III. Immunology Review

A. Immunologic Response Serves Three Functions

1. Defense - immunologically mediated resistance to infection.

2. Homeostasis - that function of the immune system which removes effete or damaged self components such as aged red cells.

3. Surveillance - that function of the immune system which detects and destroys mutant cells, thus providing protection from malignancy.

B. Components of the Immune System

1. Cells and tissues of the immune system.
   a. Consists of a number of organs and different cells types that have evolved to recognize non-self antigens.
   b. Pluripotential cells are capable of differentiation into many different cell types.
   c. Stem cells are located within the bone marrow, fetal liver and yolk sac of the fetus and give rise to both inflammatory and immune cell lines.
   d. These self replicating cells differentiate into two types of "committed" stem cells.
      1) One group eventually differentiates further and matures to become platelets, erythrocytes (red blood cells), monocytes or granulocytes.
      2) Second group produces cells of the lymphoid line only.
   e. Cells of the immune system are found within the blood, body tissues, thymus, spleen, liver, lymph nodes and those body areas exposed to the external environment.
   f. These organs comprise the reticuloendothelial system (RES).

2. Monocyte-Macrophage Cell System
   a. Derived from stem cell in the bone marrow.
   b. Monocytes circulate to sites of inflammation or migrate to various tissues.
      1) May leave the blood for the tissues and will further differentiate into tissue macrophages.
      2) Tissue macrophages found throughout the body, especially in the liver, spleen, peritoneal, pleural and synovial fluids and in lymph nodes, tonsils and gut-associated lymphoid tissues.
c. Macrophages have several cell surface receptors, one of them being a receptor for the Fc portion of the immunoglobulin molecule.

1) Cells coated with immunoglobulin can attach to macrophages when it come in contact with Fc receptor.

2) This allows removal of many cells from the circulation by macrophages.

3) The ability of macrophages to remove antibody coated cells (macrophage clearance) varies in intensity.

d. Tissue macrophages also possess a receptor for the complement component C3b.

1) Cells coated with complement may also be removed from circulation.

2) Some blood group antibodies are capable of complement activation, resulting in coating then destruction by macrophages.

e. Tissue macrophages also possess cell surface proteins of the major histocompatibility complex (MHC) which is important in transplantation immunity and presentation of antigen to helper T lymphocytes.

f. Macrophages participate in phagocytosis, inflammation, and cellular immunity.

g. Macrophages are mainly involved in nonspecific immunity and include the phagocytic cells: mononuclear phagocytes, polymorphonuclear phagocytes (neutrophils), eosinophils and mediator cells: basophils, mast cells and platelets.

3. T Lymphocytes (T cells)

a. Derived from stem cells in the bone marrow.

b. Precursor cell leaves bone marrow and travels to the thymus to mature prior to release into circulation.

c. Approximately 75 to 80% of lymphocytes are T cells.

d. T lymphocytes are important in recognizing foreign material that is fixed in the tissues of cells.

1) They are not capable of secreting antibody.

2) Examples of foreign materials are transplanted tissue, tumors and organisms causing tuberculosis.

3) When stimulated T cells differentiate further into several types of T cells with very different functions. (These will be covered in detail later).

e. T cells play an important role in regulating the production of antibodies by B cells. T-B cell cooperation is necessary for antibody production to take place.

1) Helper T cell - upon interaction with an antigenic molecule release substances which help B lymphocytes to produce antibodies against this antigen.

2) Suppressor T cell are thought to "turn off" B cells so that they can no longer cooperate with normal T cells to induce an immune response.
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3) Normal immune response probably represents a very fine balance between the action of helper and suppressor T cells.

f. Killer or Killer T cells precise identity and site of origin are unknown, but they are involved in cell mediated immunity by recognizing and destroying antibody-coated target cells.

g. T cells have surface proteins known as cluster determinants (CDs).

1) CD4 and CD8 are widely used in clinical medicine.
2) Helper T cells are CD4 positive cells enhance and promote the action of other immune cells.
3) Suppressor T cells are CD8 positive and have suppressive or cytotoxic effects.
4) Approximately 65% of T cells are helper, and 35% are suppressor giving a ration of approximately 2:1.
5) Subset analysis of the CD4:CD8 ratio is useful in following the course of AIDS.

4. B lymphocytes (B cells)

a. Derived from stem cells in the bone marrow.
   1) Name comes from discovery of these stem cells in the Bursa of Fabricus in the chicken.
   2) In humans development occurs in the bone marrow.

b. B lymphocytes are concerned with humoral immunity, i.e. they recognize certain substances as foreign.
   1) Transform into plasma cells and produce a family of proteins known as antibodies or immunoglobulins.
   2) Important in the eradication of circulating foreign material such as bacteria.

c. Activated B cells begin antibody production and undergo a process called clonal expansion.
   1) Involves commitment of B cell to production of an antibody with a single specificity with proliferation of that particular cell line.
   2) This process generates many B cell committed to production of the same antibody.
   3) Memory B cells are generated, resulting in a rapid production of antibody.

C. Immune Response

1. Consists of two types:
   a. Innate or nonspecific immune response.
   b. Adaptive or specific immune response.

2. Innate immunity involves the body’s first line of defense.

   a. Physical barriers which include intact skin and mucous membranes.

   b. Physiological factors which include hydrochloric acid in the stomach, ciliated epithelium lining the respiratory tract, flushing action of urine, large amounts of unsaturated fatty acids found in the skin, sweat, tears, and commensal normal flora.

   c. If an organism penetrates through these defense mechanisms it encounters phagocytic cells of the RES which will engulf and destroy them.
d.  *Inflammation* is the body's reaction to injury and is known as the body's second line of defense which results in:

1) Increased blood supply to the area.
2) Increased capillary permeability.
3) Migration of leukocytes into the surrounding tissue.
4) These three events manifest symptoms which include pain, heat, redness and swelling.

3. Adaptive (specific) immunity involves specificity, recognition, memory and specific reactivity.
   a. Involves ability to recognize self and non-self.
   b. Encounters with non-self or foreign materials results in production of antibodies (humoral immunity) or actions of T-cells (cell mediated immunity).
   c. Great deal of interaction exists between these two systems.
   d. Immunohematology primarily concerned with the causes and effects of humoral immunity.

D. **Antigens**

1. An antigen is any substance which is recognized as foreign by the body and is capable, under appropriate conditions, of provoking a specific immune response. It is capable of:
   a. Stimulating the formation of antibody and the development of cell-mediated immunity.
   b. Reacting specifically with the antibodies or T lymphocytes produced.

2. Physical nature of antigens
   a. **Foreign nature** - The body is tolerant of its own components and does not initiate immune response against these.
   b. **Molecular size** - molecules with a molecular weight of less than 10,000 daltons have no or weak antigenicity.
   c. **Molecular complexity and rigidity** - Complex proteins are better antigen than large repeating polymers such as lipids, carbohydrates, and nucleic acids, which are relatively poor antigens.
   d. **Genetic factors** - not all individuals within a species will show the same response to a substance - some are *responders* and some *non-responders*.
   e. **Route of administration and dose** (oral, skin, intramuscular, etc.) for stimulation of the immune response is very important and recognition may not occur if dose is too large or too small.

3. Antigenic Determinants or epitopes
   a. Structures on antigens that are recognized as foreign by the immune system.
b. Number of antigenic determinants on a molecule varies with molecular size.

c. An immune response is directed against specific determinants, and resultant antibodies will specifically bind to them.

d. Multivalent antigens may elicit antibodies of different specificities, each directed to a different epitope.

e. Some antibodies produced in response to one antigen may cross react with other antigens having the common determinant.

4. Blood group antigens are chemical structures embedded in or protruding from RBCs, WBCs, and platelets and have three common forms:

   a. Glycoproteins - HLA system.
   b. Glycolipids - ABH, Lewis, Ii, and P blood group systems.
   c. Proteins - Rh, M, N blood group systems.

5. Antigens classified into two major types:

   a. exogenous, outside
   b. endogenous, inside

6. Blood bankers concerned with allogeneic antigens (from other humans) and autologous (self) antigens.

7. Haptens are substances, usually of low molecular weight, that can combine with antibody but cannot initiate an immune response unless it is coupled to a larger carrier molecule.

E. Cellular Immunity or cell mediated immunity

1. Important defense mechanism against viral infections, some fungal infections, parasitic disease and against some bacteria, particularly those inside cells.

2. Responsible for delayed hypersensitivity, transplant rejection and possibly tumor surveillance.

3. This branch of the immune system depends on the presence of thymus-derived lymphocytes (T lymphocytes).

4. Cell-mediated reaction is initiated by the binding of the antigen with an antigen receptor on the surface of the sensitized T lymphocyte, causes stimulation of the T lymphocyte helper and suppressor T cells that regulate the intensity of the body's immune response.

5. T cells capable of direct interaction with the antigen. This group can be divided further.

   a. T cells which, on contact with the specific antigen, liberate substances called lymphokines.
   b. Cytotoxic T cells which directly attack antigen on the surface of foreign cells.

6. Lymphokines are a mixed group of proteins which have diverse properties.

   a. One function is the attraction of neutrophils and monocytes to the site of infection
b. Some lymphokines will cause aggregation of macrophages at the site of the infection, while others activate macrophages, inducing them to phagocytose and destroy foreign antigens more vigorously.

c. The end result of their combined action is an amplification of the local inflammatory reaction with recruitment of circulating cells of the immune system.

F. The Humoral Immune Response

1. Humoral immunity has to do with the production of antibodies induced when the host's immune system comes into contact with foreign antigenic substance and reacts to this antigenic stimulation.

2. Primary and Secondary Immune responses
   a. Involves humoral and cellular events, first exposure results in primary immune response, subsequent exposures result in secondary or anamnestic response.
   b. The immune response is mediated by lymphocytes which differentiate in the bone marrow into B lymphocytes or B cells.
   c. B cells produce antibodies capable of reacting with the antigen which caused their production.
   d. The primary immune response involves the first encounter with the antigen and usually involves the cellular immune system.
      1) Antigen is ingested by antigen processing cells (APCs) and presented to the helper T cell which has a receptor for the antigen on its surface.
      2) Activated helper T cell secretes lymphokines which causes activation of the B cells.
      3) B cells proliferate and differentiate into antibody secreting cells (plasma cells) and memory cells.
   e. The secondary immune response involves a later exposure to the same antigen causing activation of the memory cells to proliferate into plasma cells with immediate production of antibody.
   f. Antibody production occurs in four phases following antigen challenge:
      1) Lag phase when no antibody is detectable.
      2) Log phase in which antibody titer rises logarithmically.
      3) Plateau phase during which the antibody titer remains steady.
      4) Decline phase during which antibody levels gradually decline.

3. Responses following primary and secondary antigenic challenge differ in several major respects:
   a. Time.
      1) Primary response involves days, weeks or even months before appearance of detectable antibody (long lag phase).
      2) Secondary response results in rapidly increased antibody levels detectable in a matter of hours (short lag phase).
   b. Antibody Titer.
      1) Primary response results in generation of a pool of potential antibody-forming cells and initial low titer.
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2) Secondary response plateau levels of antibody are much greater and remains elevated longer.

c. **Antibody Class.**
   1) Primary response initial antibody produced is IgM, within 2 to 3 weeks production switches to IgG.
   2) Secondary immune response memory B cells immediately produce IgG.

d. **Antibody affinity and avidity.**
   1) Antibodies in primary response usually have low affinity (degree of fit between and antigen and antibody) for respective antigen and lower avidity (strength of bond between an antigen and its respective antibody).
   2) Secondary response has greatly increased affinity and avidity.

G. **Immunoglobulins**

1. **Introduction**
   
   a. Humans produce specific proteins or immunoglobulins which can be differentiated on the basis of:
      1) Size
      2) Biologic function
      3) biochemical properties
      4) serological activity
   
   b. Human immunoglobulins have been assigned to five classes based on the primary structure of their respective heavy chains.

2. **Basic Structure of Immunoglobulins**
   
   a. Basic Structure consists of two identical heavy chains and two light chains held together by a chemical link (disulfide bonds).
      1) Light chains are named by the greek letters *kappa* and *lambda*.
      2) Heavy chains by the greek letters: *gamma* (G), *alpha* (A), *mu* (M), *delta* (D), and *epsilon* (E).
   
   b. Antibodies are treated with enzymes to determine structure and activity of the various areas of the antibody.
   
   c. Papain breaks the Ig into three fragments: 2 Fab, containing a light chain and a portion of the heavy chain, and 1 Fc fragment.
      1) Fab contains antigen binding site.
      2) Fc is the region that determine biological properties of the Ig.

3. **IgM Class**
   
   a. Largest of all the antibody molecules and the structure consists of five of the basic units *(pentamer)* joined together by a structure known as J-chain.
   
   b. Accounts for about 5-10% of the immunoglobulin pool.
c. IgM is restricted almost entirely to the intravascular space due to its large size.

d. IgM fixes complement and is much more efficient than IgG in the activation of complement and agglutination.

e. It is the first antibody to be produced and is of greatest importance in the first few days of a primary immune response to an infecting organism. Thus it acts as an effective first line of defense against bacteria.

f. IgM does not cross the placenta.

g. Many blood group antibodies that are capable of agglutinating antigen positive RBCs suspended in saline in tests performed at 22 C are IgM causing visible agglutination.

h. IgM antibodies are potent agglutinators that activate complement very efficiently.

4. **IgG Class**

a. Most abundant of the immunoglobulins in the plasma (accounts for 70 to 80% of the total immunoglobulin pool) and because of its very small molecular weight it can diffuse into the interstitial fluid.

b. Consists of one basic structural unit, i.e. Y-shaped molecule having 2 light chains and 2 Gamma heavy chains.

c. Antibodies of this class are produced in response to a wide variety of antigens, including bacteria, viruses and RBC and WBC allo-antigens.

d. IgG can coat organisms and this enhances their phagocytosis by neutrophils and macrophages.

e. Through its ability to cross the placenta, maternal IgG provides the major line of defense against infection for the first few weeks of a baby's life.

f. It is the predominant antibody produced in the secondary response.

g. The serologic behavior and characteristics of IgG antibodies make them one of the most clinically significant in blood banking.

h. Most blood group antigens capable of eliciting an immune response result in the production of IgG antibodies.

1) These antibodies are detected by serologic test procedures based on their behavior characteristics, such as reactivity at 37 C, complement activation, indirect agglutination and hemolysis.

2) Much of routine blood banking involves serologic test procedures designed to detect and identify IgG antibodies.

i. Four subclasses which differ in their heavy chain composition and in some of their characteristics such as biologic activities. IgG1, IgG2, IgG3 and IgG4.
5. **IgA Class**

a. As well as being in the plasma, IgA is the **major immunoglobulin of the external secretory system and is found in saliva, tears, colostrum breast milk and in nasal, bronchial and intestinal secretions**. IgA is present in large quantities in colostrum and breast milk and can be transferred across the gut mucosa in the neonate and plays an important role in protecting the neonate from infection.

b. It is produced in high concentrations by lymphoid tissues lining the gastrointestinal, respiratory and genitourinary tracts.

c. It plays an important role in protection against respiratory, urinary tract and bowel infections and it is probably also important in preventing absorption of potential antigens in the food we eat.

d. IgA represents 10 to 15% of the total circulatory immunoglobulin pool.

e. In plasma IgA may exist as a single basic structural unit or as two or three basic units joined together.

f. The IgA present in secretions exists as two basic units (a dimer) attached to another molecule know as **secretory component**.

   1) This substance is produced by the cells lining the mucous membranes.
   2) It is thought to protect the IgA in secretions from destruction by digestive enzymes.

h. **IgA does not cross the placenta and does not bind complement.**

i. For blood banking, if an individual is IgA deficient they may produce anti-IgA which can cause severe, life-threatening anaphylactic reactions during transfusion. Once identified these individuals must be transfused with blood and components which lack IgA.

6. **IgE Class**

a. Trace plasma protein (only about 0.004%) in the plasma of non-parasitized individuals.

b. It is of major importance because it mediates some types of allergic reactions, allergies and anaphylaxis and is generally responsible for an individual's immunity to invading parasites.

c. IgE is unique in that its Fc region binds strongly to a receptor on mast cells and basophils and, when antigen is bound it causes the basophil (or mast cell) to release histamines and heparin from these cells, resulting in allergic symptoms.

d. Clinical effects of IgE mediated reactions include increased vascular permeability, skin rashes, respiratory tract constriction (wheezing), and increased secretions from epithelium (watery eyes, runny nose).

e. Not much else is known about its biologic role.

f. **IgE does not fix complement and does not cross the placenta.**

g. No blood group antibodies have been reported to belong to this class.
7. **IgD Class**
   a. Accounts for less than 1% of the total immunoglobulin pool.
   b. This is primarily a cell membrane immunoglobulin found on the surface of B lymphocytes.
   c. *IgD does not fix complement and does not cross the placenta.*
   d. Little is known about the function of this class of antibody.
   e. No blood group antibodies have been reported to belong to this class.

H. **Clinical Significance of Blood Group Antibodies**

1. *Clinical significance* of blood group antibodies is evaluated by their ability to *produce hemolytic transfusion reactions* or *hemolytic disease of the newborn (HDN).*

2. Transfusion reaction is the term used to describe an unfavorable response by a recipient to the infusion of blood or blood products and *include the following:*
   a. In-vivo hemolysis,
   b. Decreased survival of transfused cells,
   c. anaphylaxis,
   d. graft-versus-host disease,
   e. post-transfusion purpura,
   f. alloimmunization,
   g. sepsis due to bacterial contaminated components,
   h. and disease transmission.

3. Human red blood cells contain hundreds of blood group antigens which may be an integral part of the RBC or adsorbed onto the RBC from the plasma.
   a. At least 15 different blood group systems have been recognized in humans.
   b. Individuals may produce antibodies to these blood group antigens due to exposure to RBCs through previous transfusion or pregnancy.
   c. Transfusion of RBCs possessing antigens to which the individual has antibodies may result in immune hemolysis of the transfused cells.
   d. The severity of the reaction depends on a number of factors, including the characteristics of the antibody class involved.
      1) Antibodies to the ABO system antigens are *usually IgM*, cause complement activation and *intra vascular hemolysis.*
      2) Other RBC antigens induce formation of *IgG class* antibodies which may cause accelerated RBC destruction *extra vascularly.*
      3) Symptoms may include fever, low back pain, nausea and vomiting, circulatory shock, anemia, jaundice, and kidney failure which may ultimately result in death.
4) Secondary response symptomatic, primary response may be asymptomatic due to slow destruction of RBCs.

4. Transfusion reaction to other components of blood, WBC and platelets, are very common, but reactions are not as severe as RBC reactions.

5. An additional adverse effect is disease transmission, which can only be prevented by careful donor screening.

6. Some adverse effects can be prevented, some cannot. The primary duty of Immunohematologists is to provide serologically compatible blood and blood components for transfusion.

I. Complement

1. Introduction

a. The complement system is an integral part of the body's immune system composed of more than 25 glycoproteins which circulate in the plasma in an inactive form.

b. These proteins can be activated by two independent pathway, classic and alternative or properdin pathways.

c. Three primary functions:

   1) Lysis of antibody coated cells, such as bacteria and RBCs.
   2) Mediation of opsonization, preparation of foreign cells for phagocytosis.
   3) Generation of peptide fragments that regulate features of the inflammatory and immune response.

d. Activation of complement results in a cascade or interaction of the complement proteins, leading to generation of products that have important biologic activities which includes:

   1) opsonization,
   2) immune adherence,
   3) and chemotactic and anaphylatoxic activity.

e. In blood banking complement is important in two major areas:

   1) Some antigen-antibody complexes cause sufficient quantities of complement to be bound to RBCs to complete activation cycle, causing hemolysis.
   2) Antigen-antibody complexes initiate complement binding in such a way that allows demonstration of the existence of such complexes by the use of serologic techniques.

e. Fresh serum is necessary to ensure adequate quantities of viable complement as complement is unstable and heat labile.

2. The Classic Pathway

a. Eleven components involved, numbered C1 to C9.

b. Complement cascade requires presence of cations, both calcium and magnesium.
c. Activation of the classic pathway almost always initiated by immunoglobulin.

1) Requires only 1 molecule of IgM.
2) Requires 2 molecules of IgG.

d. Process involves cleavage of the components such that removal of part of the complement molecule leaves the residual portion enzymatically or biologically active.

1) Cleavage of most components generates small fragment, which enters plasma, and larger fragment which attaches to surface and continues in the reaction.
2) Major controls are inhibitory proteins (C1 and C3 inhibitors), spontaneous decay, and rapid clearance of active fragments.
3) Activation is divided into three phases: recognition, activation and attack.

e. Recognition Phase

1) C1 has three subcomponents, C1q, C1r, and C1s.
2) C1q binds directly to Immunoglobulin molecule, C1r and C1s and requires calcium.
3) C1r and C1s become enzymatically active.
4) C1r cleaves C1s, C1s cleaves C4 and C2 to initiate the activations phase.
5) Recognition unit: C1q,C1r,C1s.

f. Activation Phase

1) Activated C1s cleaves C4 and C2, which begins the activation phase.
2) C4 cleaved: C4a released to plasma and decays rapidly, C4b binds to target cell surface.
3) C2 cleaved: C2a released to plasma, C2b remains bound to C4b. Requires calcium and magnesium.
4) C4bC2b cleaves C3 to C3 convertase which cleaves C3 into C3a and C3b, which attaches to the target cell surface near the C4b,2b complex.
5) C3b actually a complex of C3c and C3d.
6) Sometimes C3b binds to surface whereas other times C3d binds, binding of C3b initiates phagocytosis.
7) C4b,2b,3b complex (C5 convertase) splits C5 into C5a which is released into plasma and C5b which attaches to cell membrane and is the nucleus for formation of membrane attack complex.
8) Activation Unit: C4b,C2b,C3b,C5b

g. Attack Phase

1) Once membrane bound, C5b binds C6 and C7 by adsorption.
2) This complex attaches to the cell membrane and binds C8 and a variable number of C9 molecules.
3) Components C5b,C6,C7,C8 and C9 comprise the terminal membrane attack unit.
4) Becomes embedded in the cell membrane and forms a central pore or hole, which results in a change in membrane permeability.
5) Membrane then loses its ability to retain cellular contents and prevent entrance of extracellular material such as water.
6) Loss of cellular integrity results in rapid cell death and lysis.
7) Classic pathway: C1,C4,C2,C3,C5,C6,C7,C8,C9
3. Alternative (Properdin) Pathway
   a. Proteins in the alternative pathway perform activities similar to those in the classic pathway but are usually *non-antibody* triggered.
   
   b. Any one of a variety of substances can initiate complement activation including:
      1) bacterial polysaccharides and lipopolysaccharides,
      2) endotoxins,
      3) cobra venom,
      4) trypsin like enzymes,
      5) and aggregates of IgA and IgG4 that do not activate C1.
   
   c. C1, C4 and C2 do not participate.
   
   d. Alternative pathway: C3,C5,C6,C7,C8,C9

4. Regulation of the complement cascade.
   a. Activation of complement cascade results in complex series of molecular event with potent biologic consequences.

   b. Modulating mechanisms are necessary to regulate complement activation and control production of biologically active split products.

   c. First mechanism is spontaneous decay of activated components.

   d. Second mechanism involves specific control proteins that modulate the activity of certain complement components at critical activation steps.
      1) C1 inhibitor blocks activities of C1r and C1s.
      2) Other factors inhibit activation of other complement components.

   e. A number of proteins act to control the membrane attack unit.