**LECTURE NOTES 6**

**B CELLS, ANTIBODIES AND ANTIGENS**

**B CELLS**

B cells perform two important functions

1. Theydifferentiate into plasma cells and produce antibodies

2. They can resent antigen to helper T cells

During embryogenesis B cell precursors are recognized first in the fetal liver. From there they migrate to the bone marrow, which is their main location during adult life.

Unlike T cells, they do not require they thymus for maturation. Pre B cells lack surface immunoglobulins and light chains but do have µ heavy chains in the cytoplasm.

The maturation of B cellshas two phases, antigen independent phase consists of stem cells, pre B cells and B cells, whereas the antigen dependent hase consists of the cells that arise subsequent to the interaction of antigen with the B cells e.g. activated B cells and plasma cells.

B cells display surface IgM, which serves as a receptor for antigens. This surface IgM is a monomer, in contrast to circulating IgM, which is a pentamer. The Monomeric IgM on the surface has an extra transmembrane domain that anchors the protein in the cell membrane that is not present in the circulating pentameric form of IgM.

Surface IgD on some B cells may also be an antigen receptor. Re B cells are found in the bone marrow, whereas B cells circulate in the bloodstream.

B cells constitute about 30% of the recirculating pool of small lymphocytes and their life span is short, i.e. days or weeks. Approximately 109 B cells are produced each day. Within lymph nodes they are located in germinal centers within the spleen, they are found in the white pulp. They are also found in the gut associated lymphoid tissue e.g. payer's patches.

**Clonal selection**

Clonal selection accounts for antibody formation. Each individual has a large pool of B lymphocytes (about 107). Each immunologically responsive B cell bears asurface receptor (Either IgM orIgD) that can react with one antigen (orclosely related group of antigens). There are about 107 different specificities. An antigen interacts with the B lymphocyte that shows the best fit with its immunoglobulin surface receptor. After the antigen binds, the B cell is stimulated to proliferate and form a clone of cells. These selected B cells soon become plasma cells and secrete antibody specific for the antigen. Plasma cells synthesize the immunoglobulins with the same antigenic specificity (they have the same heavy chain and the same light chain) as those carried by the selected B cell. Antigenic specificity does not change when heavy chain class switching occurs.

Clonal selection also occurs with T cells. The antigen interacts with a specific receptor located on the surface of either a CD4 positive or a CD8 positive T cell. This selects this cell and activates it to expand into a clone of cells with the same specificity.

**Activation of B cells**

Multivalent antigens binds to surface IgM or IgD and crosslink adjacent immunoglobulin molecules. The immunoglobulins aggregate to form atches and eventually migrate to one pole of the cell to form a cap.

Endocytosis of the capped material follows, the antigen is processed and epitopes appear on the surface in conjunction with class II MHC proteins. This complex is recognized by a helper T cell with a receptor for the antigen on its surface,The T cell now produces various interleukins (IL 2, IL 4, and IL 5) that stimulate the growth and differentiation of the B cell.

The activation of B cells to produce the full range ofantibodies requires two other interactions in addition to recognition of the epitope by the T cell antigen receptor and the production of IL 4 and IL 5 by the helper T cell.

These costimulatory interactions which occur between surface proteins on the T and B cells are as follows

1. CD28 on the T cell must interact with B7 on the B cell
2. CD40L and the T cell must interact with CD40 on the B cell. The CD28-B7 interaction is required for activation of the T cell to produce IL 2 and the CD40L –CD40 interaction is required for class switching from IgM to IgG and other immunoglobulin classes to occur.

**Effector functions of B cells**

The end result of the activation process is the production of many plasma cells that produce large amounts of immunoglobulins specific for the epitope. Plasma cells secrete thousands of antibody molecules per second for a few days and then die. Some activated B cells form memory cells, which can remain quiescent for long periods but are capable of being activated rapidly upon re exposure to antigens.

Most memory cells have surface IgG that serve as the antigen receptor but some have IgM. Memory T cells secrete interleukins that enhance antibody production by the memory B cells. The presence of these cells explains the rapid appearance of antibody in the secondary response.

**ANTIBODIES**

Antibodies are globulin proteins (immunoglobulins) that react specifically with the antigen that stimulated their production. They make up to 20% of the protein in blood plasma. Blood contains three types of globulins, alpha, beta and gamma based on their electrophoretic migration rate.

Antibodies are gamma globulins. There are five classes of antibodies; IgG, IgM, IgA, IgD and IgE. Antibodies are subdivided into these classes based on differences in their heavy chains.

The structure and characteristics of antibody molecules are critical for their functional properties. Recognizing these features will help you understand their essential roles in the host defences.

**Structure and properties of Antibodies**

Antibodies, also called immunoglobulins, are Y shaped proteins that have two general parts; The arm and the stem.

The two identical arms, called the Fab regions, bind antigens. The stem is the Fc region. These names were assigned following early studies that showed that enzymatic digestion of antibodies yielded two types of fragments ˗ fragments that were antigen binding (Fab) and fragments that could be crystallized (Fc). All antibodies have the same basic Y shaped structure called an antibody monomer.

It consists of two copies of high molecular weight polypeptide chain called the heavy chain .and two copies of a lower molecular weight polypeptide chain called the light chain. The amino acids in the chains fold into characteristic domains, referred to as immunoglobulin domains, the light chains have two domains each and most heavy chains have four. Each light chain is linked to a heavy chain by a disulfide bond. The fork of the Y is a flexible stretch called the hinge region and one or more disulfide bonds link the two heavy chains there.

**Constant Region** When the amino acid sequences of antibody molecules that bind to different epitopes are compared there is tremendous variation in the portion referred to as the variable region. The other portion is known as the constant region.

**Variable region**

The variable region is the portion at the ends of fab region it accounts for the antigen binding specificity.Part of the antigen binding site,the portion that attaches to a specific epitope.The fit needs to be precise,because the interaction depends on numerous non˗ covalent bonds to keepthe molecules together. Nevertheless, the antigen˗ antibody interaction is reversible, and the molecules can separate, leaving bothantigen and antibody unchanged.

The constant region includes the entire Fc region, as well as part of the two Fab regions. The consistent nature of this region allows other components of the immune system to recognize the otherwise diverse antibody molecules.

There are five general types of constant regions, and these are correspond to major classes of Immunoglobulin (Ig molecules, i.e. IgM, IgG, IgA, IgD and IgE. Each class has distinct functions and properties of antibodies.

**Protective outcomes of Antibody Antigen Binding**

The protective outcomes of antibody antigen binding depends on the antibody class and include;

**Neutralization**

Toxins and viruses must bind specific molecules on a cell surface before they can damage that cell. A toxin or virus coated with antibodies cannot attach to cells and said to be neutralized.

**Opsonization**

Phagocytic cells have receptors for the Fc region of IgG molecules, making it easier for the phagocyte to engulf antibody coated antigens. Recall from chapter 14 that the complement protein C3b opsonizes antigens; IgG molecules have a similar effect.

**Complement system activation**

Antigen antibody complexes(commonly called immune complexes) can trigger the classical pathway of complement system activation.

When multiple molecules of certain antibody classes are bound to a cell surface, a specific complement system protein attaches to their Fc regions, initiating the cascade.

Recall that activation of the complement system results in production of the opsonin C3b, initiation of an inflammatory response and formation of membrane attack complexes.

**Immobilization and prevention of adherence**

Binding of antibodies to flagella interferes with a microbes ability to move, binding to pili prevents it from attaching to surface.

These capabilities are often necessary for a pathogen to infect a host, so antibodies that bind to flagella or pili prevent infection.

**Cross linking**

The two arms of an antibody can bind separate but identical antigen molecules, linking them. The overall effect is that large antigen antibody complexes form, creating big mouthfuls of antigens for phagocytic cells to engulf.

**Antibody dependent cellular cytotoxicity (ADCC)**

When multiple IgG molecules bind to a virally infected cell or a tumor cell, that cell becomes a target for destruction by natural killer (NK) cells. The NK cell attaches to the Fc regions of IgG and once attached, kills the target cell by delivering compounds directly to it.

**Immunoglobulin classes**

All five major classes of immunoglobulins have the same basic monomeric structure, but each class has a different constant portion of the heavy chain. Some of the immunoglobulins form multimers of the basic monomeric structure.

**Characteristics of the various immunoglobulin**

**IgM**

IgM accounts for 5% to 13% of the circulating antibodies and is the first class produced during the primary response to an antigen. It is the principal class produced in response tosome T independent antigens.

IgM is a pentamer. Its large size prevents it from crossing from the bloodstream into tissues, so its primary role is to control bloodstream infections. The five monomeric subunits give IgM a total of 10 antigen binding sites, so it cross links antigens very effectively. It is the most efficient class in triggering the classical pathway of complement system activation.

A fetus is normally sterile until the birth membrane is raptured, so IgM generally begins being made about the time of birth. However, a fetus infected in utero can make IgM antibodies.

**IgG**

IgG accounts for about 80% to 85% of the total serum immunoglobulin (serum is the liquid portion of blood). It circulates in the blood but exists the vessels to enter the tissues as well.

IgG provides the longest term protection of any antibody class, its half-life is 21 days, meaning that a given number of IgG molecules will be reduced by about 50% after 21 days.

In addition, IgG is generally the first and the most abundant circulating class produced during the secondary response. The basis for this will be discussed later in the chapter. IgG providesprotection by neutralization,opsonization, complement activation,immobilization and prevention of adherence,cross linking and ADCC

An important characteristic of IgG is that it is transported across the placenta into the fetus bloodstream, so it protects the developing fetus against infections. Women who are notalready immune to a given pathogen lack IgG against the microbe, so they are warned to take extra precautions duringpregnancy toavoid pathogens that can infect and damage a fetus. For example,pregnant women are advised nottoeat raw meat or become first timecat owners to avoid primary infection by *Toxoplasmagondii*, a parasite found in steak tartare and other raw meat as well as the feces of infected cats.

Maternal IgG notonly protects the developing fetus, but also the newborn. The maternal antibodies resent at birth gradually degrade over a period of about 6 months, but during this time the infant begins producingprotective antibodies.

IgG is also in colostrum, the first breast milk produced after giving birth. The newborns intestinal tract absorbs this antibody.

**IgA**

The monomeric formof IgA accounts for about 10% to 13% of antibodies in the serum. MostIgA however is the secreted form,a dimer called secretory IgA (sIgA). In fact, IgA is the most abundant immunoglobulin class produced. The secreted form is important in mucosal immunity and is found on the mucous membranes that line the gastrointestinal, genitourinary and respiratory tracts. It is also in secretions such as saliva, tears and breast milk. Secretory IgA in breast milk protects breast fed infants against intestinal pathogens.

Protection by secretory IgA is primarily due to the direct effect of its binding.These include neutralizing toxins and viruses and interfering with the attachment of microbes to host cells.

IgA is produced by the plasma cells that reside in the mucosa associated lymphoid tissues (MALT. Recall that plasma cells are the antibody secreting form of Bcells. As IgA is transported across the mucosa (mucous membrane), a polypeptide called the secretory component is added. This attaches the antibody to the layer of mucus that coats the mucosal surface and protects it from destruction by enzymes there.

**IgD**

IgD accounts for less than 1% of all serum immunoglobulins. It is involved with the development and maturation of the antibody response, but its functions in blood have not been clearly defined.

**IgE**

IgE is barely detectable in blood, because most is tightly bound via the Fc region to basophils and mast cells, rather than being free in circulation.The boundIgEmolecules allow these cells to detect and respond to antigens. For example, when antigen binds to two adjacent IgE molecules carried by a mast cell, the cell releases histamine and other inflammatory mediators. IgE mediated responses seem to be important in eliminating parasites, particularly helminths.

Unfortunately for allergy sufferers, basophils and mast cells also release their chemicals when IgE binds to normally harmless materials such as food, dusts and pollens, leading to immediate reactions such as coughing, sneezing and tissue swelling. In some cases these allergic or hypersensitivity, reactions can be life threatening.

**NATURE OF ANTIGENS**

The term antigen was initially coined in reference to compounds that induce antibody production, it is derived from the descriptive expression antibody generator. Today, the term is used more broadly to describe any molecule that reacts specifically withan antibody,a Bcell receptor or a T cell receptor,itdoes notnecessarily imply that the molecule can induce an immune response. When referring specifically toan antigen thatelicits an immune response inagiven situation,the more restrictive term immunogen may be used. The distinction between the terms antigen and immunogen helps clarify discussions in which a normal protein from host A elicits an immune response when transplanted into host B the protein isanantigen because it can react with an antibody or lymphocyte but it is an immunogen only for host B not for host A.

Antigens include an enormous variety ofmaterials, from invading microbes andtheir products to plant pollens, but they fall into two general categories. Most antigens are T dependent antigens,meaning that the responding B cell requires assistance froma THcell in order to become activated. T dependent antigens characteristically have a protein component. In contrast, T independent antigens can activate B cells without TH cell help. They include lipopolysaccharides (LPS) and molecules with repeating subunits such as somecarbohydrates.

Various antigens differ intheir effectiveness in stimulating an immune response. Proteins generally induce a strong response,whereas lipids often do not. The terms antigenic and immunogenic are used interchangeably to describe the ability of an antigen toelicit an immune response. Small molecules are usually not antigenic.

Although antigens are generally large molecules, the adaptive immune system recognizes discrete regions of the molecule known as epitopes, or antigenic determinants. Some epitopes are stretches of 10 or so amino acids, whereas others are three dimensional shapes such as a region that sticks out in a molecule. A bacterial cell usually has a diverse assortment of macro molecules on its surface, each with a number of distinct epitopes, so the entire cell has a multitude of different epitopes.

**The features of molecules (antigens) that determine immunogenicity**

**Foreignness**

In general molecules recognized as "Self" are not immunogenic i.e. we are tolerant to those self-molecules. Tobe immunogenic, molecules must be recognized as "non˗self" i.e. foreign.

**Molecular size**

The most potent immunogen are proteins with high molecular weight i.e. above 100,000. Generally, molecules with molecular weight below 10,000 are weakly immunogenic and very small ones e.g. an amino acid are non˗immunogenic. Certain small molecules e.g. haptens become immunogenic only when linked to a carrier protein.

**Chemical structural complexity**

A certain amount of chemical complexity is required e.g. amino acid homopolymers are less immunogenic than heteropolymers containing two or three different amino acids.

**Antigen determinants (Epitopes)**

Epitopes are small chemical groups on the antigen molecule that can elicit and react with antibody. An antigen can have one or more determinants (epitopes). Most antigens have many determinants i.e. they are multivalent. In general, a determinant is roughly five amino acids or sugars in size. The overall three dimensional structure is the main criterion of antigenic specificity.

**Dosage, Route and Timing of Antigen Administration**

These factors also affect immunogenicity. In addition, the genetic constitution of the host determines whether a molecule is immunogenic. Different strains of the same species of animal may respond differently to the same antigen.

**Adjuvants**

Adjuvants enhance the immune response to an immunogen. They are chemically unrelated to the immunogen and differ from a carrier protein because the adjuvant is not covalently bound to the immunogen, whereas the carrier protein is. Adjuvants can act in a variety of ways: cause slow release of immunogen, thereby prolonging the stimulus, enhance uptake of immunogen by antigen resenting cells and induce costimulatory molecules ("second signals").

Another important mechanism of action of some adjuvants is to stimulate Toll Like receptors on the surface of macrophages, which results in cytokine production that enhances the response of T cells and B cells to the immunogen (antigen).Some human vaccines contain adjuvants such as aluminum hydroxide or lipids.

**B Lymphocytes and the antibody response**

Most antigens are T dependent antigens, meaning the B cells that recognize them require help from TH cells, the response to these antigens will be the primary focus of this section. The B cell response to T independent antigens will be covered at the end of this section.

**B cell activation**

Naïve B cells gather in the secondary lymphoid organs to encounter antigens. When a B cell's antigen receptor (B cell receptor) binds to a T dependent antigen, the B cell takes the antigen in by endocytosis, enclosing it within an endosome. There, the antigen is degraded into peptide fragments that are then delivered to protein structures called MHC Class II molecules. These moves to the B cell surface, where they present pieces of the antigen for inspection by TH cells a process called antigen presentation.

TH cells which also gather in the secondary lymphoid organs, scan the naïve B cells there to determine if any encountered an antigen they recognize. If a TH cells antigen receptor, TCR, binds one of the peptide fragments being resented by a B cell, then that T cell activates the B cell. It does this by delivering cytokines to the B cell, initiating the process of clonal expansion of that articular B cell.

If no TH cell recognize the peptides presented by a B cell, that B cell may become anergic (unresponsive to future exposure to the antigen). This results in tolerance to that antigen, a mechanism the adaptive immune system uses to avoid responses against self and other harmless antigens.

**Characteristics of the primary response**

In the first (primary) exposure to an antigen, it takes about 10 to 14 days for a substantial amount of antibodies to accumulate. During this delay, the person might experience signs and symptoms of an infection, which could be life threatening. The immune system however, is actively responding.

Naïve B cells that bind the antigen present the peptide fragments to TH cells. Once activated, those B cells multiply, generating a population of cells that recognize the antigen. As some of the activated B cells continue dividing, others differentiate to form antibody secreting plasma cells.

Each plasma cell generally undergoes apoptosis after several days, but activated B cells continue multiplying and differentiating, generating increasing numbers of plasma cells as long as antigen is present. The result is a slow but steady increase in the titer (concentration) of antibody molecules.

Over time, the proliferating B cells undergo changes that improve the immune response.

**These include,**

**Affinity maturation**

This is a form of natural selection among proliferating B cells. As activated B cells multiply, spontaneous mutations commonly occur in certain regions of the antibody genes. Some of these result in slight changes in the antigen binding site of the antibody and therefore the B cell receptor. B cells that bind antigen for the longest duration are most likely to proliferate.

**Class switching**

All B cells are initially programmed to differentiate into plasma cells that secrete IgM. Cytokines produced by TH cells, however, induce some activated B cells to switch that genetic program, causing them to differentiate into plasma cells that secrete other antibody classes. B cells in the lymph nodes most commonly switch to IgG production. B cells in the mucosa associated lymphoid tissues generally switch to IgA production, providing mucosal immunity.

**Characteristics of Secondary response**

The secondary response is significantly faster and more effective than the primary response. Repeat invaders are generally eliminated before they cause noticeable harm. This is why a person who has recovered from a particular disease generally has long lasting immunity in that disease.

Vaccination takes advantage of this naturally occurring phenomenon.

**The response to T independent antigens**

T independent antigens can activate B cells without the aid of TH cells. Relatively few antigens are T independent, but they can be very important medically.

Polysaccharides and other molecules that have numerous identical evenly spaced epitopes are one type of T independent antigen. Because of the arrangement of epitopes, clusters of B cell receptors bind the antigen simultaneously, leading to activation of that B cell without the involvement of helper T cells.

These T independent antigens are significant because they are not immunogenic in young children. This is why children less than 2 years of age are more susceptible to pathogens such as *Streptococcus pneumonia* and *Haemophilus influenza* which cloak themselves in polysaccharide capsules.

Lipopolysaccharides, which are a component of the outer membrane of gram negative bacteria, is another type of T independent antigen.