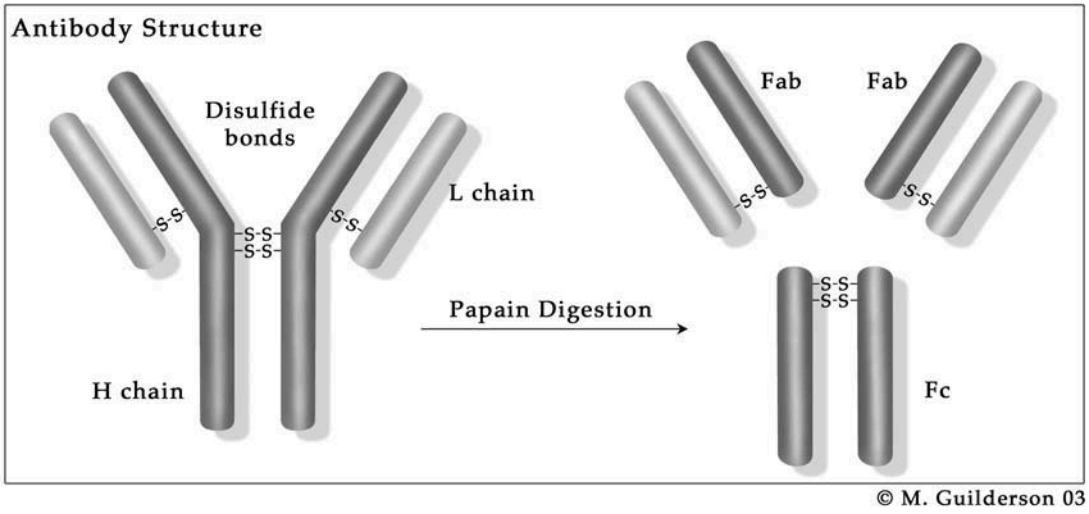
2. **Activating Complement**: This refers to the process by which antibody binds to an antigen on the surface of a pathogen by its Fab section. This leaves the Fc section free to bind the first component of Complement (C1). This binding of C1 (often called "fixing Complement") activates an enzyme cascade which results in lysis of the organism by the membrane attack complex (the MAC attack, see section on Complement). IgM and IgG are very good at this.

3. **Neutralization of Toxins:** Some pathogens secrete dangerous toxins (such as tetanus toxin). In the circulation, tissues and secretions (such as mucus), antibody will bind to these toxins and neutralize their activity. Antibodies also form easily recognized antibody-toxin complexes which are removed from the body by phagocytosis.

4. **Blocking Attachment:** Antibodies can immobilize and agglutinate infectious agents by binding to their surface antigens and preventing them from attaching to tissues like the intestinal mucosa or from penetrating cells (in the case of viruses).

2.1 Antibody Structure

Antibody is made up of four polypeptides or "chains". Actually, each antibody



molecule is composed of two sets of two chains. The general structure is shown below:

The heavy chain is the largest (and thus the heaviest). There are two identical heavy chains and two identical light chains in each antibody. The heavy and light chains are folded into domains and are held together by disulphide bonds. In humans there are two different kinds of light chain (kappa and lambda) and five different kinds of heavy chains (μ, δ, γ, α, ε; more about these below).

Once the heavy and light chains are assembled, however, the areas or “regions” of the antibody become more important than the chains.

The two important regions of the antibody are:

. the Fab region
. the Fc region

The antigen binding region (Fab) is the recognition region of the antibody. It has great diversity so that antibodies can be made to recognize any and every antigen the body encounters. The Fc region is responsible for the effector function of the antibody (such as opsonization or Complement activation) and also defines the class of antibody (IgG versus IgA for example).

2.2.1 Fab Region of Antibodies

The Fab region of antibody is involved in antigen binding. This region is composed of the entire light chain (either kappa or lambda) and a part of the heavy chain. Often this region is called $F(ab)_2$ because the two Fab regions of the antibody (like the two arms of a human) are joined together in the middle by bonds.

The N-terminal end of the Fab region consists of a "hypervariable" area of the heavy chain and a hypervariable region of the light chain. This hypervariable region is the area encoded by the gene elements that undergo random gene rearrangement, thereby generating amino acids and thus structural diversity.

2.2.2 Fc Region of Antibodies

The Fc region of the antibody is limited in variability and is responsible for the biological activity of the antibody (as opposed to antigen binding). The Fc region is made up of only heavy chain elements. There is no light chain in the Fc region. The Fc region varies between antibody classes (and subclasses) but is identical within that class. The Fc region of an IgA molecule that reacts with an antigen on E. coli will be identical to the Fc region of an IgA molecule that reacts with an antigen on Salmonella but the Fab regions of these two antibodies will be different. However, the Fc region of an IgG molecule that reacts with a Salmonella antigen and an IgA molecule that reacts with the same antigen would be different. The C-terminal ends of the heavy chains form the Fc region. The Fc region plays an important role as a receptor binding portion and in binding Complement.

The Fc portion of antibodies will bind to Fc receptors in two different ways. For example, IgG binds first to a pathogen by its Fab portion before its Fc portion can bind to receptors on phagocytic cells (like macrophages) inducing phagocytosis. In contrast, the Fc portion of free floating IgE binds to Fc receptors (FcE) on mast cells before the antibody reacts with antigen.

The Fc portion of antibody also binds Complement and initiates the classical pathway of Complement activation which leads to lysis of the pathogen by the MAC attack.

2.3 Antibody Diversity

One question immunologists have struggled to answer is how antibodies can be made to recognize virtually any and every foreign antigen the body is confronted with. Extensive work in molecular genetics has revealed how this comes about. A brief discussion of the gene rearrangements responsible will be included here but a more complete discussion can be found in immunology texts.

Each antigen binding region (Fab) is made of a variable domain of a heavy chain and a variable domain of a light chain. It is these variable domains that create the diversity in antibodies.

Let us consider the heavy chain first. In the gene for the heavy chain, there are many segments. From the 5' end to the 3' end, the segments are called variable heavy (V_H), diversity heavy (D_H) and joining heavy (J_H), then the constant (C) regions.

In the human immunoglobulin gene locus there are 300-1000 different V_H segments in tandem, 13 different D_H's and 4 J_H's. During the development of young B cells they undergo a process called “immunoglobulin gene rearrangement”. In this process most of the gene segments in the immunoglobulin gene locus are randomly spliced out and deleted and the remaining segments are recombined so that one V_H segment is combined with one D_H segment and one J_H segment. The rest of the gene segments are discarded. Since there are so many different segments to choose from, many thousands of different combinations can be made. Each combination has a slightly different gene sequence.

In addition to the segment selection, the unique mechanism for splicing the segments together adds further diversity by randomly adding or removing nucleotides to the segments as they recombine. Even one nucleotide difference can have dramatic effects on the final antigen binding structure. Both of these processes result in many different amino acid sequences of the mature variable region protein, resulting in many unique conformations.

The same segment selection and recombination process takes place to create the variable region of the light chains except that there are no D segments in the light chains.

The result of all this random recombination is the ability to produce a tremendous spectrum of antibodies because many thousands of possible heavy chains can combine with thousands of possible light chains. This is reflected in the fact that millions of different B cells exist in our bodies at any one time and within that B cell population is the ability to produce antibodies able to recognize a huge diversity of antigens.

2.4 Antibody Isotypes (Classes)

IgM

This antibody class has the mu (μ) Heavy Chain. It can be found as "surface antibody" on the surface membrane of B cells or as a 5-subunit macromolecule secreted into the blood by activated B cells and plasma cells. Surface IgM (sIgM) is structurally different in the Fc region from secreted IgM because to be stably expressed on the surface of the B cell it must be tethered to the B cell surface membrane. Surface IgM binds directly as an integral membrane protein
so it has a “transmembrane domain” attached to the bottom of the Fc region. sIgM does not bind to an FcR like IgE or IgG does.

Secreted IgM is found as a "pentameric" molecule in blood at moderate levels (1-3g/l in adults). The five IgM subunits are held together by a polypeptide (Jchain, for joining). Because each IgM subunit has two antigen binding sites, this means that the IgM pentamer has 10 binding sites for antigen. Pentameric IgM binds to antigens on the surface of a pathogen like a spider. Because of its pentameric configuration, IgM is particularly good at activating Complement, via its Fc regions, and causing agglutination but it is very poor at opsonization. IgM is the first antibody to be produced in response to infection since it does not require "class switch" to another antibody class.

IgG

This antibody class has the gamma (γ) Heavy Chain. It is the most abundant class of antibody in the blood (serum concentration is 8-16 g/l !). There are four subclasses of IgG (with slightly differing _ chains). They are all monomeric and they usually have a very high affinity for antigen. Unlike IgM, IgG is able to rapidly leave the blood stream and enter tissues, especially at sites of inflammation. This is primarily because pentameric IgM is very large (about 900kDa) and IgG is much smaller (about 160kDa).

IgG is also the only class of antibody to be actively transported across the placental barrier. Therefore IgG provides the only antibody protection for newborns until their own immune system begins to produce antibodies in response to antigen.

The subclass of IgG produced is dependent on the cytokines present (especially IL-4 and IL-2) and each class has its own special activity. In general though, IgG is very good at activating Complement, and is the best antibody for opsonization using Fc receptors on phagocytes. This is because phagocytes have a large number of Fc gamma receptors (Fc_R) on their surface. IgG also
plays an important role in neutralizing toxins produced by pathogens in the blood and tissues.

IgA

This antibody class has the alpha (α) Heavy Chain. IgA is found in low levels in the blood (1.5-4 g/l) in both monomeric and dimeric forms but IgA is most abundant and most active in secretions at mucosal surfaces where it appears as a dimeric protein. The two IgA molecules are held together by J chain polypeptide (not the same as the J region of the antibody gene). To pass through epithelial surfaces, a secretory component is transiently attached to dimeric IgA. The dimeric IgA provides the primary defense at mucosal surfaces such as bronchioles, nasal mucosa, prostate, vagina, and intestine. IgA is also abundant in saliva, tears and breast milk, especially colostrum.

IgE

This antibody class has the epsilon (ε) Heavy Chain. The blood concentration of this antibody is normally very low as most IgE is tightly bound to its Fc epsilon receptors (Fc_R) on mast cells and basophils. The production of IgE is controlled by specific cytokines and this class of antibody is responsible for Type I hypersensitivity reactions (allergy, asthma and anaphylaxis). IgE is increased greatly in response to helminth (worm) parasite infection.

IgD

This type of antibody is found on the surface of most B lymphocytes just like sIgM. So far, the exact function of this antibody is unknown but it appears that it acts as an antigen receptor and that it is needed for B cell activation. A very small amount of IgD is secreted, and its functions as a secreted antibody are unclear.

2.5 The Cells that Make Antibody - B cells

2.5.1 B Cell Development

Pre B Cell to B Cell

For a B cell to be functional it needs to have a functional antibody on its surface. The stages in B cell development are concerned with the generation of that surface antibody and testing to make sure it works (it can activate the B cell signaling pathways).

B cells develop from stem cells in the bone marrow. At the youngest stages in the bone marrow, pre-B cells rearrange the gene for antibody heavy chain. If they successfully transcribe and translate the heavy chain protein they attach two heavy chain molecules together as a "dimer". Then they attach to this dimer two molecules of a light chain substitute called "surrogate light chain". At this stage this composite molecule looks a lot like normal antibody. They bring this composite molecule to the surface of the cell to be sure that it will insert properly into the membrane using the transmembrane region of the heavy chain. They also test to make sure that all the right membrane signal transduction elements associate with the heavy chain. This is essentially a test of the heavy chain. So much gene rearrangement goes on that you have to be sure that the end result has an Fc region that works and that the other end will bind to light chain.

Heavy chain passes the test if the composite molecule on the surface activates Bruton's tyrosine kinase (BTK). Activation of this signal transduction molecule will ensure that a cascade of events will occur that leads to continued life and happiness for the pre-B cell. Failure to activate BTK will lead to pre-B cell death. Remember that the B cell gets a second chance at this process because there are always two alleles (maternal and paternal) for every gene. It can try again on the second allele. If the first allele is successful the other one is shut down by a process called "allelic exclusion".

If the heavy chain looks OK then the pre-B cell will go ahead and rearrange the light chain gene (again it has two tries because it has two alleles.) Successful light chain gene rearrangement leads to the expression of fully developed IgM (and IgD) on the surface of the B cell. These surface antibodies have a transmembrane domain so they are attached directly into the membrane, not to Fc receptors. The expression of surface antibody marks the transition from "pre-B cell" to a mature naive B cell.

Naive B Cells

The new (naive) B cells then enter the circulation and travel through the blood to lymphoid organs. The B cells are called naive because they have not seen antigen yet.

It is important to know that each and every naive B cell (and there are many millions in the body at any one time) has a different antibody on its surface. Actually, each B cell has hundreds of thousands of antibodies on its surface but for each individual B cell all the surface antibodies are identical and all bind to the same site on the recognized antigen. In contrast, surface antibody varies from B cell to B cell. Thus the surface antibodies on all the millions of B cells are not identical. The B cell population achieves this "diversity" by random gene re-arrangement. This concept was discussed earlier in the antibody section but essentially it means that every B cell makes changes to the DNA that code for the heavy and light chains which form an immunoglobulin molecule. Since there are millions of such changes that could be made, and they are random changes, then the resulting antibody produced by each B cell is different.

In addition, once a B cell makes surface IgM it can then make a different class of antibody (class switch). However, class switch only involves a change in the Fc region of the antibody, not in the antigen binding (Fab) region. That means class switch changes the class (and function) of the antibody but not its antigen specificity.

In the lymph nodes, naive B cells may encounter an antigen recognized by their surface antibodies. As you might imagine, there are many (more than 99%) that circulate their entire life span without encountering antigen. These cells die within a few days. That's not nearly as exciting as what happens to B cells that do encounter antigen. The results are:

- B cell activation
- B and T cell interaction
- Differentiation into plasma cells
- Antibody secretion and class switch
- Production of B memory cells

see B cell Malignancies Clinical Notes

2.5.2 B Cell Activation

B cells can recognize antigen in its native form as soluble protein, unlike T cells which require antigen to be degraded and presented on the surface of an antigen presenting cell in the context of MHC (see section on T cell activation). The antigen recognition unit of the B cell is the surface immunoglobulin. Surface antibodies are antigen specific but, remember, the naive B cell has never been in contact with an antigen. The antigen-specificity arises from random gene rearrangements (antibody diversity) in the cells while in the bone marrow. These naive B cells leave the bone marrow and migrate to the spleen and lymph nodes. If the B cell comes in contact with the antigen, the B cell becomes activated.

After antigen recognition, the B cell ingests the whole protein antigen and processes it into peptides for presentation to activated T cells. The peptides are placed into the open grooves of specialized molecules called Class II MHC molecules (see Chapter 6) held within vesicles in the B cell. The MHC/peptide is then brought to the surface for presentation to T cells. In addition, the B cell increases the expression on its surface of molecules involved in B cell/T cell interaction – called co-stimulatory molecules.

2.5.3 T and B Cell Interaction

B cell clonal expansion and the production of plasma cells and memory cells require T cell help. This help is in the form of cytokines. The steps that must happen are:

1. **The B cell must present processed antigen plus MHC class II to an activated Th cell specific for that antigen.**

2. **The B and T cells must form a conjugate, and cytokines must be produced and released by T cells.**

3. **Cytokine induced signals in the B cell must stimulate proliferation and differentiation.**

Let us consider each step in more detail.

1. B cells present antigen plus MHC II.

After the antigen is recognized and binds to the surface antibody on the B cells, a process called receptor-mediated endocytosis takes place. The antibody and antigen are internalized by the B cell in an endocytic vesicle. This vesicle is merged with a lysosomal vesicle which contains digestive enzymes which break the antigen down into individual peptides. This endo-lysosome then merges with another vesicle which contains "empty" Class II MHC molecules attached to the vesicle membrane. When these vesicles merge the free peptides bind to the MHC in the empty pocket (groove) of the MHC molecule. Think of a hot dog slipping nicely into a hot dog bun – it is a very close analogy. The peptides are now bound to MHC II on the vesicle membrane and when this vesicle is brought to the cell surface the MHC/peptide complex appears on the B cell surface ready for presentation to activated CD4+ T helper (Th) cells.

There are a couple of important points to remember about antigen presentation by B cells. The first is that B cells do not phagocytose whole bacteria like macrophages do. They bring in soluble protein antigen by receptor-mediated endocytosis. The second, and very important point, is that B cells only successfully present antigen to activated (or memory) Th cells. They cannot activate naive Th cells like dendritic cells do.

2. Formation of the T and B cell conjugate. When an activated Th cell recognizes the B cell-displayed peptide plus MHC II, the Th cell and the B cell form a conjugate. This means that the T cell receptor (TcR) and accessory molecules bind to the antigen-MHC on the B cell.

The T-B contact sends signals to both the Th and B cell. In the Th cell, the receptor-binding stimulates the production of cytokines and receptors. This Th cell activation is covered in more detail in the section on T helper cell activation.

3. Proliferation of activated B cells.
The release of cytokines from the activated T cells provides an important second signal to the B cell. The contact signal and the cytokine signals are both required for the B cell to become activated to grow and divide or proliferate. The cytokine that is particularly important for proliferation is IL-4. Other cytokines are involved and some are necessary for the proliferating B cells to differentiate.

2.5.4 B Cell Differentiation into Plasma Cells

B cells are small lymphocytes and have a thin rim of cytoplasm around the nucleus. They look very much like T cells. Plasma cells, on the other hand, are much larger and contain abundant endoplasmic reticulum in the cytoplasm. Cells with abundant endoplasmic reticulum are involved in the production and secretion of large amounts of protein. In the case of the plasma cell this protein is antibody (immunoglobulin).

The differentiation of B cells into plasma cells occurs as the cell divides in the presence of cytokines. Some daughter cells continue to divide, many differentiate into plasma cells and a few differentiate into B memory cells. Different cytokines are known to stimulate B cells to become plasma cells secreting different classes of antibodies such as IgG, IgA or IgM etc. This change of antibody production from IgM to other classes is called "class switch".

2.6 Antibody Secretion

Plasma cells are the final stage of development of B cells which have recognized antigen and been stimulated by T cell-derived cytokines. These plasma cells reside in the spleen, lymph nodes, mucosal lymphoid tissue and bone marrow and secrete the antibodies found in the circulation. The first time antigen is seen, the antibody response is called the primary response. The level of antibodies in the blood takes a few days to increase and

the first antibodies detected are usually IgM. The primary response also includes some IgG. Persistence of the antigen, or re-exposure to the same antigen, amplifies the IgG response. The secondary response is much faster and more robust than the primary response and IgG predominates.

The antibody in blood to a given pathogen is a mixture of antibodies produced by a large variety of B cells that have responded to this pathogen. This pooled product from a variety of B cell clones is called "polyclonal antibody". In fact, since there are a number of different sites on any given antigen which can be recognized as foreign (called epitopes), even the same antigen will elicit a variety of B cells to produce different antibodies, all directed to the same antigen but a different epitope on that antigen. This is also called a polyclonal response. However, one activated B cell and its clonal progeny will all produce the same antibody directed to the same site (epitope) on the antigen. This is referred to as a "monoclonal" response and is the basis for the production of monoclonal antibodies for research and clinical therapies.

2.7 Class Switch

Class switch is a process whereby the B cell, as it develops into a plasma cell, can switch the immunoglobulin class (also called isotype) of antibody it produces while retaining the same antigen specificity.

The class of antibody (IgG or IgM etc.) is defined by the Fc portion of the heavy chain. Class switch involves rearrangement of this area of the immunoglobulin gene. Other rearrangements that take place in the Fab regions of the genes of all the naive B cells in a person define antibody diversity (breadth of antigen recognition). Class switch does not occur until after B cell activation and proliferation. It is under the control of cytokines such as IL-4 and IFN-γ.

Since B cells initially express IgM they are initially using the constant (C) heavy chain gene for IgM (the mu gene). The immunoglobulin heavy chain gene locus has the constant region genes for mu (μ), delta (δ), gamma (γ), epsilon (ε) and alpha (α) in tandem. In class switch to IgG for example the μ gene and the δ gene are spliced out such that the portion of the gene that defines the variable region of the heavy chain (VDJ) is brought into apposition with the γ constant region gene. This results in IgG being produced.

2.8 B Memory Cells

The secondary response is dependent on a population of long-lived B memory cells. These cells are generated in lymphoid tissue after B cell activation and proliferation and reside in the bone marrow, the lymph nodes, the lymphoid tissue in mucosal sites and the spleen. They express high affinity surface immunoglobulins which enable them to be activated by lower levels of antigen than naive B cells.

2.9 Antigens and Antigen-Antibody Interaction

2.9.1 Antigens

An antigen is a substance capable of inducing a specific immune response. The term is derived from the generation of antibodies to such substances. Specific immune responses require recognition molecules like the T cell receptor on T cells or surface antibodies on B cells. These molecules recognize the antigen, or parts of it, and stimulate a response by the specific arm of the immune response (T or B cells).

Antigens are usually foreign proteins (or parts of them) that enter the body via an infection. Sometimes, however, the body's own proteins are treated like antigens by the immune system and the result is autoimmune disease.

It is important to recognize that bacteria or viruses are not themselves antigens but they contain antigens both on their surface and inside them. Such antigens can be isolated and used to safely vaccinate against infection by the whole organism (see Vaccine section). It is also important to recognize that the T cell receptor on T cells and the B cell receptor (surface antibody) on B cells recognize different forms of antigen. T cells recognize only small peptides derived from a protein antigen by digestion. B cells generally recognize antigen motifs caused by the tertiary folding of whole soluble protein.

Each part of the antigen that is recognized by either an antibody or a T cell receptor is known as an epitope. Depending on the size of the protein or polysaccharide, there may be hundreds of B cell epitopes (recognized by different antibodies) or T cell epitopes (presented by antigen presenting cells to different T cells) in the same molecule. This actually helps the body have a better response to the antigen as many T and B cells can be activated to respond to one antigen.

2.9.2 Antigen-Antibody Interaction

The antibodies on the surface of B cells and the soluble antibodies in the blood and tissues recognize antigens in the native form. This means that antibodies can recognize antigen on the surface of bacteria or viruses as well as antigen free-floating in the tissues (for example bacterial toxins). For example, an HIV-infected person will develop a vigorous antibody response to the gp120 glycoprotein on the surface of the HIV virus. Antibodies of this type help prevent viral spread by blocking attachment of viruses to their target cells and are often called "neutralizing" antibodies.

In addition to interacting with antigen on the surface of pathogens, antibodies can also interact with free antigen in the blood or tissues. This antigen is usually released by the pathogen or the result of pathogen lysis by the other immune components. Antibody binds to this free antigen and creates antigen-antibody complexes (immune complexes) of various sizes. Most immune complexes are taken out of circulation in the liver by phagocytic cells but some can be deposited in tissues and initiate inflammatory responses which can lead to significant tissue pathology and chronic inflammatory conditions (discussed in Chapters 8 and 9).

See Clinical Note: B Cell Malignancies (Appendix 6)

Chapter 3: Complement

3.1 Complement Activity

Complement activation is a cascade of events which resembles in some ways the coagulation pathway (see Appendix 2). In fact, coagulation often results in the Complement cascade being activated. The Complement cascade consists of a series of protein proenzymes, present in the blood, that are converted to active enzymes by interaction with pathogens and with each other. It's a bit confusing and often hard to remember, but still important because the Complement cascade has a number of very important roles in combating infection and in inflammatory disease.

There are two major ways of activating the Complement cascade, by the "classical pathway" or the "alternate" pathway. The initial components of the classical pathway (C1,C4,C2) are activated by the Fc regions of IgG or IgM antibody, but only after antibody has bound to antigen (usually to a pathogen). The initial components of the alternate pathway (Factor B, D and P) are directly activated by certain pathogens. Either pathway can be partly activated by blood coagulation.

There are four major outcomes of activating this cascade:

1. local vasodilation and increase in vascular permeability
2. attraction of immune cells, especially phagocytes (chemotaxis)
3. opsonization (or tagging) of foreign organisms for phagocytosis
4. destruction of invading organisms by the membrane attack complex (MAC attack)

Each of these outcomes will be discussed in more detail below.

Although all the molecules in the cascade are important, the third component, called C3 protein, stands out as of special interest because both the classical and alternate pathways converge on C3. It is an enzyme that is split into two fragments (C3a and C3b) by components of either the classical pathway (C4b, C2a) or the alternative pathway (factor Bb, C3b, and P). C3a is primarily a very powerful activator of mast cells to release mediators which cause local vascular permeability and vasodilation. C3a is also a chemotactic factor for phagocytic immune cells. C3b, on the other hand, binds directly to the surface of foreign organisms (or immune complexes) and acts to 'tag' these for destruction by phagocytic cells like macrophages and neutrophils which have specific receptors for C3b. C3b also reacts directly with the other components of the Complement cascade (C5-9) to produce the MAC attack.

Much more information about the Complement pathway is available in textbooks but if you understand how Complement is triggered by antibodies or invading organisms, and that the end result of the pathway becoming activated is:

1. vasodilation and increased vascular permeability
2. attraction of immune cells to the site
3. opsonization for phagocytosis
4. interacting with other components of Complement to initiate the formation of the MAC attack

then that is just about all you really need to know about Complement.

3.2 Complement-Induced Vasodilation and Increased Vascular Permeability

The Complement components responsible for vasodilation, and vascular permeability, are the "a" subunits of C3, C4 and C5 (C3a, C4a and C5a). These three proteins are called anaphylatoxins. There are receptors for these proteins on the surface of mast cells and basophils. Binding of the anaphylatoxins to their receptors induces the cell to degranulate, releasing mediators like histamine. Such mediators induce smooth-muscle dilation on the arteriolar side in the vasculature making the blood vessels wider and allowing more blood to flow to the capillaries in the area. They also result in an increase in the permeability of the endothelial lining of the blood vessels. Increased vascular permeability allows fluid as well as macromolecules like IgG and Complement to flow out of the blood plasma into the tissue. Vasodilation results in redness and increased permeability results in swelling (edema).

The advantage of these functions of the Complement system is to increase blood flow to the site of infection and allow antibodies, more Complement and immune cells to enter the tissues to "scale up" the attack.

3.3 Chemotaxis

Chemotaxis is the process whereby an immune cell is attracted to, and moves toward, a soluble factor. Usually the cell being attracted is a phagocyte (like a neutrophil). One of the most potent chemotactic agents is C5a. C5a is formed when the Complement component C5 is cleaved to form C5a and C5b. Other substances that have chemotactic ability are secreted by immune cells and released during inflammatory responses. A number of these are small protein molecules called “chemokines”. Chemotaxis of phagocytic cells is important because it "recruits" the cells to the tissue where they are needed to ingest the invading organism or antigens or debris from the inflammation. It is important to remember that immune cells do not “leak out” of the blood because of increased vascular permeability like plasma proteins and water do. They must be attracted to the site by chemotaxis and penetrate through the endothelial lining of the vasculature in an active process of “transmigration”. After they transmigrate they essentially “crawl” through the extravascular tissue to the site of the infection or inflammation.

3.4 Opsonization

Opsonization is the process where particles such as microorganisms become coated with molecules which bind to specific receptors on phagocytes. IgG and Complement proteins like C3b can opsonize and are therefore referred to as "opsonins".

The Complement fragment C3b nonspecifically binds to foreign organisms or immune complexes during Complement activation. Since phagocytes have receptors for C3b on their surface (CR3) the binding of C3b to a microorganism or an immune complex tags it for ingestion and degradation by the phagocytes

3.5 Membrane Attack Complex -MAC

Complement activation by either the classical or alternative pathway will result in the formation of the membrane attack complex or MAC. The last steps in the Complement cascade, after the activation after C3b, involve C5b, C6, C7 C8 and C9 which complex to form the MAC.

Briefly, C5 is broken down into C5a and C5b after binding to C5 convertase, an enzyme formed by other fragmented Complement proteins. C5a diffuses away and has anaphylatoxin and chemotactic activity (discussed above). The C5b fragment binds directly to the target organism and becomes the binding site for other Complement components. C5b will quickly degrade

unless C6 binds to it creating C5b6, to which C7 will then bind. The addition of C7 changes the conformation of the proteins so that they are able to insert into lipid membranes. Next, C8 and finally C9 bind to the complex creating the MAC. C5b678 will bind up to 16 molecules of C9.

The MAC looks like a tube with a pore in the centre (like a very short straw) Because of its structure and hydrophobic nature, the MAC inserts into the membrane of the organism or cell and allows ions, water and other small molecules to freely pass through the pore (the MAC attack). As a result, the organism will not be able to maintain osmotic integrity and will quickly die. Cells without a cell wall will often burst (lyse).

3.6 They Fight Back

On a closing note it is important to recognize that the immune response to pathogens is like a war and we don't always win easily. The pathogens have ways of avoiding our defenses. Some microbes evade destruction by having surfaces that interfere with opsonization by C3b or insertion of the MAC. Others inhibit Complement activation (they have proteins that bind IgGFc such as protein A and protein G). Still others cause Complement degradation by elaborating enzymes that degrade Complement components. Not surprisingly, such microbes are pathogenic (meaning they cause disease).

Chapter 4: T Cells

Preamble

It is very difficult to discuss T cell activation without knowledge of the presentation of antigen by protein molecules coded for by the genes in the major histocompatibility complex. These molecules, MHC Class I and MHC Class II are pivotal for T cell activation. As a result a short synopsis of these molecules precedes the discussion about T cells.

MHC stands for major histocompatibility complex. In humans the same proteins are often referred to as human leukocyte antigens or HLA. Since MHC is a more specific term (there are lots of antigens on human leukocytes, not all of them are MHC antigens) it will be used in this resource.

MHC-Class I

All cells in the body except red blood cells have MHC class I protein on the surface. Your finger cells, your liver cells, your B cells, your gut cells - they all have MHC class I on the surface. Everyone has MHC class I proteins but only identical twins will have identical MHC class I. The genes for the MHC proteins show considerable diversity between people. Class I MHC is a complex of the Class I protein and another protein called β2 microglobulin.

Since everyone has a different MHC, this allows your body to tell what cells belong to it and what cells are foreign. The function of MHC-I is to sample the internal contents of the cell and show them to the immune system.

MHC-Class II

Class II MHC is a complex of proteins only expressed by antigen presenting cells (APC). The function of these proteins is to present an antigen to T helper cells to activate an immune response which will provide both humoral (antibody) and cell mediated immunity.

The class II MHC consists of an alpha and a beta chain with a transmembrane segment to hold them on the surface of the cell. At the end farthest from the cell is a cleft where the processed antigen sits. The processed antigen consists of a small peptide of about 13-16 amino acids. MHC-II picks up the antigen that has been ingested (phagocytosed) by the APC.

4.1 T Cells

T cells are lymphocytes which develop in the thymus. T cells have antigen recognition molecules (T cell receptor, TcR) on the surface which are composed of two different polypeptide chains; alpha and beta chains. The alpha and beta chains are associated with a group of five linked proteins collectively referred to as CD3. A minor population of T cells has two different chains, a gamma chain and a delta chain. Little is known of the activities of these gamma-delta T cells. Like antibody, the TcR has both a variable and a constant region. As described for antibodies, the variable region of the TcR is created by gene rearrangement.

The “T cell receptor complex” includes the alpha and beta chain of the TcR and the associated CD3 proteins. The recognition of antigen-MHC by the TcR-CD3 complex does not require any other molecules. However, other proteins on T cells are important in the interaction leading to T cell activation. These "accessory " or “co-stimulatory” molecules on T cells bind to ligands (surface molecules which function as a lock and key binding pair) on antigen presenting cells to give the 'second signal' required for T cell activation.

The TcR will only recognize antigen as a peptide in the groove of MHC. This is because part of the TcR actually binds to part of the MHC molecule during recognition. The TcR will not bind to free antigen.

Recent evidence also suggests that some T cells can recognize antigen presented by a molecule called CD1. This molecule is similar in general shape to Class I MHC but is capable of presenting lipid and carbohydrate antigen to T cells. CD1 presentation is particularly important in the activation of gamma/delta T cells but there is evidence that alpha/beta T cells can also be activated in this manner.

4.2 T Cell Development

For a T cell to be functional it needs to have a functional T cell receptor on its surface. That T cell receptor must be able to recognize antigen as a peptide in the groove of self MHC. The T cell receptor must be appropriately linked to CD3 for proper signal transduction. Finally, you don’t want the T cell to be self-reactive. The development of the T cell receptor and the testing of the reaction of the receptor occur in the thymus.

Pre-T cells (like pre-B cells) arise from lymphoid stem cell precursors in the bone marrow. They leave the bone marrow and transit, via the blood stream, to the thymus where they develop and undergo “thymic education”. The first thing to happen is that the _ chain of the TcR undergoes gene rearrangement. Just like in surface antibody development in B cells this _ chain in T cells has
to be tested for functional activity before the _ chain is rearranged. The _ chain is brought to the surface with a surrogate (pseudo) _ chain and tested. If it links properly with the signal transduction components then the T cell goes on to rearrange _ chain genes and put a mature T cell receptor on its surface. At this stage the T cell is both CD4+ and CD8+ (called a double positive cell).

Putting a T cell receptor on the surface is only the first stage. T cells are very specific in that not only do they have to recognize specific antigen, they have to recognize it in a specific context, as a peptide in the groove of self MHC. Therefore the next step is to make sure the T cell can recognize self MHC. The T cells interact with MHC bearing cells in the thymus and if the TcR binds weakly to them the cell goes on to further development. If the T cell gives the MHC bearing cells the cold shoulder then it does not receive the life prolonging signals that come from this interaction and it dies. Most of the T cells in the thymus will fail this test and die.

If they pass this test they then have to be tested for self reactivity. If they interact with MHC bearing cells very strongly, indicating that the self peptide and the self MHC is their chosen antigen to respond to, then they will be killed.

If they pass both of these tests they will become mature double positive T cells. These cells will then lose either CD4 or CD8 to become naive CD4+ cells or CD8+ cells. The cells with a CD4 marker are called helper T cells (Th cells). The CD8 positive cells that develop are cytotoxic T cells (Tc cells). Th and Tc cells both have a TcR, but they perform very different functions in the immune system.

4.3 Th cell activation

T cell activation is, in general, similar for both Th and Tc cells but each are considered separately for clarity. Th cell activation is initiated by the interaction of TcR-CD3 complex with antigen-MHC class II molecules on the surface of an antigen presenting cell. This interaction initiates a cascade of biochemical events in the T cell that eventually results in growth and proliferation of the T cell. This occurs primarily through an increase in IL-2 secretion by the T cell and an increase in IL-2 receptors on the T cell surface. IL-2 is a potent T cell growth cytokine which, in T cell activation, acts in an autocrine

(self-stimulating) fashion to promote the growth, clonal proliferation and differentiation of the T cell recently stimulated by antigen. The T cell receptor is an antigen recognition molecule and therefore the T cell that best responds to the antigen presented is the one that gets turned on. All its progeny will recognize the same “T cell epitope” of the original antigen, since they will all have identical T cell receptors. Naive, bystander T cells have no IL-2 receptors and thus cannot be involved.

The activation takes place through the T cell receptor complex and is aided by the CD4 molecule on Th cells as well as other accessory molecules, such as CD45, CD28 and CD2. The APC also provides a co-stimulatory signal through molecules such as B7 and by the secretion of co-stimulatory cytokines (such as IL-1 or IL-12).

Activated Th cells then continue to become effector cells whose role includes B cell help and activation of effector cells such as macrophages.

4.4 Antigen Presenting Cells (APC)

Antigen presenting cells are a functionally defined group of cells which are able to take up antigens and present them to T lymphocytes in a recognizable form (in the groove of an MHC class II molecule). Although many cells can do this, the cells which are most efficient, the so-called "professional antigen presenting cells", are dendritic cells. These are professional cells because they are highly effective at producing the "second signal" required for naive T cell activation. The APC first internalizes the antigen (maybe in the form of a bacteria or bacterial product), processes it (breaks it down into antigenic peptides by digesting it with enzymes [lysosyme]) and then expresses the antigen fragment (peptide) on its surface in the groove of an MHC class II molecule. Now the antigen is in the form recognizable by T cells. Antigen presentation by APC is a required first step in Th cell activation. Presentation to naive T cells like this happens almost exclusively in the lymph nodes and spleen. This means the dendritic cells have to leave the site of infection and travel to the local draining node to accomplish this.

Dendritic cells are the only cells that can present antigen to naive T cells but macrophages and B cells can present antigen to activated or memory T cells. It is thought that antigen presentation to memory T cells by macrophages is very important the second time you see an infection since this can happen at the site
of infection.

4.5 T Cell Help -Th Cells

The generation of an effective immune response depends on the activation of Th cells. Both humoral and cell-mediated responses are initiated or amplified by Th cells. The importance of these CD4+ cells to a functioning immune system is clearly demonstrated by the impact of the HIV virus. This virus preferentially kills CD4+ T cells and the devastating immune deficiency syndrome resulting from this is the disease known as AIDS.

Mature B cells that have already seen antigen require contact with a T cell in order to become plasma cells or memory cells (T-B cell interaction). The T cells provide signals to the B cell through contact of the TcR complex with processed antigen presented on Class II MHC by the B cell. In addition, the activated T cell produces cytokines which stimulate B cell proliferation and the differentiation of these cells into antibody secreting plasma cells. Th cell cytokines are required for “class switch” so that isotypes other than IgM can be produced.

4.6 T Helper Cell Subsets

Our understanding of how and why T cells behave in an immune response has markedly increased with the discovery that there are at least two subsets of T helper cells. The two subsets look the same and have the same T cell markers and receptors. However, they secrete very different cytokines upon activation. In addition to other cytokines, the Th1 subset produces abundant IL-2 and IFN gamma. These cytokines are particularly important in cell-mediated immunity. The Th2 subset is very good at providing B cell help by secreting abundant IL
4. Because the various cytokines have different effects in an immune response, sometimes activation of one subset may be preferable to the other. For example, virus infection is best combated with cytotoxic T cells. This response is more likely to be stimulated by Th1 cytokines. Although there is evidence that these subsets are cross-regulatory (activation of one downregulates the other) it is likely that they work together in normal immune responses. To use the example above, viral infection is also marked by anti-viral antibody. There are, however, some instances where only Th1 immunity is useful, as in the case of tuberculosis infection, where few antibodies are made to the pathogen and they have no role in dealing with the infection.

4.7 CD8+ T Cytotoxic Cells

Cytotoxic T cells (Tc) are derived from a lymphocyte stem cell matured in the thymus. These cells are characterized by the presence of the CD8 protein on their surface, and an antigen-specific T cell receptor which recognizes antigens in the context of MHC class I.

The main role of the cytotoxic T cell, as the name suggests, is to kill other cells. The requirement for MHC class I on the target cell means that the Tc cell is very important in recognizing and destroying self-cells that have been altered or infected. For maximal killing potential the Tc cell must first become activated and mature into a cytotoxic T lymphocyte (CTL).

Activation of the resting Tc cell is a two step process. First, the TcR on the CD8+ cell must interact with processed antigen on MHC (class I) on the surface of an APC. Secondly, the CD8+ Tc cell must be stimulated by cytokines (IL-2 especially). In most cases the IL-2 is supplied by activated Th cells in the vicinity but in heavy viral infections, for example, the CD8+ cells produce enough IL-2 themselves for activation. Like resting Th cells, resting Tc do not express IL-2 receptors. Antigen stimulation increases the expression of IL-2 receptors on the cell surface. This ensures that only the cells recognizing the antigen will become activated and proliferate.

4.8 Cytotoxic T Lymphocyte Activity

Activated CD8+ T cells (CTL's) are very effective at destroying target cells, especially virus-infected cells and tumor cells. The killing happens in three steps:

1. Conjugate formation between the CTL and the target cell.
2. Membrane attack on the target cell.
3. Target cell death.

1. Conjugate formation

Through the interaction of the TcR-CD3 complex and processed antigen presented by MHC I, the target cell and the CTL form a conjugate (they adhere to each other). Tc cells will not form a stable conjugate with cells lacking MHC Class I or with MHC bearing cells presenting processed antigen they do not recognize.

2. Membrane attack

Immediately following the formation of the conjugate (within 2 minutes!), granules in the CTL start to move through the cytoplasm towards the zone of conjugation. The CTL changes shape and becomes flattened towards the target allowing an area of contact between the two cells. Contents of the granules are released from the CTL (exocytosis) and are responsible for damage to the target cell membrane. The granules release cytokines, enzymes and a molecule called perforin that is able to poke small holes (perforations) in the target cell membrane. This membrane damage does not kill the cell but allows killing molecules to be introduced into the cell.

3. Target cell death

Following perforin-mediated damage to the cell membrane the molecule Granzyme is transported into the target cell. This molecule activates a cell suicide pathway which involves a cascade of molecules called caspases. The result is to induce "**apoptosis**" or programmed cell death in the target cell. CTL also have a back-up plan. They express a molecule called Fas ligand (FasL) which binds to Fas on the target cell. The binding of FasL to Fas on the target cell also activates the caspase pathway and results in apoptosis. In experimental systems interruption of both of these killing pathways results in the nearly complete ablation of CTL-mediated killing of target cells.

Chapter 5: Effector Cells of the Immune System

5.1 Monocytes and Macrophages

Monocytes circulate in the blood after leaving the bone marrow. Monocytes usually circulate in the blood for only a day or so before they enter the tissue to mature into macrophages. Monocyte production and release from the bone marrow is increased during an immune response. Under normal conditions, monocytes enter the tissues as resident macrophages in various locations (such as the skin, lung, liver, spleen, bone marrow and peritoneal cavity). These fixed, resident macrophages play an important role in keeping the tissues clear of antigen and debris. More monocytes are rapidly recruited as needed to these and other sites.

When monocytes enter the tissues and become macrophages they undergo several changes. The cells enlarge, allowing greater phagocytosis and they increase the amount of digestive enzymes (lysosyme) in their intracellular vesicles (lysosomes) thus facilitating microbe degradation. In the tissues, macrophages live for months and are motile (using pseudopods to move like amoebae).

Macrophages are usually in the resting state unless activated during an immune response. Activation of these cells may happen in response to Th-derived cytokines (especially IFN_) or from contact with bacteria or bacterial products. Phagocytosis of pathogens also stimulates activation. The activated state is characterized by more efficient phagocytosis and killing of microbes.

There are three major roles that macrophages play in the immune response to pathogens. The first is their very important role in phagocytosis. In this role they recognize and remove unwanted particulate matter including products of inflammation and invading organisms, immune complexes, toxins and dying cells. The large number of macrophages in the spleen and liver (where they are called Kupffer cells) are particularly important for removal of bacteria from the bloodstream.

The second important role macrophages play is as antigen presenting cells (APC) during secondary immune responses. Although they are very poor at activating naive T cells they are very good at activating memory T cells. The great advantage of this is that circulating memory T cells which are rapidly drawn to the site of infection can be immediately activated by macrophages without antigen being transported to the local draining node for presentation to T cells. Their third role is cytokine secretion. After activation, these cells secrete important inflammatory cytokines such as IL-1, IL-6 and TNF-_. IL-1 and TNF act to recruit neutrophils and more monocytes from the circulation as well as having systemic effects (such as fever). In

chronic inflammation, macrophages act as scavengers and can become giant cells (via cell fusion) which help form granulomas.

5.2 Natural Killer Cells

These cells are sometimes called large granular lymphocytes (LGL's) because they are large, granular and lymphocytes (immunologists are so imaginative!). NK cells have some surface markers in common with T cells, and they are also functionally similar to cytotoxic T lymphocytes (CTL). Like CTL, NK cells are particularly important in the killing of cellular targets (such as tumor cells or virus-infected cells). Unlike CTL, however, the killing by NK cells is not antigen specific, they do not need to recognize specific antigen presented by MHC on the target cell. In fact, it is the very presence or absence of Class I MHC that appears to be involved in NK cell activation. It is thought that many tumor cells are too busy proliferating to bother about expressing the normal surface molecules at normal levels. The lack of normal levels of Class I MHC on the surface of tumor cells is sufficient to activate NK cells to kill them.

NK cells do not have a T cell receptor and are not T cells but they kill target cells in the same manner as CTL kill targets (see above). However, they also produce large amounts of tumor necrosis factor alpha (TNF-_). This factor has many functions but one important one in this context is that it binds to the TNF receptor on target cells and induces apoptosis.

Recent data have shown that NK cells also produce a lot of IFN-_, which is very interesting since this cytokine activates macrophages and stimulates them to produce large amounts of TNF-_.

5.3 Neutrophils

Neutrophils are produced in the bone marrow from the granulocyte-monocyte stem cell. These cells are often called polymorphonuclear cells (PMN's). This is because of the polymorphic shape of the nucleus. Sometimes the terms neutrophil and PMN are used interchangeably. Neutrophils are the most common white blood cells in the circulation, making up about 60-70% of the total WBC count. They are very short-lived cells, circulating in the blood for about 8 hours after their release from the bone marrow. If induced to migrate out of the blood into the tissues, they will engage in a variety of effector functions before dying by apoptosis within 1-2 days.

Neutrophils are attracted into the tissue by chemotactic factors that include Complement proteins, clotting proteins, cytokines and chemokines. They are the first cells to arrive at the site of inflammation by leaving the blood, through the endothelium into the tissue (called “transmigration” or “emigration”). The appearance of neutrophils in the tissue is associated with bacterial infection, acute tissue injury, immune complex-Complement activation, necrosis and tissue remodeling. In the tissues, neutrophils are very active phagocytic cells. They are the most effective at killing ingested microorganisms and can do this by oxygen dependent pathways (such as superoxide anion [O_2^-] and hydrogen peroxide [H_2O_2]), nitrogen dependent pathways (nitric oxide [NO]) or independent pathways (such as defensins and digestive enzymes). Neutrophils, however, do not normally act as antigen presenting cells.

5.4 Eosinophils

Eosinophils are named because of their intense staining with 'eosin'. Under the microscope, eosinophils typically have a bi-lobed nucleus and contain many basic crystal granules in their cytoplasm. The granules are eosinophil mediators that are toxic to many organisms and also to tissues. Eosinophils circulate in the blood and emigrate into tissues, are phagocytic, and have been linked with anti-parasite immunity. Recently, eosinophils have been suggested to play a major role in the lung pathology associated with the late phase of asthma. There is also some evidence that they may be involved in immune responses against breast and colon tumors.

5.5 Mast Cells

Mast cells are formed in the tissue from undifferentiated precursor cells released into the blood from the bone marrow. They are not the tissue counterparts of basophils but they are similar in many respects. Mast cells contain numerous granules with preformed mediators which can be released from mast cells after stimulation. The preformed mediators include histamine and other active substances, including some cytokines (such TNF-α).

Stimulation of mast cells also results in the production of newly formed mediators such as prostaglandins and leukotrienes. Stimulation of mast cells occurs in several ways such as by the anaphylatoxins (C3a and C5a) of the Complement system or by the cross-linking of surface IgE. Mast cells have high affinity Fc receptors for the IgE that is produced against an allergen. As a result, mast cell release is most significant in either acute inflammation or in allergic responses.

5.6 Basophils

Basophils are found in low numbers in the blood. Their functions are not well understood but they are known to be involved in Type I hypersensitivity (allergic) responses. These cells have high affinity Fc receptors for IgE on their surface. Cross-linking of the IgE causes the basophils to release pharmacologically active mediators such as heparin and histamine. Basophils, therefore, act very much like mast cells except that they are in the blood instead of the tissues.

Chapter 6: Cytokines

The immune system has many different types of cells acting together to take care of unwanted infections and altered cells. Cytokines are the protein messenger molecules produced by these cells in order to communicate and orchestrate the attack. Cytokines also maintain the normal growth, migration and survival of immune cells in a physiologic state. Chemokines, interleukins and growth factors are subfamilies of the cytokine family. Just as hormones in the endocrine system can produce an effect on other cells, so cytokines can act on other immune cells, especially cells that are close by. Cytokines also act on non-immune cells, such as the blood vessel endothelium.

Cytokines have several important characteristics:

> _ the same cytokine may be made by a number of different cells. _ the same cytokine may have different effects in different circumstances (this is called '*pleotropy*')
> _ different cytokines may have the same activity depending on the situation (*'redundancy'*). _ cytokines often act together and increase the effects of one another ('*synergy*'). They may also act as antagonists. _ most cytokines have either paracrine or autocrine effects. *Paracrine* means they act on cells near to them or that they are actually touching. The *autocrine* function of IL-2 is well known because, when a T cell is stimulated to make IL-2, it stimulates itself via the IL-2 receptor to proliferate. An example of an uncommon *endocrine* function for cytokines is IL-1 which can cause fever by stimulating the hypothalamus.

Originally, the cytokines were named according to their function (like T cell growth factor, now called IL-2) but then the pleotropy of cytokines was observed, making function-specific names confusing. After more and more cytokines were identified, and in order to avoid confusion, immunologists started naming some of the cytokines 'interleukins' (or IL for short) and numbering them as they were found. The first interleukin identified therefore was IL-1 and we are now over 30.

You will find that some cytokines are more important than others in basic immunology. The functions of cytokines are best studied within the context of an actual immune response but a short synopsis is given for some of the important ones below.

6.1.1 Interleukin 1 (IL-1)

Interleukin 1 has many functions on many different cells and is secreted by a number of cells including macrophages, monocytes and dendritic cells. An important stimulus for IL-1 production by the macrophage is the presence of microbial products. IL-1 (originally described as T cell activation factor) helps to activate T helper cells by acting as a co-stimulator with the antigen presenting cell receptors. It also helps promote the maturation and clonal proliferation of B cells. IL-1 is an important part of the inflammatory response. One way it mediates this is by increasing the expression of specific cell adhesion molecules on the endothelial cells lining the blood vessels and thus facilitating the transmigration of immune cells from the blood into the tissue.

One very interesting action of IL-1 is its action on the hypothalamus. Here IL-1, and some other cytokines (including IL-6, the IFNs and TNFs), bind to receptors on the endothelial cells within the hypothalamus and appear to 'reset' the thermoregulatory centre, increasing the core body temperature causing fever. IL-1, therefore, has been called an "endogenous pyrogen".

6.1.2 Interleukin 2 (IL-2)

This cytokine was originally described as "T cell growth factor" and is secreted primarily by T helper cells. It acts on both T cells and NK cells. In an autocrine fashion, the antigen-primed T helper cell secretes IL-2, stimulating itself as well as other neighboring antigen-primed T cells, to proliferate (T cell activation). Growing T cells in long-term culture require IL-2 as a growth factor. Also, Natural Killer (NK) cell activity and CTL activity is maximal only after cytokine help provided by IL-2. This has been used as the basis of some new cancer immunotherapies.

IL-2, in combination with IL-4, causes activated B cells to produce IgM and resist class switch to IgG.

6.1.3 Interleukin 4 (IL-4)

This cytokine is released by T helper cells of the Th2 subtype and is particularly active on resting and active B cells. It was originally called B cell stimulating factor. IL-4 increases MHC II expression on resting B cells and on macrophages. On activated B cells, proliferation and differentiation is stimulated and an antibody class switch is induced. A B cell stimulated with IL-4 alone becomes a plasma cell secreting IgE and other allergy related antibodies. IL-4 acts

with IL-10 in an immunoregulatory manner to decrease the activity of activated macrophages.

6.1.4 Interleukin 5 (IL-5)

Like IL-4, this cytokine is secreted by the Th2 type of T helper cell. In mice it also stimulates the proliferation and differentiation of activated B cells but in humans it does not have this effect. In humans IL-5 is also very important in stimulating the growth and differentiation of eosinophils.

6.1.5 Interleukin 6 (IL-6)

Monocytes, macrophages and bone marrow cells secrete this cytokine but the major producer is the Th2 type of T helper cells. IL-6 acts on proliferating B cells to promote differentiation into plasma cells and it stimulates antibody secretion. Myeloid stem cells are helped to differentiate by IL-6. IL-6 has been described as "hepatocyte stimulating factor" and strongly stimulates hepatocytes to make acute phase proteins in response to inflammation. One of these acute phase proteins, C-reactive protein, is used as a clinical indicator of inflammation or infection.

This cytokine also induces fever and is always found in increased levels in sites of inflammation. It is very important in the regulation of the inflammatory response.

6.1.6 Interleukin 8 (IL-8)

IL-8 is a powerful chemotactic factor for neutrophils and was the first identified “chemokine”. Macrophages and endothelial cells secrete IL-8 when stimulated by IL-1, TNF-_ or bacterial products in order to attract neutrophils and allow them to adhere to vascular endothelial cells. This helps the neutrophils marginate on local blood vessels and enter the tissue where they are needed at sites of inflammation and infection. Neutrophils are the first line of defense against invading bacteria. Look to see them in most types of infection, especially in the early (acute) stage.

6.1.7 Interleukin 10 (IL-10)

This interesting cytokine was originally described as "cytokine synthesis inhibitory factor" because of its important inhibitory role. It acts on macrophages to inhibit cytokine production in order to down-regulate the Th1 type of T helper cell. It is released by Th2 cells and also down-regulates MHC II expression on antigen presenting cells. It has been shown to act with IL-4 to decrease macrophage inflammatory activity and may be an important cytokine in immune regulation.

6.1.8 Interleukin 12 (IL-12)

It is now clear that IL-12 is the major cytokine used by antigen presenting cells to activate T cells and drive them down the Th1 pathway. Secretion of IL-12 is a critical co-stimulation signal for T cells of this pathway. IL-12 tends to oppose the effects of IL-4 on APC-antigen stimulated T cells.

6.2 Interferon Gamma (IFN-γ)

Activated T cells (cytotoxic and Th1) and Natural Killer cells secrete IFN-_. Its major functions are to activate macrophages (they become angry macs) and to increase the expression of class II MHC on APC. IFN-_ stimulated macrophages are more phagocytic and they are more capable of killing intracellular pathogens due to increased production of H_2O_2, NO and lysozymes. They also have increased ability to present antigen. IFN-_ secreted by Th1 cells has a down-regulatory effect on Th2 function, and will induce a class switch to IgG. It can actually inhibit development along the Th2 pathway by inducing IL-12 production by macrophages. This cytokine has a role in many different types of immune responses such as delayed type hypersensitivity, inflammation, antibody production and viral infection. IFN-γ, along with TNF-α, is the cytokine involved in mediating the macrophage influx seen in many chronic infections such as tuberculosis. The importance of IFN-γ is evident in individuals with deficiency of IFN-γor of its receptor. In both cases overwhelming viral and mycobacterial infection is common.

6.3 Tumor Necrosis Factor Alpha (TNF-α)

Like IL-1, TNF-α is a pleotropic cytokine that has many different effects in different circumstances, on different cells and in conjunction with other cytokines. TNF derives its name from the fact that it binds to TNF receptors on cells (tumor cells in particular) and activates the caspase pathway which leads to cell death (apoptosis). In addition to this killing effect however, TNF has a powerful inflammatory effect and is involved in recruiting neutrophils, monocytes, T cells and NK cells into sites of inflammation

by inducing changes in the vascular endothelial cell adhesion molecule profile. Like IL-1, TNF-α has systemic effects in that it acts on the hypothalamus to induce fever (it is also an endogenous pyrogen). IFN-γ potentiates many of the effects of TNF-α (synergy). However, TNF overproduction can lead to excessive, tissue-destructive inflammation (for example in arthritis and septic shock).

6.4 Transforming Growth Factor beta (TGF-β)

TGFβ is secreted by platelets, macrophages and lymphocytes. It has many functions which include increasing IL-1 production by activated macrophages, down-regulating excess macrophage and T cell activation/proliferation, inducing a class switch to IgA by proliferating B cells and acting as a chemoattractant for monocytes and macrophages. TGFβ actually aids in wound healing because it limits the inflammation caused by injury.

Chapter 7: Transplantation and Major Histocompatibility Complex

7.1 Preamble

Long before our understanding of the complex mechanisms of the large variety of immune responses we knew that organs transplanted from one individual into another undergo vigorous immune attack and become irreparably damaged. The term "rejection" was coined to refer to the fact that the recipient body would not tolerate the existence of a donor organ. In fact, this finding was surprising at the time. There was no reason to believe that an organ from one human was fundamentally different from another. It soon became clear that the perception of the donor organ as "foreign" was related to surface antigens on the donor cells. These antigens were called the "major histocompatibility antigens" and because there are a number of them located in a grouped gene locus they were called the major histocompatibility complex (MHC) now called the human leukocyte antigens (HLA) in humans.

The reasons behind organ rejection became better understood when it was ascertained that these MHC antigens were the molecules involved in antigen presentation to T cells. T cells look to these molecules and the peptides they present to distinguish self from foreign. It is their interaction with T cells that makes these molecules the major barrier to transplantation within a species.

7.2 Class I MHC

Class I MHC has long been suggested to be the major target of the immune response in transplantation. All cells possess Class I MHC on their surface and the purpose of this molecule is to sample the internal contents of cells and bring them to the surface for presentation to the immune system. If, for example, there is a viral infection in the cell, then peptides derived from the viral proteins in the cytoplasm will be brought to the surface in the groove of the Class I MHC molecule. These viral peptides, in the groove of self MHC Class I will be seen as foreign by CD8+ cytotoxic T lymphocytes (CTL) and the infected cell will be killed in an effort to limit viral replication within the cell.

In transplantation, the Class I MHC on the surface of donor cells is not the same as the Class I on the recipient cells. This is because there are a large variety of Class I variants in the population and it is very unlikely that any two individuals (except for identical twins) would have inherited exactly the same set of Class I molecules as each other. For some reason, not yet clearly understood, the CD8+ CTL cell population (which sees antigen in the groove of class I MHC normally) recognizes this donor MHC as foreign. The result is the death of the cell as if it

was a virally infected cell. This is called the “direct” response since it requires only the recognition of foreign class I by the CTL and then direct killing of the donor cell by the CTL.

7.3 Class II MHC

Class II MHC is present only on antigen presenting cells (APC) and is involved in antigen presentation to CD4+ T cells, rather than CD8+ T cells. Like Class I MHC, there are a large variety of Class II molecules in the population and it is very unlikely that any two people would have inherited the same set of Class II molecules. Thus, like Class I MHC, Class II MHC will be recognized as foreign. However, the direct recognition of Class II on the surface of donor APC will not lead to the direct destruction of the APC because the vast majority of CD4+T cells are helper cells rather than killer cells. The recognition of foreign Class II will lead to the local elaboration of cytokines, such as IFN-_ which will activate local responses such as macrophage activation and macrophage secretion of TNF-_ which will damage the donor cells. This damage is indirect since it was not due directly to T cell killing. In addition, the activation of CD4+ T cells will enhance the development of a B cell response to the transplant so that anti-donor antibody is produced.

7.4 Indirect Presentation

The two scenarios described above deal with the direct recognition by recipient T cells of MHC molecules on the surface of donor cells. However, in addition to this, there is another way in which the recipient sees the donor antigen. This is by indirect presentation, and some say this is the major way the anti-transplant response is activated.

For indirect presentation to occur some of the donor cells have to be damaged during the transplant procedure. This is very common since the organ is deprived of oxygen the whole time the transplant operation is being performed and, in the case of kidneys, the organs are often kept in preservative solution for a number of hours (up to 24 hours) before transplantation. This time without oxygen causes “ischemic” damage to the tissues of the organ, including a significant amount of cell death of the endothelial cells lining the vasculature. When the organ is reperfused with blood, additional damage due to reperfusion injury occurs. These both provide a ready source of dead and dying cells within the organ, many of which are transported out by the blood flow.

These dead cells are a rich source of antigens but most of the proteins in the donor cells are the same as those in the recipient and they are ignored. The donor MHC antigens, however, are seen as different (foreign) and dealt with as though they came from a pathogen. They are ingested by recipient dendritic cells, processed, and presented to T cells. Both Class I and Class II peptides can be presented by recipient dendritic cells in the groove of dendritic cell Class I and Class II as foreign peptides (note that dendritic cells are the only APC that can take in protein from the outside of the cell and present it in both Class I and Class II MHC; other APC can only present this "exogenous" antigen in Class II MHC). In this manner the recipient dendritic cells can activate recipient CD4+ and CD8+ T cells that will subsequently migrate to the transplant (the activation probably happens in the spleen) and cause damage to the transplant in the manner described above.

7.5 Clinical Note: HLA typing

In humans the MHC molecules are referred to as the Human Leukocyte Antigens or HLA antigens. The HLA family is divided into several different genes; the genes encoding for Class I molecules are the A, B and C genes and the genes encoding the Class II molecules as DR, DQ and DP. A given individual will have two copies out of a large variety of possible variants at each of these genes.

HLA Typing is used to identify the HLA genes in a given individual. This is useful in kidney transplantation where the closer the match between the kidney donor and the recipient, the better the chance of survival of the kidney transplant. When a donor organ becomes available the donor is HLA-typed and compared to the HLA types of the patients waiting for a transplant. The patient who has the most HLA A, B and DR genes in common with the donor kidney is selected to receive the transplant. Specific diseases are also associated with specific HLA types. For example, 95% of patients who develop ankylosing spondylitis have HLA B27. HLA typing can be used in these circumstances to support a clinical diagnosis.

Traditionally, HLA typing was performed using panels of antibodies that react with known HLA proteins. The peripheral blood lymphocytes from the individual are incubated with the antibodies in the presence of Complement. If there is lysis of the cells, that individual carries the HLA protein recognized by that specific antibody.

Molecular (DNA) techniques can now be used to type HLA molecules. PCR using primers that amplify the DNA of specific HLA genes allow accurate identification of HLA types. This has the advantage of not requiring the preparation of live cells from the individual to be typed.

Chapter 8: Hypersensitivity

What is Hypersensitivity?

Hypersensitivity is a clinical term which refers to an inappropriate reaction to antigen. The term actually means an over (hyper) sensitivity to antigen. In fact, this term is a misnomer because the reaction is not characterized by an overreaction but by a reaction that you don't want, usually because it is accompanied by pathology of some kind. Hypersensitivity reactions result from immune responses which are essential to combat disease, the recognition of antigen and the reaction to that antigen. However, not all antigens are products of invading microbes. Ragweed and poison ivy are not dangerous infectious pathogens but the immune response to antigens in these plants can be as vigorous as it is to pathogenic microbes.

Hypersensitivity reactions have been classified into four categories:

- Type I: IgE-mediated Hypersensitivity (Allergy and Asthma)
- Type II: Antibody-mediated Cell Death
- Type III: Immune Complex Disease
- Type IV: Delayed Type Hypersensitivity

The first three of these categories are mediated by antibody in some form or another but the fourth type of hypersensitivity is "cell-mediated" in that antibody plays no part. Each of these will be discussed in detail below.

8.1 Type I Hypersensitivity: Allergy and Asthma

Anyone who suffers from hay fever or allergies knows how inappropriate this type of immune response can be. Type I hypersensitivity is also called IgEmediated and may result in local or systemic anaphylaxis. Systemic anaphylaxis is a potentially lethal reaction.

This type of hypersensitivity response is induced by a certain type of antigen called an allergen. It isn't really understood what makes a protein like ragweed pollen (for example) just an antigen to one person and a potent allergen to another, although genetic predisposition is one known important determinant. What distinguishes Type I hypersensitivity from other antigen-induced antibody responses is the stimulation of IgE production (instead of IgG production).

The allergen is presented to naive T cells in the normal manner and T cell/ B cell interaction occurs (see above). During this interaction abundant IL-4 is released by the T helper cell. The release of IL-4 by T cells is critical in stimulating a class switch to IgE. The maturing B cell clonally expands then differentiates into an IgE-secreting plasma cell. The secreted IgE binds by its Fc portion to high affinity Fc receptors for IgE (FcER or FcεR) on the surface
of mast cells and basophils. Now these cells are "sensitized".

The mast cells reside in tissues while basophils circulate in the blood. These cells stay sensitized for a long time and when they come into contact with the allergen they degranulate. The allergen cross-links two surface IgE molecules sending a signal into the cell to release mediators.

That signal involves complicated signal transduction pathways. There are a few important aspects of these pathways that need to be discussed because they relate to treatment of allergy and asthma. The cross-linking of the FcER immediately causes the activation of adenylate cyclase which results in a transient rise in intracellular cAMP. This transient rise is critical to mediator release from mast cells. If this increased level of cAMP lasts too long the mast cell will shut down. Drugs such as Theophylline prolong the increase in cAMP and thus inhibit mast cell degranulation. Another important part of the activation cascade is the requirement to mobilize calcium from intracellular stores and to import calcium into the cell from outside. If this rise in calcium is blocked the mast cell cannot degranulate. Drugs such as Disodium Cromogylcate (cromolyn sodium) prevent the entry of calcium into mast cells and inhibit degranulation.

The mediators released by the mast cells and basophils are active on local tissues and on other immune cells. Some are released immediately because they are stored in the granules ready for release. These are often referred to as pre-formed or primary mediators. These mediators are responsible for the immediate (within seconds) effects that are seen when one encounters an allergen. Because of this rapid release of mediators and their immediate effects, this type of hypersensitivity is often referred to as “Immediate Hypersensitivity”.

In addition to the rapid release of granule stored mediators, a variety of events occur in the cell which result in the production of new mediators for release after activation. These are called the newly formed or secondary mediators. These later mediators tend to have more long-lasting effects than the pre-formed mediators and are responsible for late phase events.

Preformed mediators:

The most important pre-formed mediator is histamine. Its main effects are to increase vascular permeability leading to local edema (swelling), to increase blood flow to the area by inducing vasodilation (redness), to activate cells in the mucosa to release mucus and to mediate constriction of the smooth muscle cells of the bronchioles (broncho-constriction) leading to shortness of breath. Histamine binds to histamine receptors on a variety of cells and the effects of the compound depends on which receptor (H1, H2 or H3) that particular cell is bearing.

In addition to histamine, there are a number of important cytokines that are stored in the granules ready for release. Mast cells are one of the very few cells that store cytokines; most cells make them on demand when needed. The three most important of these cytokines are eosinophil chemotactic factor, eotaxin and IL-5. All of these cytokines have the effect of attracting eosinophils to the site and activating them. In asthma this potent triumvirate, along with the IL-5 produced by activated T cells, is responsible for the substantial influx of eosinophils to the lung and the subsequent damage to the lung tissue.

Newly formed mediators:

Some of the most important newly formed mediators released by mast cells are the products of the breakdown of plasma membrane phospholipids. The liberation of arachidonic acid from the plasma membrane leads to the production of prostaglandins (PG) and leukotrienes (LT). The breakdown of the phospholipid backbone leads to the production of platelet activating factor (PAF). Both PAF and LT are potent mediators of allergy and have many of the same effects as histamine but they are longer lasting. These lipid mediators are produced within minutes of mast cell activation, and continue to be produced for hours.

In addition to the lipid mediators, mast cells also transcribe, translate and secrete numerous cytokines. Although a large variety of cytokines have been reported to be released by mast cells, many of them have been reported at very low levels and are of questionable biological relevance. Some cytokines however, are clearly important. The importance of the secretion of IL-5 is

discussed above. Two other important cytokines, IL-1 and TNF-_ are of special interest because they have potent systemic, as well as local, effects.

In asthma these newly formed mediators have very significant direct effects on the airway (bronchial tree) as well as having very significant indirect effects by their recruitment and activation of other leukocytes (including Th2 cells) to the lungs. Because of these longer term direct and indirect effects they cumulatively overshadow the immediate, but transient, effects of histamine.

Treatments:

There are three ways to treat IgE-mediated hypersensitivity reactions. The first is to try to block mast cell degranulation with drugs such as theophylline, cromolyn or corticosteroids. The second is to try to block mediator receptors on the target cells to block the effects. Antihistamines or drugs to block leukotriene receptors have this effect. The third is to treat with drugs which reverse the effects of the mediators (i.e. produce bronchodilation and vasoconstriction). Epinephrine and $\alpha 2$ adrenergic agonists have this effect.

In asthma the anti-inflammatory effects of corticosteroids (reduce mast cell activation and mediator release, reduce eosinophil and T cell recruitment and activation, and reduce inflammatory cytokine synthesis and release) have made them the mainstay of long term asthma therapy. In asthma they are used locally by inhalation.

8.1.1 Anaphylaxis

Anaphylaxis is caused by the systemic release of mast cell and basophil mediators. The result is a “whole body” allergic response with disastrous consequences. Histamine, for example, will increase vascular permeability and induce vasodilation. The resultant plasma leakage and increased vascular bed will rapidly cause a dramatic drop in blood pressure. In addition, other mediators will cause systemic shock-like symptoms and, in some cases, death. Thus, the treatment of anaphylaxis with anti-histamine has to be supplemented with adrenergic agents such as epinephrine to reverse vasodilation, and with corticosteroids to inhibit further mast cell activation (as well as directly inhibit excess vascular permeability).

8.2 Type II Hypersensitivity: Antibody Mediated Cell Lysis

This type of hypersensitivity is antibody working at its best but with unpleasant results. Type II hypersensitivity is simply antibody fixing Complement and opsonizing for phagocytosis. Both of these processes are very efficient for killing targets. The reason the results are unpleasant is because we don't want these particular targets killed.

The best example of Type II hypersensitivity is Erythroblastosis fetalis (also called hemolytic disease of the newborn or "blue baby" syndrome). This disease is a problem when an Rh- mother has a child by an Rh+ father. The Rhesus (Rh) antigens are antigens on the surface of red blood cells and the Rhesus D antigen is the one implicated in this disease. Some people have these RhD antigens on their cells (Rh+) and some people don't. About one in six individuals are homozygous negative for the Rhesus D antigen and are called Rh-.

Now if an Rh- mother has a child by an Rh+ father there should be no problem in the first pregnancy. The mother will have no pre-existing antibody to RhD antigens since she has never seen them before. Even though there will be a few fetal blood cells making their way into the mothers circulation they will not be sufficient to cause enough of an immune response to damage the fetus. (Remember also that pregnancy makes the mother somewhat immunosuppressed). In contrast, at birth, there will be significant mixing of blood and the mother will become exposed to the RhD antigens present on the baby's red blood cells during a traumatic event. In response to this, the mother will make an IgG antibody response to the RhD antigens.

During a second pregnancy the baby is in trouble. Now the mother has significant levels of circulating IgG against the RhD antigen on the baby's blood cells. Since, during the second half of the pregnancy, IgG is actively transported across the placenta, the IgG will bind to the baby's red blood cells and initiate Complement-mediated lysis by the classical pathway and opsonization for phagocytosis by phagocytic cells in the liver and spleen. The result will be severe anemia *in utero* and will be potentially fatal for the baby.

Treatment:

This disease is best prevented rather than treated. The way to do this is post-natal treatment of the mother with an antibody to the RhD antigen as soon as possible (ideally within 24-48 hours) after the first birth (even a miscarriage or stillbirth). The product RhoGAM is an IgG antibody directed against RhD and this will quickly clear the fetal Rh+ red blood cells from the system and prevent sensitization of the mother. In addition, pre-natal treatment can be used late in the first pregnancy (and in subsequent pregnancies). This concept is a bit confusing since you would think that administration of an IgG antibody to RhD at 28 weeks of pregnancy

would harm the Rh+ fetus (since it can cross the placenta). The general view is that the amount of antibody administered is enough to mop up the small number of fetal red blood cells that make it into the mothers circulation during pregnancy (and thus help reduce the possibility of sensitization) but not enough to seriously harm the fetus (although some fetal cells will likely be killed).

If prevention is not possible the baby may require intra-uterine blood transfusion or to be delivered as soon as is feasible and a blood transfusion performed. In both cases the strategy is to provide adequate red blood cells for the baby and in both cases the blood transfusion would be with Rh- red blood cells to prevent reaction with the anti-Rh antibody.

8.3 Type III Hypersensitivity: Immune Complex Disease

Again, this disease is a result of a normal protective response by antibody. When antibody in the circulation encounters free soluble antigen (for example, a bacterial toxin) then antibody (usually IgG but also IgM) will bind to the free antigen. This will target the antigen for clearance by the liver. This process works most efficiently if more than one antibody molecule and more than one antigen molecule are involved so that an "immune complex" is formed. These immune complexes exist in a variety of sizes and are normally cleared from the blood by phagocytic cells in the liver and spleen. Immune complexes also activate the Complement system (C3b binds to the complexes) and this enhances phagocytosis of the complexes.

However, some smaller immune complexes are inefficiently cleared and can get deposited in various organs and tissues where they cause inflammation and subsequent pathology. For example, immune complexes may get deposited in the joints where they will initiate Complement activation and an inflammatory response which can lead to arthritis.

Perhaps the best example of immune complex disease is "immune complex glomerulonephritis" (sometimes referred to as "auto-immune glomerulonephritis"). In this disease immune complexes are deposited in the glomeruli of the kidney. They become wedged between the vascular endothelial cells at permeable filtration sites in the glomerulus. When they attach to the basement membrane they activate Complement. This activation of Complement results in the presence of the Complement split products C5a (which attracts neutrophils to the site) and C3b bound to the complexes. Since neutrophils have receptors for both C3b and the Fc portion of the antibody part of the immune complex, neutrophils will bind to the site. Neutrophils will engage in "frustrated phagocytosis" as they attempt to kill whatever has attracted their attention. This will cause a dramatic inflammatory response in the vessel wall and surrounding tissue and

lead to enough tissue damage to compromise normal kidney function.

8.4 Type IV Hypersensitivity: Delayed Type Hypersensitivity

Type IV hypersensitivity is called delayed type hypersensitivity (DTH) because of the fact that the expression of this response takes time, usually 24-48 hours. In some ways it is very much like IgE-mediated hypersensitivity in that you must be exposed to an antigen and then, on re-exposure, there is a response characterized by inflammation and edema. However, these responses are very different.

DTH is a cell-mediated response and antibody plays no role in it. The best example of the DTH response is the response to purified protein derivative (PPD), from *Mycobacterium tuberculosis* bacteria, which is used to test for prior exposure to tuberculosis. Because the mycobacterium that causes tuberculosis lives within cells, especially macrophages, the body mounts a very poor antibody response to it. The response to *M. tuberculosis* is almost entirely cellular. As such, to test for prior exposure to *M. tuberculosis* one tests for a memory T cell response.

When PPD is introduced into the skin of the patient it is immediately taken up by local resident macrophages and dendritic cells. The trauma to the skin and the activation of the macrophages by phagocytosis of the PPD sets off a minor local inflammatory response which will lead to an influx of circulating lymphocytes. In the circulating lymphocyte population will be memory Th1 cells reactive to *M. tuberculosis* antigen, resulting from the previous exposure to *M. tuberculosis* (because of a previous infection).

So now in the skin you have activated macrophages and memory T cells. In this situation macrophages will digest the PPD and express PPD peptide on their surface in the context of Class II MHC for presentation to local T cells. Macrophages are not efficient at presenting antigen to naive T cells but are very good at activating memory T cells. Thus, these macrophages will activate the memory T cells. Since they are memory Th1 cells (sometimes referred to as T_{DTH} cells) they will immediately secrete large amounts of IFN-γ. The IFN-γ will further activate the local macrophages and cause the secretion of large amounts of TNF-α and IL-1. These cytokines will recruit many more macrophages to the local site and cause more severe inflammation. Unlike IgE-mediated hypersensitivity, where edema and redness can be seen within minutes, the DTH response takes time to develop. Therefore, a “positive” response is seen 24-48 hours after introduction of the PPD.

What is important about DTH is that the general process of macrophage activation by Th1 cells resulting in inflammation is a process that the immune system uses to combat infections that are not readily cleared by antibody. For example, the response to the TB bug in the lung is almost identical to the response to PPD in the skin.

Other common bacteria such as *Salmonella* and *Listeria* also survive in macrophages after phagocytosis and cause disease (these are called intracellular pathogens). In addition, intracellular parasites such as *Leishmania* and intracellular fungi such as *Pneumocystis carinii* are fought by a DTH-like response involving Th1 cells and macrophages.

A DTH-type response is responsible for the inflammation resulting from exposure to poison ivy, poison oak and some cosmetics. In these cases there is no prior infection but a small molecule (hapten) in the plant oil (or cosmetic) binds itself to self proteins in the skin. This self protein-hapten complex can activate an immune response and result in hapten specific Th1 memory cells which mount a response very similar to the response described above. This reaction is called “contact hypersensitivity” or “contact dermatitis”. Contact dermatitis can be elicited by numerous small molecules in cosmetics, soaps, solvents and even some metals.

Chapter 9: Autoimmunity

Although the majority of self-reactive T cells have been deleted in the thymus this process is far from complete. Moreover, selective elimination of B cells with surface immunoglobulin recognizing self antigen is not an efficient process during development. Thus, a further level of control is required to limit reaction to self antigens. This is called "peripheral tolerance" and it limits the activation of self reactive cells. However, this peripheral tolerance can be bypassed or subverted under certain circumstances. The result is autoimmune disease.

The etiology of most of the autoimmune diseases is unknown but there are a number of theories about how the normally tightly controlled immune response goes awry.

The three most accepted theories are:

1. **The release of sequestered antigen.**
2. **Molecular (or antigenic) mimicry.**
3. **Polyclonal B cell activation.**

9.1 Release of Sequestered Antigen

Sequestered antigen is antigen that is normally hidden from the immune response. A good example would be myelin basic protein (MBP) which is a large component of the myelin sheath which surrounds nerves. This protein is unlikely to be in the thymus to mediate negative selection of T cells but also it is unlikely to be floating around in the circulation for presentation to T cells. Even if some of it is present in the circulation as soluble protein there is no reason for the immune system to mount an immune response to it since it presents no danger to the body. If MBP is picked up by dendritic cells during routine pinocytosis they would not provide potent co-stimulatory signals to T cells in the absence of infection or trauma (danger) since there would be no inflammatory mediators produced in the local area.

If, however, a viral infection damages the nervous tissue, now MBP could be released in large quantities and under conditions of inflammation. The local dendritic cells would now be seeing large amounts of a protein they are not used to seeing in an environment where damage is occurring and an active antiviral immune response is being initiated. In this context the dendritic cells could present the MBP antigen to T cells with the appropriate co-stimulation to initiate an immune response to MBP. The immune response initiated would then cause more damage to the nerves releasing more MBP antigen in the context of more inflammation. This will result in a chronic response which will damage the nerve sheath.

There is also evidence that the expression of "unexpected" antigens can lead to autoimmunity, especially antigens out of sync with normal development. This may relate to the fact that some tumors express antigens which are normally expressed only in fetal life (so called "onco-fetal" antigens). If normal tissue expresses such out of sync antigens then they could be exposed to the same killing mechanisms as tumors are.

9.2 Molecular (Antigenic Mimicry)

When the immune response makes a vigorous response to a pathogen, antibodies and cell-mediated responses are highly activated. These responses are specific to a particular antigen but it is possible that other antigens, some of them self antigens, could look so similar to some of the antigens from the pathogen that "cross-reaction" occurs.

One of the most obvious examples of this cross-reaction is rheumatic fever. When a person is infected with Streptococcus bacteria a vigorous anti-Strep antibody response is mounted. This helps clear the bacteria and prevent reinfection. Unfortunately, one of the dominant Strep antigens is very similar to an antigen on human cardiac myocytes. Thus, when a very vigorous antibody response is made to Strep then the antibody can also recognize the cardiac myocytes and cause myocyte cell death.

Celiac disease is another example of cross-reactivity causing pathology. Celiac disease is an "autoimmune enteropathy" triggered by interesting double cross reactivity. Celiac patients have mounted a particularly vigorous antibody and T cell-mediated response to a gastro-intestinal viral infection. Unfortunately for them, the dominant peptide sequence in the virus is almost identical to a peptide sequence in gliadin, a protein in wheat gluten. Thus, consumption of wheat is perceived as re-infection with the virus. Unfortunately, the protein gliadin cross-reacts with certain epithelial cell proteins (such as tissue transglutaminase) and the reaction to gliadin causes epithelial and villous damage. Every time they eat gluten in wheat (or barley and rye) the gut immune response is reactivated and creates chronic inflammation and damage. There are a number of other cell-mediated autoimmune diseases, such as multiple sclerosis and diabetes that have been postulated to be the result of antigen mimicry.

9.3. Polyclonal B Cell Activation

Although T cells undergo a very stringent selection process in the thymus to weed out potentially self-reactive cells, B cells are not subject to such close scrutiny. This is not usually a problem since B cells generally depend on T cell help to become activated and differentiate into plasma cells. There are, however, some agents that non-specifically activate B cells to progress into IgM producing plasma cells (no class switch is possible without T cell help). If this happens it is entirely possible that antibody to self antigen could be made with pathological consequences.

It is well known that Epstein Barr Virus (EBV; the cause of mononucleosis) is such a polyclonal activator and antibodies are often made to a variety of self antigens during EBV infection. This type of activation has been suggested to account for some of the autoantibodies seen in rheumatoid arthritis, systemic lupus erythematosis, autoimmune hemolytic anemia and thrombocytopenia.

9.4 Common Autoimmune Diseases

9.4.1 Diabetes

Type I diabetes is a potentially fatal autoimmune disease that develops in children. The basis of the disease is the immune destruction of the insulin producing β cells in the islets of Langerhans (in the pancreas). Without these cells the body cannot produce insulin and blood glucose levels go sky high. Untreated, this is fatal. Fortunately, insulin can be injected on a regular basis and glucose homeostasis restored to some level. However, bolus delivery of insulin is not without risk and glucose homeostasis is never as good as can be achieved with functioning β cells. This leads to chronic problems, most importantly vascular disease due partly to excessive fluctuations in blood glucose and lipids. With poor glucose control, declining peripheral circulation in the extremities can lead to necrosis, gangrene and amputation. Similar processes in the eye can result in blindness. Arteriosclerosis is accelerated with resulting premature coronary disease.

The reason for the destruction of the β cells is unclear. There is clearly a genetic effect but no specific genes have yet been identified. Both antigenic mimicry and expression of normally hidden (or fetal) antigens have been proposed. What is clear is that a response very similar to a transplant rejection response is mounted against the β cells. CTL contribute but the main player is the macrophage which, in a DTH-like response, releases the potent cytokine TNF-α. This leads to β cell death.

9.4.2 Systemic Lupus Erythematosus (SLE)

SLE, also called "Lupus" gets its name from the fact that it is often characterized by a rash on the face that makes the patient look almost "wolflike" (lupus is Latin for wolf). Like many autoimmune diseases this disease is much more common in women than in man (ratio is 10:1). The major symptoms are arthritis, skin rashes and kidney dysfunction. All of these symptoms relate to the deposition of immune complexes in the blood vessels and tissues causing local inflammation. The immune complexes are complexes of normal self antigen and "autoimmune" antibody. A number of self antigens have been identified as targets, including antigens derived from the nucleosome. Antibodies to these antigens are often called "anti-nuclear antibodies". These anti-nuclear antibodies are seen in some other autoimmune diseases but antibody to double stranded DNA is the most specific for SLE and is used as a diagnostic test. The reason for the induction of these autoimmune antibodies is unknown.

9.4.3 Rheumatoid Arthritis (RA)

Rheumatoid arthritis is an autoimmune disease characterized by pain and inflammation in the joints. In its most severe form bone and tissue destruction occurs due to chronic inflammation. The disease is characterized by invasion of the joint lining tissue (the synovium) and joint fluid by neutrophils, monocytes, macrophages and T cells. The continued overproduction of cytokines such as IL-1 and TNF-α by these cells, and the activation of osteoclasts, leads to cartilage and bone degradation. This is thought to be a Th1 mediated process, although the antigen recognized by the Th cells is unknown. There is a genetic predisposition with increased risk for people with certain MHC Class II molecules (such as HLA-DR3 or 4).

As the disease progresses, autoantibodies to cartilage also appear. IgM antibodies (rheumatoid factor) develop against self IgG Fc for reasons that remain unclear. Thus, immune complex deposition and Complement activation also contribute to the joint inflammation. Rheumatoid factor in the serum is a useful diagnostic test.

The disease is episodic and difficult to treat. Severe cases are treated with corticosteroids to block inflammation but these drugs are not without significant side effects. Like SLE, this is primarily a disease of women and should not be confused with osteoarthritis which has a different etiology.

Because RA is a chronic, progressive long term disease which is episodic in nature and the current treatments are not without significant side effects it is not surprising that many patients with RA turn to alternative sources of treatment such as herbal remedies. The Arthritis Society of Canada estimates that at least 60% of patients with this disease regularly take some form of alternative therapy. Family practitioners should be aware that their RA patients are probably taking other therapies with unknown effects with respect to interaction with prescription drugs.

A major, recent advance has been the use of therapies such as antibodies to human TNF-_ to neutralize the excess amounts of this key inflammatory cytokine. This is effective in up to 70% of patients, with relief of inflammation, restoration of joint function and well being. The treatment is well-tolerated with few reported side effects to date. As expected, infection is one occasional problem but is manageable if recognized and treated early.

9.4.4 Multiple Sclerosis (MS)

MS is a devastating disease which destroys the myelin sheath around nerves in the central nervous system and thus blocks nerve function in the brain and spinal column. The result is a loss of sensation and/or muscle movement (paralysis). It is a relapsing, remitting disease with partial recovery between attacks. Most patients progressively lose muscle function and over 20-25 years succumb to the disease.

It is very clear that the destruction of the myelin sheath is mediated by Th1 responses with macrophage activation being the primary pathway of pathology. It appears that myelin basic protein (MBP) is the immunodominant antigen. In animal models purified MBP injected with a suitable adjuvant will produce a disease (experimental allergic encephalomyelitis) which is almost indistinguishable from MS. The reasons behind the activation of robust immune responses to MBP in patients with MS are unclear but the fact that some viral peptide sequences bear a remarkable resemblance to MBP, at both the DNA and protein level, suggests that molecular mimicry may play a significant role. Recent work has shown that T cell clones isolated from MS patients react both with EBV and with MBP, strongly implicating cross reactivity in the etiology of MS.

9.4.5 Graves Disease and Autoimmune Thyroiditis

Graves disease is a very straightforward disease in which autoantibodies are made to the thyroid stimulating hormone (TSH) receptor. These are agonist antibodies in that when they bind to the receptors, the receptors think they have been engaged by TSH and the cells produce more thyroid hormones. The overproduction of thyroid hormones is responsible for the symptoms of this disease.

In contrast to Graves disease, one of the most common autoimmune diseases affecting older women is Autoimmune (or Hashimoto's) Thyroiditis. Here the thyroid gland is destroyed due to autoantibodies to thyroid epithelium as well as T cell-mediated, DTH-like responses directed partly at antigens such as thyroglobulin. The result is hypothyroidism requiring thyroxine replacement therapy.

9.4.6 Myasthenia Gravis

The antibodies produced in myasthenia gravis are antagonist antibodies in that when they bind they block activation. In myasthenia gravis the autoantibodies are directed to the acetylcholine receptors. Blocking acetylcholine activation of its receptor blocks normal nerve-to-muscle transmission leading to problems with muscular movement and eventually to wasting of muscles. In addition to blocking activation, the autoantibodies eventually activate Complement and lead to muscle cell death. In severe cases the resulting weakness of respiratory muscles is life threatening.

Chapter 10: Immune Deficiency

The immune system is a multi-component, often interdependent, system which has evolved over many years to deal with a variety of challenges. However, genetic defects often result in loss of function of one or more of the components of the immune system. Sometimes external events create immune deficient states of varying degrees such as viral infection (HIV, for example) or immunosuppressive treatment of transplant patients. The most obvious result of this immune system compromise is persistent and recurrent infection.

The type of infection often gives a clue to the type of immune deficiency. Deficiencies in antibody usually cause chronic recurring bacterial infections which are difficult to treat. Deficiencies in cell mediated responses result in increased susceptibility to fungal and viral infection.

10.1 Antibody Deficiencies

10.1.1 IgA Deficiency

This is the most common antibody deficiency and results in chronic infections at mucosal surfaces since IgA is the secretory antibody which protects these surfaces. However, since IgG and IgM can compensate, to a significant degree, for the lack of IgA, this deficiency often goes unnoticed since it usually does not cause serious problems. A few patients with IgA deficiency may also develop additional antibody deficiencies (for example deficiencies in IgG) and/or autoimmune disease with time.

10.1.2 X-linked Agammaglobulinemia (XLA)

Also called X-linked hypogammaglobulinemia this disease results from a lack of B cells and, thus, no antibody in the circulation. The result is a profound inability to respond to extracellular bacterial infection so that chronic, recurrent upper respiratory tract bacterial infections are very common. Infection may also disseminate via blood (bacteremia) to distant sites (meninges, bones, joints). This disease is diagnosed early in children because once the maternal antibody has waned from their circulation they have no antibody against bacteria. The bacterial infections are hard to clear with antibiotics and the patients do very poorly without immune therapy. Treatment is with pooled gammaglobulin (IgG). Although this is a blood product, it is the safest as far as transmissible infection. Current production methods eliminate all known infectious agents.

The root of this disease lies in the lack of a particular signal transduction element crucial for B cell development. As covered above, pre-B cells must put a form of surface antibody (heavy chain with surrogate light chain) on their surface successfully to progress to the next stage. The presence of this early antibody construct activates Bruton's Tyrosine Kinase (BTK) which sends the signal to the B cell nucleus to progress. These patients lack a functional BTK molecule, their B cells do not progress and they are therefore B cell (and antibody) deficient. T cell function and cell-mediated immunity is normal.

10.1.3 Common Variable Immunodeficiency (CVI)

CVI can develop at any age. The underlying cause is not known but it is clear that there is a defect in the T cell – B cell cooperation that is needed for B cells to differentiate into plasma cells. This results in very low levels of antibody in the circulation but a normal B cell count. The result of the very low level of antibody is the same as in XLA and the treatment is the same. However, unlike XLA, these patients may develop T cell deficiencies as well, making them susceptible to additional infections (such as viral infections). Ironically, this imbalance of T cell and B cell function can result in a loss of self tolerance resulting in autoimmune disease.

10.2 Deficiencies in Cell Mediated Immunity

10.2.1 SCID

SCID refers to severe combined immune deficiency which results in no, or low numbers of, functional B and T cells. By far the most profound effect on the immune response is the lack of T cells. Children with SCID are usually diagnosed in their first six months of life because of severe bacterial, viral and fungal infections. They must be kept isolated from any potential for infection. This is the classic "baby in the bubble" syndrome. The prognosis is very poor unless their immune system can be reconstituted by providing normal bone marrow stem cells through transplantation from an HLA-matched donor.

Since the development of B cells and T cells depends on a number of critical steps, all going according to plan, a defect in any of these critical steps will result in SCID. For example, a defect in the RAG genes which are required for gene rearrangement of both the T cell receptor and antibody will result in an inability of either T cells or B cells to reach maturity.

A common genetic defect leading to SCID is a problem in the gene coding for the gamma chain of the IL-2 receptor. This chain is also part of the receptors for IL-4, IL-7, IL-9 and IL-15. A defect in the gamma chain knocks out the activity of all of these important cytokines and renders the immune response completely ineffective. A defect in the JAK kinase signal transduction pathway activated by these cytokines has a similar effect.

An interesting, and common, defect leading to SCID is a lack of adenosine deaminase. The lack of ability to deaminate adenosine (to produce inosine) results in a toxic accumulation of this molecule. For some reason lymphocytes are particularly susceptible to the toxicity of adenosine.

Chapter 11: Host Defence

Although the immune system has many responsibilities its primary job is to fight off pathogenic infectious agents. Any observation of patients with genetic or acquired immune deficiency will show quite clearly that their major problem is with infection. SCID children and AIDS patients die from overwhelming infections that do not respond adequately to antibiotic treatment. The complexity of the immune system is due, for the most part, to the wide spectrum of infectious agents that it must respond to. Although there is overlap in responses to a given infectious microbe, one or two specific mechanisms are usually critical for the response to a particular pathogen. How the immune system knows how to activate the most effective response is a subject of much interest for immunologists. In this chapter we will cover the various types of infectious agents and the kinds of responses to them.

11.1 Extracellular Bacterial Pathogens

Many bacteria grow well in fluids but, fortunately, in body fluids such as blood plasma and tissue fluid in the extracellular space, a lot of species are killed by direct activation of the alternate pathway of Complement. This initiates the MAC attack and also opsonizes the bacteria for phagocytosis and killing by macrophages and neutrophils. Such bacteria, therefore, usually do not cause disease unless a person's Complement system is seriously defective. Some bacteria have evolved strategies to evade these "innate" protective mechanisms. One example is the production of a polysaccharide capsule which interferes with MAC insertion into the bacterial membrane and inhibits phagocytosis. A number of common infection causing bacteria invade mucosal surfaces (streptococci, *E. coli*) or skin (staphylococci). Often such bacteria also produce toxins which kill host cells (including phagocytes) and even damage distant organs.

To deal with these invaders, antibody must be produced to the capsule antigens and to the toxins to neutralize them. This allows efficient opsonization, and the activation of Complement, via Fc of IgG. Toxins are neutralized by antibody. Once these infectious bacteria are taken up by phagocytes they are killed within the phagosome by oxygen dependent (H_2O_2 and HOCl) and nitrogen dependent (NO) mechanisms as well as by digestive enzymes and defensin proteins. Thus, this class of bacteria causes disease as long as they can avoid phagocytosis. Antibody is critical for recovery from such infection and protection from repeat infection by the same strain. This is best exemplified by patients who don't make adequate antibody responses and thus have serious problems with extracellular bacterial pathogens (see above).

11.2 Intracellular Bacterial Pathogens

This group of bacteria are resistant to direct Complement attack and are able to survive in phagocytic cells. They are much harder for the immune system to deal with because they are not free in the tissues where they could be attacked by antibody-mediated processes (antibody cannot diffuse into the phagocytic cell).

Intracellular bacteria get into cells by phagocytosis, not by specific receptor interaction like viruses. Once inside the cell in a phagosome some of the bacteria are killed in the normal manner but many remain alive and can multiply. Some of these bacteria are capable of preventing phagosome/lysosome fusion, which protects the bacteria in the phagosome from destruction. Some types of intracellular bacteria (such as *Listeria*) can escape the phagosome and live free in the cytoplasm, while some types (like *M. tuberculosis*) remain in the phagosome. The immune system deals with these two types differently.

Intracellular bacteria that do not escape the phagosome are the hardest to deal with. The immune system has no way of getting at the bacteria because bacterial antigens are not shown in the context of Class I MHC on the surface of the cell (usually a macrophage). The only way to deal with this situation is to wall off the infected cells. This is a version of the DTH response and is mediated by IFN_-producing Th1 cells which recruit and activate macrophages. The macrophages secrete IL-1 and TNF-α which, in turn, recruits more macrophages. The resulting influx of cells walls off the infected cells and creates a "granuloma". This is the way in which tuberculosis is fought in the lung and other organs (brain, kidney, bone). This granuloma formation is also a big reason for the significant pathology of TB in the lung and other organs since both the bug, and the immune response to the bug, cause pathology. The TB skin test for prior exposure to *M. tuberculosis* uses a bacterial protein (PPD) to re-activate memory Th1 cells created by prior infection. These memory cells induce a similar DTH-like response at the site of PPD introduction but a full granuloma is not seen because of the absence of persistent antigen produced by live mycobacteria.

Intracellular bacteria that escape the phagosome (like *Listeria*) produce bacterial antigens in the cytoplasm. This results in antigen presentation in the context of Class I MHC on the surface of the infected cell. The role of Class I MHC is to sample peptides from the cytoplasm of the cell and bring them to the cell surface for examination by the immune system. The presence of bacterial proteins in the cytoplasm will thus bring the cell to the attention of CD8+ killer T cells (CTL) which specifically recognize the bacterial peptide in the context of Class I MHC. The result is death of the cell and release of the bacteria making them accessible to antibody and to "activated" macrophages with increased killing potential. The activated macrophages are the result of the production of IFN-γ by Th1 cells generated after initial infection. The end result in healthy individuals is bacterial death several days to 2 weeks after initial infection, recovery and Th1 memory (giving immunity to re-infection).

11.3 Viral Infections

There are three main ways in which the immune system deals with viral infections. The first is by the production of IFNα and IFNβ. These are produced by many tissue cell types and work at the local site by inhibiting viral replication in the infected cell. The second, and more important, way the immune system deals with viral infection is by the production of neutralizing antibody which prevents viral attachment to, and infection of, the target cell.

The third and most important way the immune system deals with viral infection is by killing the infected cell by means of CTL. When the virus enters the cell viral proteins are manufactured in the cytoplasm. As such they are sampled, like all cytoplasmic protein, and brought to the surface in the context of Class I MHC. Viral proteins in the cytoplasm of cells results in viral peptides being presented in the context of Class I MHC on the surface of the infected cell. This leads to recognition of the virally infected cells by CD8+ T cells with TcR specific for the viral peptide. These cells develop into CTL and will kill the target cell. Killing the target cell does not actually kill the virus but it inhibits viral replication because the virus producing factory (the infected cell) has been terminated.

11.4 Extracellular Protozoa

Extracellular protozoa, like extracellular bacteria, exist "in the open" and are thus highly susceptible to the activities of antibody. The type of antibody and the type of antibody activity depends on the site of the infectious agent. For example, *Giardia lamblia*, a common intestinal protozoan infection in North America, attaches to the epithelial lining of the gastrointestinal tract by two large suckers. IgA, secreted into the gut, will coat these organisms and block attachment to the gut wall causing expulsion. Because they live in the gut lumen, these protozoa are less likely to encounter IgG or IgM.

Trypanosomes, in contrast, live in the blood and are susceptible to IgG activity (opsonization for phagocytosis and Complement-mediated lysis). In fact, a very robust IgG response is created to these organisms, which is sufficient to clear most of the parasites from the blood. Unfortunately, some of the remaining organisms change their surface coat and are no longer susceptible to the antibody response. They flourish in the blood until a new antibody response is launched at which point they are mostly killed off (but not all and the remaining ones change their surface coat etc.). Trypanosomes are the causative agent of "African Sleeping Sickness".

11.5 Intracellular Protozoa

Some intracellular protozoa, like intracellular bacteria, are taken up by macrophages by phagocytosis (*Leishmania* is an example). Others actively penetrate the cells (malaria). The ones that live in macrophages act to prevent phagosome/lysosome fusion. In addition, since IFN-γ activates macrophages to be better killers, and the bugs obviously don't want this, they decrease macrophage responsiveness to IFNγ.

Leishmania infections are extremely difficult to treat. Antibiotics have no effect and they are hidden from the immune system. *Leishmania* lives inside a vesicle and thus CTL activity is not activated. DTH-like responses are not sufficient to clear it. Visceral leishmaniasis is usually fatal.

One of the first recorded uses of a vaccine was to actively immunize children by using cutaneous *Leishmania* organisms, which share antigens with the organisms that cause the more serious visceral disease. Cutaneous lesions from infected individuals were scraped and then applied to abrasions on the back of naive children. This not only protected them from cutaneous lesions on the face but protected them from visceral leishmaniasis.

Malaria evades the immune system in a number of ways. First, there are a variety of life cycle stages that do not share dominant antigen epitopes. Thus, a robust antibody response to the sporozoite stage does not have an effect on the merozoites. In addition, the various life cycle stages spend very little time in the blood stream where they would be open to attack. The infectious sporozoite, for example, finds a happy home in a liver cell within minutes of infection. If antibody is made to some of the stages, they simply slough off their surface coat before Complement fixation or phagocytosis can occur.

Further, the most important stage of the parasite life cycle, the merozoite, lives inside red blood cells which are non-nucleated and have no chance of killing the parasite and do not express Class I or II MHC.

Although there are treatments for malaria, multi-drug resistant forms are already spreading throughout Southeast Asia. Attempts to reduce levels of the mosquito host have failed since DDT was banned. Vaccine development has concentrated on trying to prevent infection by obtaining an effective antisporozoite antibody response. Although you don't hear about this disease much, it still kills millions of children a year in developing countries.

11.6 Helminth Parasites

There are a very large variety of helminth (worm) parasites which affect humans. There appears to be very little immune response to tapeworm (cestode) infection and, unless the worm load is enormous, very little pathology is associated with it. However, there can be significant pathology associated with roundworm (nematode) infection and with the various flukes (trematodes).

The most common roundworm infection in the world is *Ascaris lumbricoides*. This intestinal parasite infects nearly one billion people, mostly in third world countries. It is about the size of a pencil and some children have up to 200 worms in their guts. Morbidity and mortality are largely due to intestinal blockage from high worm burdens. The worms persist for a long time which suggests a limited response but anti-worm antibody and cells reactive with worm antigens can be isolated from infected patients. However, the size of the worm, the environment it lives in and its thick chitin surface probably protect it well from immune responses. While in the gut it is most likely that gut inflammation, with excessive mucus secretion and increased peristalsis, may play a significant role in fighting these lumen dwelling worms by hastening expulsion.

Many nematodes have life cycle stages that spend time in the tissues. These can activate responses that can impede the development of similar larval stages after re-infection. This may involve eosinophils and be the reason that high levels of eosinophils are seen in the blood of patients with helminth infections. Some nematodes, such as *Trichinella spiralis* (the causative agent of trichinosis from eating infected, undercooked pork) have life cycle stages that spend a lot of time in the tissues. These initiate DTH-like responses and sometimes granuloma.

One of the most important trematode infections is *Schistosoma mansoni*, the causative agent of schistosomiasis. This parasite lives in the portal system. Eggs produced by this parasite are destined to go to the intestine and thus out into the world to produce the next stage. However, given the direction of blood flow in the portal vessels it is not surprising that many of them end up in the liver where they cause extensive inflammation, hepatomegaly and death. Because they live in the portal system the immune system has a very difficult time with them because antigens introduced into the portal circulation tend to induce immune tolerance. This is probably because antigens in the portal blood are usually food antigens from the intestine and "oral tolerance" mechanisms prevent these antigens from being seen as foreign.

One of the most striking characteristics of the immune response to helminths is the dramatic activation of Th2 type responses including high IgE and eosinophil levels in the blood. This Th2 response is so robust that it will change the normal response to other non-helminth antigens if they are delivered at the same time. This has suggested to some that the activation of the type 2 response by helminths is an evasion strategy.

Clinical Note – IgE and Eosinophil Response to Parasitic Infection

Chapter 12: Vaccines

In the Western world, almost all children will be vaccinated against common childhood diseases such as measles and mumps. In some cases, re-vaccination will continue on a regular basis throughout adult life against the potential of contracting diseases such as diphtheria or tetanus. Vaccination is also widely used to protect people of all ages when they travel to countries where pathogenic diseases are common. The development of effective vaccines against fatal, intractable diseases such as HIV infection and malaria is an ongoing process but is proving very difficult.

On a population basis, vaccination is critical to reduce the level of disease in the population. However, as many childhood diseases have reached record low levels of incidence in North America, individual parents have come to view the risks of vaccination of their children as unacceptably high in comparison to the potential threat of infection (and the complications of the infectious disease). This view is becoming more widespread in the individual-centered society of the USA. The risk it poses to the population in general (and to the individual children) is the resurgence of large outbreaks of childhood diseases with significant mortality or permanent damage as outcomes. This issue will present itself in the offices of family practitioners in both Canada in the USA increasingly often.

Vaccination, or immunization, refers to the introduction of a modified infectious agent, or a derivative of the infectious agent, into the body to create an active immune response. In North America children are now routinely vaccinated against diphtheria, tetanus, pertussis, polio and *H. influenzae* (Pentacel or PPTPActHib); against measles, mumps and rubella (MMR); against Varicella (chickenpox); and against hepatitis B. Most places are now also routinely immunizing against pneumococcus and meningococcus infections in infancy. In most cases immunization must be repeated to get the best protection. The repeat immunizations are often referred to as “booster” shots because they boost protection.

There are a large variety of vaccine types which vary in their efficacy and safety. In general, the closer the vaccine agent is to the original infectious pathogen the better the vaccination efficiency but the less safe the vaccine.

Vaccines can be roughly broken down into two main groupings

1. **Whole organism vaccines**
2. **Subunit vaccines**

12.1 Whole Organism Vaccines

These vaccines use the infectious pathogen itself for immunization. However, the pathogen is either in a killed form or so modified (attenuated) that it does not cause the full natural ("wild type") disease.

12.1.1 Live Vaccines

The most effective vaccine is usually a live infectious agent that has been somehow modified (attenuated) to make it non-pathogenic. Originally this was done by chemical means but more recently genetically manipulated (crippled) microorganisms have been used which lack the ability to live for long periods or cause significant disease. These vaccine agents bear the same broad spectrum of antigens as the pathogen. Because they are alive and they replicate for a period in the host they persist for longer periods than killed vaccine agents. For this reason they stimulate the most effective and long lasting immunity to the natural infection. They generate both antibody (including secretory IgA if the vaccine agent is inhaled or ingested) and cell-mediated responses to the agent.

The down side of live vaccines is that, on rare occasions, they revert back to the pathogenic form. Furthermore, in a patient with an unrecognized immune deficiency, even the "attenuated" vaccine agent may cause serious disease. Thus, these vaccines cause significant disease, even death, in a small but predictable number of vaccinated people every year (usually less than 1or 2 per million). While the incidence of serious morbidity or mortality is extremely low, in a large country the number of cases can be significant. Obviously, these vaccines should never be used in patients with known or suspected immune deficiency (for example, in children having frequent infections already).

12.1.2 Killed Vaccines

Killed vaccines have the advantage of being much safer than live attenuated vaccines. They cannot revert back to the pathogenic form if they are killed properly. Although safer than attenuated vaccines, killed vaccines are less effective. Killed "whole cell" (whole organism) vaccines generally retain the full spectrum of antigens of the pathogen, although some of these antigens are damaged by the killing process. In addition, because the agent is not alive and cannot replicate it is quickly cleared from the body and does not activate the immune response as well as a live organism. Although killed vaccines are not as effective as live attenuated vaccines they may give better protection than isolated component (sub-unit) vaccines because they present a wider spectrum of antigens and activate a greater inflammatory response which induces more effective antigen presentation by dendritic cells. However, because of this, such vaccines have more side effects such as local pain, fever, allergic and hypersensitivity reactions.

12.2 Sub-unit Vaccines

Sub-unit vaccines refer to vaccine agents that are a component of the pathogen rather than the entire pathogen. In general, they are very safe because the immunizing agent is not a whole organism and cannot cause disease and there are usually fewer unpleasant side effects.

12.2.1 Isolated Protein Vaccines

These vaccines consist of a potentially immunodominant protein isolated from the pathogen. They have the advantage of being well defined and characterized as to any adverse biological activity. They also allow for the targeting of the immune response to specific immunodominant surface antigens which will lead to effective immune clearance of the infectious agent upon challenge. But they have serious limitations. Because they are isolated protein they do not activate a response as broad or as robust as the whole organism vaccines do. Activation of cell-mediated immunity is particularly difficult to achieve. The development of the immune response to these proteins can be enhanced by the use of substances called "adjuvants" which initiate inflammation and cause prolonged antigen release. In animals, robust responses can be achieved by using adjuvants containing killed mycobacterium in oil to initiate significant inflammatory responses. These powerful adjuvants cannot be used in humans. In humans, adsorption of the protein antigen to alum, which is composed of aluminum hydroxide crystals, is usually used to initiate a mild local inflammatory response that will enhance antigen uptake and presentation by APCs.

Recently, researchers have been using soluble protein vaccines delivered using liposomes (microscopic lipid vesicles) to overcome some of the problems above. Liposomes are readily taken up by antigen presenting cells and engender a good immune response. In addition, the use of recombinant DNA technology has helped solve the problem of the availability of pure protein.

One area where isolated protein vaccines remain essential is the immunization against microbial toxins. Immunization with inactivated toxin, called a toxoid, generates a response directed at a particular pathogenic product of an infectious agent. The aim here is to inactivate (neutralize) the toxin by generating antibody which binds to it and then clears it from the system. The aim is not to immunize against the infectious agent because it is only their toxin that causes disease. Immunization with tetanus toxoid and diphtheria toxoid is routine and protects against the potentially fatal effects of tetanus toxin and diphtheria toxin during infection with *Clostridium tetani* or *Corynebacterium diphtheriae*. More recently, the new pertussis (whooping cough) vaccine employs (*Bordetella*) pertussis toxoid to generate a protective response without the serious side effects of the pertussis whole-organism vaccine

used for over 40 years.

12.2.2 Vector Vaccines

In these vaccines the DNA of the infectious agent is placed into a live viral (or bacterial) vector to make a chimeric (hybrid) agent. The idea is to use a nonpathogenic virus (or bacteria) which replicates, but is self-limiting, in the patient and to design that agent so that a non-pathogenic, but antigenic, protein from the pathogen of interest will be produced after immunization. These vaccines represent the best of both worlds in that the vaccinating agent is alive and producing the protein of the pathogen of interest but is unrelated to the pathogen, cannot cause disease and is, in itself, benign, even though it can temporarily replicate. These vaccines can stimulate good antibody mediated and cell mediated immunity. However, they do not deliver a wide spectrum of antigens so the response is quite restricted.

12.2.3 Polysaccharide Conjugate Vaccines

Basic principles suggest that immunization with polysaccharides will give you, at best, an IgM response. This is because a robust IgG response cannot happen without class switch and class switch depends on T cell activation. T cell activation cannot occur unless antigenic peptide is presented to T cells. There is no antigenic peptide in polysaccharide vaccines.

However, these vaccines do work in humans, although only after the age of 2-3 years. The reasons for this are just beginning to be known. The first is that the polysaccharides probably bind to self proteins known as "haptens". This creates a self protein/polysaccharide chimera which can be taken up by dendritic cells for activation. In addition, and more likely, we now know that an MHC-like molecule called CD1 is capable of presenting non-peptide antigens to some T cells. It may be that these polysaccharide agents are presented in this manner.

Activation of effective IgG immune responses to polysaccharides is not just cause for an interesting immunological discussion. In children, IgG antibody to polysaccharides is the last to develop during immune maturation yet polysaccharide encapsulated bacteria (such as *Haemophilus influenzae* and *Streptococcus pneumoniae*) are major causes of serious and even fatal infections (meningitis, bacteremia, pneumonia) in the first two years of life. To address this challenge, polysaccharides are now chemically coupled to proteins to form so-called "conjugate" vaccines. Current examples are *H. influenzae* polysaccharide conjugated with diphtheria toxoid (Act-Hib) and *S. pneumoniae* polysaccharides conjugated to diphtheria toxoid (Prevnar). These can induce specific IgG to the polysaccharide component following immunization starting as early as 2 months of age (although boosting is required). These vaccines are now part of the recommended primary immunization schedule for babies.

These new vaccines have dramatically decreased the frequency of the infections mentioned above. This is a major advance because it decreases the need for antibiotic use in an era of increasing problems of evolution of antibiotic resistant bacteria. A similar polysaccharide conjugate vaccine against the most common strain (C) of *Neisseria meningitides*, which causes not only meningitis but also lethal bacteremia and septic shock, is now available and recommended for children.

Pure polysaccharide vaccines may still be used for adults (such as Pneumovax23 that contains 23 different polysaccharides from *S. pneumoniae*) but even in adults the conjugate vaccines induce a longer lived response and better IgG memory than the pure polysaccharide vaccines.

12.3 Passive Immunity

Passive immunity refers to the transfer of antibody from one individual to another. This does not confer long-term protection but is useful for situations of imminent danger when there is no time to generate antibody by the patient and in cases of immune deficiency where antibody cannot be made. The isotype transferred is IgG because it is the most long lasting (with a half life of 28 days) and it confers the best protection.

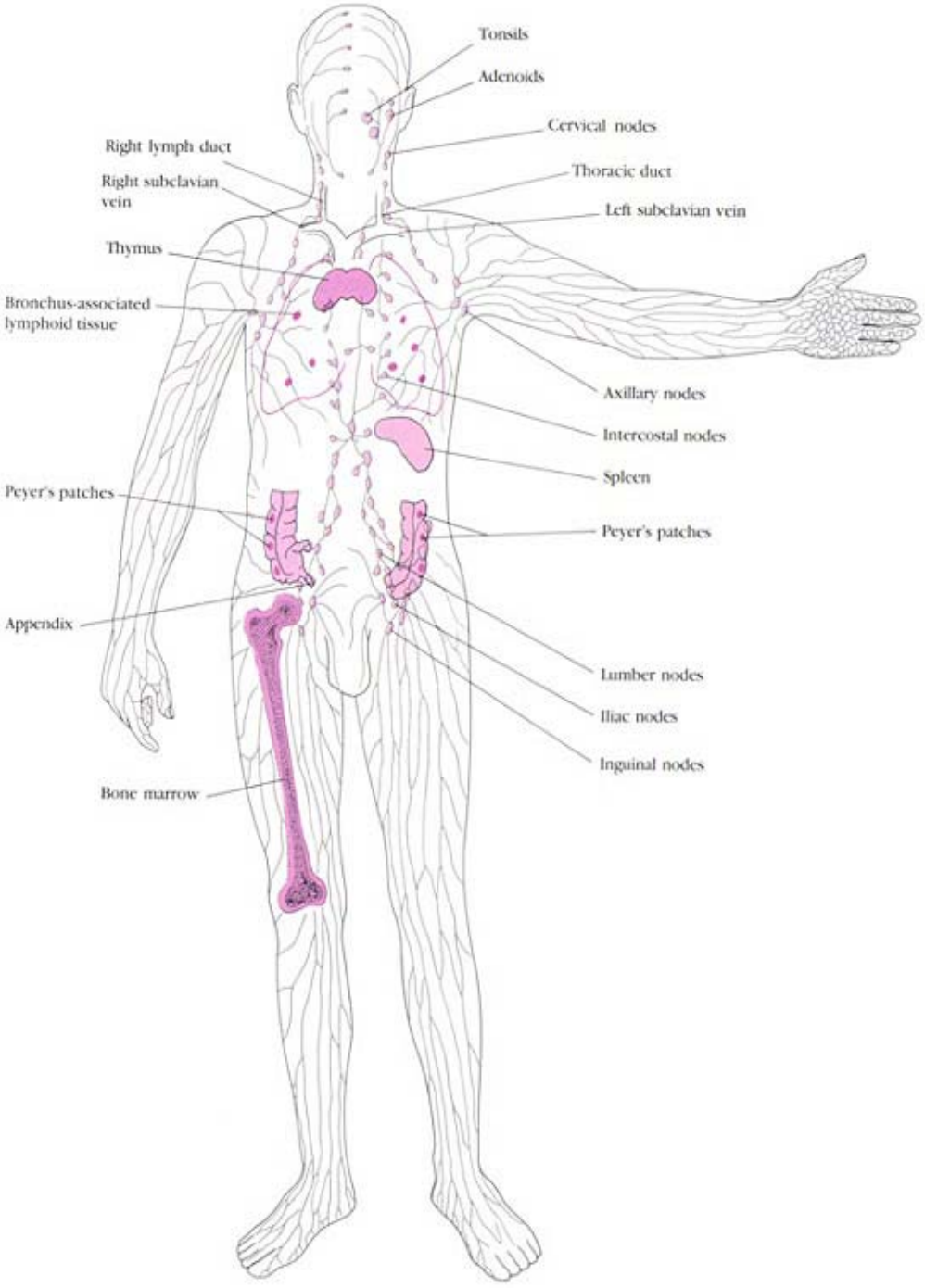
All of us received passive immunity (IgG only) from our mothers *in utero*. Trans-placental passage of IgG from mother to fetus confers protection for the first few months after birth. Passive immunity is also used in the case of tetanus infection. If active protection against tetanus has not been induced due to lack of immunization, or has waned because it has been many years since the last booster immunization then immediate protection in the form of anti-tetanus IgG antibody will be required. Passive immunization with anti-rabies IgG is used after an animal bite (such as dog, raccoon, and bat) where rabies is prevalent. In both of these examples, active immunization should also commence immediately to provide continuing protection (by the patient's own antibody) once the passive IgG is gone after a few weeks. Similarly, antivenin antibodies are used for spider bites or snake bites. Often these antibodies are produced in other species such as horses and the IgG from their blood is purified.

Patients who cannot make adequate IgG antibody responses and thus have humoral immune deficiency usually require monthly administration of pooled IgG (also called "gammaglobulin" or "immune globulin") to protect them against many types of infections. This is usually given intravenously. In other cases, passive protection against a specific infection needs to be given when someone is at high risk of severe disease (for example, chicken pox in an immuno-suppressed cancer patient who never had chicken

pox or a baby during birth to a hepatitis B-carrying mother). Disease-specific IgG should be administered intramuscularly in such settings.

Immune globulin (pooled IgG) is made from the blood plasma of thousands of human donors and thus confers protection against infections experienced by the population as long as IgG is sufficient for protection. It is a relatively safe product with no transmission of known infectious agents (for example HIV, hepatitis A, B or C) using current production methods. However, caution must be shown with its use because of allergic and other reactions in a few percent of patients.

Appendices Appendix 1: Lymphoid Tissue



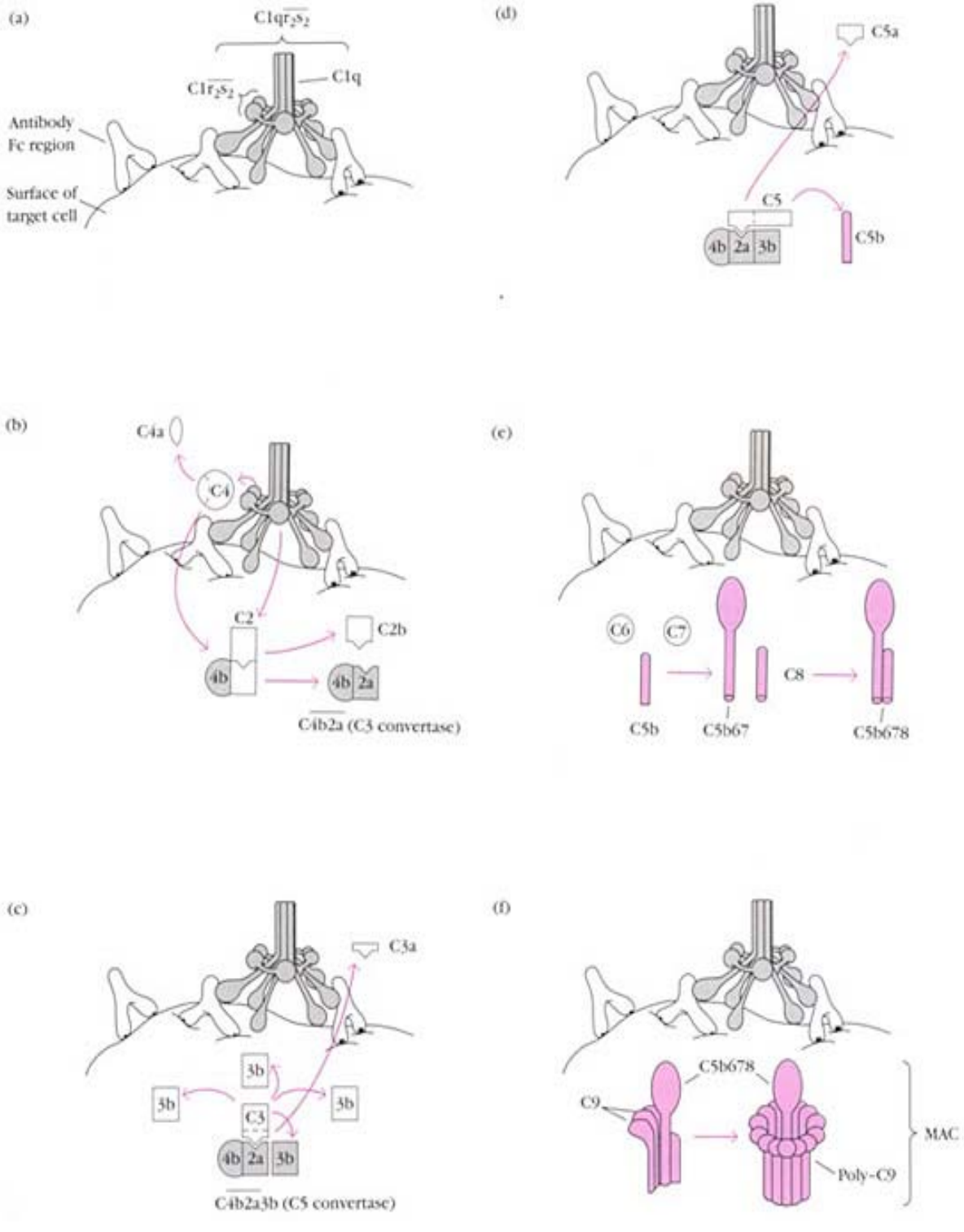
Appendix 2: Complement

Complement has an important role in clearing antigen. This is done by the four major functions of Complement activation:

1 Local vasodilation
2 Attraction of immune cells, especially phagocytes (chemotaxis)
3 Opsonization (or tagging) of foreign organisms for phagocytosis
4 Destruction of invading organisms by the membrane attack complex (MAC attack)

A simplified version of the Complement pathway is shown on the next page.

As you can see, the central molecule is the C3 protein. The products of the C3 split include a small peptide C3a which is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5. The other part of C3, C3b coats antigens on the surface of foreign organisms and acts to opsonize or 'tag' the organism for destruction. C3b also reacts with other components of the Complement system to form the membrane attack complex (MAC) consisting of C5b, C6, C7, C8 and C9.


(a)
C1qr2s2
C1q
C1r2s2
Antibody
Fc region
Surface of
target cell
(b)
C4a
C4
C2
C2b
4b
4b 2a
C4b2a (C3 convertase)
(c)
C3a
3b
3b
3b
C3
4b 2a 3b
C4b2a3b (C5 convertase)
(d)
C5a
C5
4b 2a 3b
C5b
(e)
C6
C7
C8
C5b
C5b67
C5b678
(f)
C5b678
C9
MAC
Poly-C9

Appendix 3: Clinical Note - Normal Antibody Levels

IgG 1-3 mos 1.46 - 6,48 g/L 7-12 mos 2.69 - 9.13 g/L 3-5 yrs 5.18 - 14.47 g/L 9-11 yrs 7.74 - 16.41 g/L Adult 5.64 - 17.65 g/L	**IgA** 0 - 0.23 g/L 0.08 - 0.54 g/L 0.23 - 1.37 g/L 0.52 - 2.56 g/L 0.85 - 3.85 g/L	**IgM** 0.19 - 1.30 g/L 0.27 - 0.80 g/L 0.43 - 1.98 g/L 0.41 - 2.47 g/L 0.53 - 3.75 g/L

Appendix 4: Clinical Note Immunization

The term immunization, which is derived from the Latin *immunitas*, meaning immunity, refers to the process of rendering a patient immune to a particular pathogen or foreign substance. There are two types of immunization, passive and active. Passive immunization involves the transfer of antibodies from an immune individual to a non-immune individual. An example of passive immunization is the immunity acquired *in utero* from antibodies which pass from the mother to the fetus through the placenta. This form of immunity is relatively short-lived because the transferred antibodies survive in the circulation for only a few weeks before they are degraded and removed. Active immunization involves the deliberate exposure of a patient's immune system to killed or attenuated live microorganisms in the form of a vaccine in order to induce a state of specific immunity. Immunity acquired in this fashion is long-lasting because active immunization creates a pool of memory lymphocytes (T cells and antibody-producing B cells) that are specific for the immunizing antigen. Active immunization can also result from natural infections, explaining the resistance of previously infected individuals to further exposure to the pathogen.

Appendix 5: Clinical Note - IgE and Eosinophil Response to Parasite Infection

Helminth (worm) infection leads to a dramatic increase in IgE and eosinophils in the circulation. There is a fundamental question at the moment as to whether this is a protective response against the helminths or a "red herring" (irrelevant) response induced by the parasite to escape a more effective immune response. Most of these parasites are, in fact, more avidly killed by IgG and macrophages than by IgE and eosinophils. In addition, only a small part of the IgE induced during helminth infection is directed at the worm. The rest is "non-specific" and does not recognize worm antigens. Stay tuned to this one.

Appendix 6: Clinical Note - B cell Malignancies

B cell malignancies or neoplasms may arise in all lymphoid tissues where B cells are normally being produced. Most patients with B cell neoplasms are initially diagnosed with disease involving bone marrow or lymph nodes. In the case of bone marrow involvement, the transformed B cells frequently circulate through the blood and become widely disseminated throughout peripheral lymphoid tissues. However, B cell malignancies may also arise in some non-lymphoid tissues such as the thyroid, gastrointestinal tract, salivary glands and conjunctiva. In North America and Europe, B cell neoplasms are more common that T cell neoplasms. Diagnosis is usually based on morphologic criteria, immunophenotyping, and the presence of monoclonal immunoglobulins in serum and/or urine and on the surface or cytoplasm of lymphocytes. Chromosome abnormalities may also be present. The transformation of B cells from small resting lymphocytes to large proliferating (transformed) lymphocytes, and the resulting displacement of normally functioning cells in the bone marrow and other lymphoid tissues relate to the clinical features of the disease. Multiple myeloma and Burkitt's lymphoma are two examples of B cell neoplasms.