Immunoglobulin A

- Immunoglobulin A represents 15% to 20% of the total circulatory Ig pool. It is the predominant immunoglobulin in secretions such as tears, saliva, colostrum, milk, and intestinal fluids.
- IgA is synthesized largely by plasma cells located on body surfaces. If produced by cells in the intestinal wall, IgA may pass directly into the intestinal lumen or diffuse into the blood circulation. As IgA is transported through intestinal epithelial cells or hepatocytes, it binds to a glycoprotein called the secretory component. The secretory piece protects IgA from digestion by gastrointestinal proteolytic enzymes. It forms a complex molecule termed *secretory IgA,* which is critical in protecting body surfaces against invading microorganisms because of its presence in seromucous secretions (e.g., tears, saliva, nasal fluids, colostrum).
- IgA monomer is present in relatively high concentrations in human serum; it has a concentration of 90 to 450 mg/dL (55 to 270 IU/mL) in normal adult humans. At the end of the first year of life, 25% of the adult IgA level is reached, and 50% at 3.5 years of age. The average adult level is attained by age 16 years.

Immunoglobulin M

- Immunoglobulin M accounts for about 10% of the Ig pool and is largely confined to the intravascular pool because of its large size.
- This antibody is produced early in an immune response and is largely confined to the blood.
- IgM is effective in agglutination and cytolytic reactions. In humans, IgM is found in smaller concentrations than IgG or IgA. The molecule has five individual heavy chains

Immunoglobulin G

- The major immunoglobulin in normal serum is IgG. It diffuses more readily than other immunoglobulins into the extravascular spaces and neutralizes toxins or binds to microorganisms in extravascular spaces.
- IgG can cross the placenta. In addition, when IgG complexes are formed, complement can be activated. IgG accounts for 70% to 75% of the total Ig pool

Table 2-2 Characteristics of Immunoglobulin Classes

	IgM	IgG	IgA	IgE	IgD
Molecular weight (daltons, Da)	900,000	160,000	360,000	200,000	160,000
Sedimentation coefficient (Σ)	19	7	11	8	7
Carbohydrate (%)	12	8	7	12	12
Subclasses	—	IgG1-4	α1, α2	—	—
Serum concentration, adults (mg/mL)	1.5	13.5	3.5	0.05	Trace
Serum half-life (days)*	5	23	6	2.5	3

*Half life (days) = the amount of time to reach ½ activity concentration. Serum values are average concentrations in normal, healthy individuals. Adapted from Peakman M, Vergani D: Basic and clinical immunology, St Louis, 2009, Elsevier, p 41.

Immunoglobulin D

Immunoglobulin D is found in very low concentrations in plasma, accounting for less than 1% of the total Ig pool. IgD is extremely susceptible to proteolysis and is primarily a cell membrane Ig found on the surface of B lymphocytes in association with IgM.

Immunoglobulin E

- Immunoglobulin E is a trace plasma protein found in the blood plasma of unparasitized individuals (MW, 188,000 Da). IgE is crucial because it mediates some types of hypersensitivity (allergic) reactions, allergies, and anaphylaxis and is generally responsible for an individual's immunity to invading parasites.
- The IgE molecule is unique in that it binds strongly to a receptor on mast cells and basophils and, together with antigen, mediates the release of histamines and heparin from these cells.

Allotype Determinants

The second principal group of determinants is found on the immunoglobulins of some, but not all, animals of a species.

Antibodies to these allotypes **(alloantibodies)** may be produced by injecting the immunoglobulins of one animal into another member of the same species.

The allotypic determinants are genetically determined variations representing the presence of allelic genes at a single locus within a species. Typical allotypes in humans are the Gm specificities on IgG (Gm is a marker on IgG). In humans, five sets of allotypic markers have been found—Gm, Km, Mm, Am, and Hv.

Idiotype Determinants

A result of the unique structures on light and heavy chains, individual determinants characteristic of each antibody are called **idiotypes.** The idiotypic determinants are located in the variable part of the antibody associated with the hypervariable regions that form the antigen-combining site

IMMUNOGLOBULIN VARIANTS

- An antigenic determinant is the specific chemical determinant group or molecular configuration against which the immune response is directed. Because they are proteins, immunoglobulins themselves can function as effective antigens when used to immunize mammals of a different species.
- When the resulting anti immunoglobulins or antiglobulins are analyzed, three principal categories of antigenic determinants can be recognized—isotype, allotype, and idiotype (Fig. 2-13; Table 2-3).
- **Isotype Determinants**
- The isotypic class of antigenic determinants is the dominant type found on the immunoglobulins of all animals of a species.

ANTIBODY SYNTHESIS

the production of antibodies (B lymphocytes and plasma cells) directed against the antigen. Production of antibodies is induced when the host's lymphocytes come into contact with a foreign antigenic substance that binds to its receptor. This triggers activation and proliferation, or **clonal selection.** Clonal expansion of lymphocytes in response to infection is necessary for an effective immune response (Fig. 2-14). However, it requires 3 to 5 days for a sufficient number of clones to be produced and to differentiate into antibody-producing cells. This allows time for most pathogens to damage host tissues and cells Whether a cell-mediated response or an antibody response takes place depends on how the antigen is presented to the lymphocytes; many immune reactions display both types of responses.

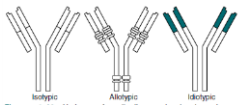

Figure 2-13 Variants of antibodies—antigenic determinants. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1989, Williams & Wilkins.)*

2. Type of antibody. IgM-type antibodies are the principal class formed in the primary response. Although some IgM antibody is formed in a secondary response, the IgG class is the predominant type formed.
3. Antibody titer. In a secondary response, antibody levels attain a higher titer. The plateau levels in a secondary response are typically 10-fold or greater than the plateau levels in the primary response.
An example of an anamnestic response can be observed in hemolytic disease, when an Rh-negative mother is pregnant with an Rh-positive baby (see Chapter 26). During the mother's first exposure, the Rh-positive RBCs of the fetus leak into the maternal circulation and elicit a primary response. Subsequent pregnancies with an Rh-positive fetus will elicit a secondary (anamnestic) response.

Subsequent exposure to the same antigen produces a memory response, or **anamnestic response,** and reflects the outcome of the initial challenge. In the case of antibody production, the quantity of IgM-IgG varies.

Primary Antibody Response
Although the duration and levels of antibody (titer) depend on the characteristics of the antigen and the individual, an IgM antibody response proceeds in the following four phases after a foreign antigen challenge (see Fig. 2-14):
1. Lag phase—no antibody is detectable.
2. Log phase—the antibody titer increases logarithmically.
3. Plateau phase—the antibody titer stabilizes.
4. Decline phase—the antibody is catabolized.

Secondary (Anamnestic) Response
Subsequent exposure to the same antigenic stimulus produces an antibody response that exhibits the same four phases as the primary response (see Fig. 2-14). Repeated exposure to an antigen can occur many years after the initial exposure, but clones of memory cells will be stimulated to proliferate, with subsequent production of antibody by the individual. An anamnestic response differs from a primary response as follows:

The Complement System

- The term **complement** (spelled with an *e*) refers to a set of serum proteins that cooperates with both the innate and the adaptive immune systems to eliminate blood and tissue pathogens.
- Like the components of the blood clotting system, complement proteins interact with one another in catalytic cascades.
- researchers have discovered that the action of complement is the result of interactions among a complex group of more than 30 glycoproteins.
- Most complement components are synthesized in the liver by hepatocytes, although some are also produced by other cell types, including blood monocytes, tissue macrophages, fibroblasts, and epithelial cells of the gastrointestinal and genitourinary tracts.

Vaccination is the application of primary and second responses. Humans can become immune to microbial antigens through artificial and natural exposure. A vaccine is designed to provide artificially acquired active immunity to a specific disease (e.g., hepatitis B). Booster vaccine (repeated antigen exposure) allows for an anamnestic response, with an increase in antibody titer and clones of memory cells (see Chapter 16).

The Major Pathways of Complement Activation

The Classical Pathway Is Initiated by Antibody Binding

The classic pathway has three major stages:

1. Recognition
2. Amplification of proteolytic complement cascade
3. Membrane attack complex (MAC)

Recognition

- The recognition unit of the complement system is the C1 complex— C1q, C1r, and C1s, an interlocking enzyme system.
- In the classic pathway, the first step is initiation of the pathway triggered by recognition by complement factor C1 of antigen antibody complexes on the cell surface.
- When C1 complex interacts with aggregates of immunoglobulin G (IgG) with antigen on a cell's surface, two C1-associated proteases, C1r and C1s, are activated.
- A single IgM molecule is potentially able to fix C1, but at least two IgG molecules are required for this purpose.

- Complement components constitute approximately 15% of the globulin protein fraction in plasma, and their combined concentration can be as high as 3 mg/ml.
- In addition, several of the regulatory components of the system exist on cell membranes, so the term *complement* therefore now embraces glycoproteins distributed among the blood plasma and cell membranes.
- C3 is present in the plasma in the largest quantities; fixation of C3 is the major quantitative reaction of the complement cascade.
- Although the principal source of synthesis of complement in vivo is debatable, the majority of the plasma complement components are made in hepatic parenchymal cells, except for C1 (a calcium-dependent complex of the three glycoproteins C1q, C1r, and C1s), which is primarily synthesized in the epithelium of the gastrointestinal and urogenital tracts.

Amplification of Proteolytic Complement Cascade

- Once C1s is activated, the **proteolytic** complement cascade is amplified on the cell membrane through sequential cleavage of complement factors and recruitment of new factors until a cell surface complex containing C5b, C6, C7, and C8 is formed.
- The complement cascade reaches its full amplitude at the C3 stage, which represents the heart of the system. The C4bC2a complex, the classic pathway C3 convertase, activates C3 molecules by splitting the peptide, C3 anaphylatoxin, from the N-terminal end of the peptide of C3. This exposes a reactive binding site on the larger fragment, C3b.
- Consequently, clusters of C3b molecules are activated and bound near the C4bC2a complex. Each catalytic site can bind several hundred C3b molecules, even though the reaction is very efficient because C3 is present in high concentration. Only one C3b molecule combines with C4bC2a to form the final proteolytic complex of the complement cascade.

- The amount of C1 fixed is directly proportional to the concentration of IgM antibodies, although this is not true of IgG molecules.
- C1s is weakly proteolytic for free intact C2, but is highly active against C2 that has complexed with C4b molecules in the presence of magnesium (Mg^{2+}) ions. This reaction will occur only if the C4bC2 complex forms close to the C1s.
- The resultant C2a fragment joins with C4b to form the new C4bC2a enzyme, or classic pathway C3 **convertase.** The catalytic site of the C4bC2a complex is probably in the C2a **peptide.**
- A smaller C2b fragment from the C2 component is lost to the surrounding environment.

- The C5bC6C7C8 complex polymerizes C9 to form a tubule (pore), which spans the membrane of the cell being attacked, allowing ions to flow freely between the cellular interior and exterior. By complexing with C9, the **osmotic cytolytic reaction** is accelerated. This tubule is a hollow cylinder with one end inserted into the lipid bilayer and the other projecting from the membrane.
- A structure of this form can be assumed to disturb the lipid bilayer sufficiently to allow the free exchange of ions and water molecules across the membrane. Ions flow out, but large molecules stay in, causing water to flood into the cell. The consequence in a living cell is that the influx of sodium (Na+) ions and H2O leads to disruption of osmotic balance, which produces cell lysis.

Membrane Attack Complex

- The **membrane attack complex** (MAC) is a unique system that builds up a lipophilic complex in cell membranes from several plasma proteins. To initiate C5b fixation and the MAC, C3b splits C5a from the alpha chain of C5. No further proteinases are generated in the classic complement sequence. Other bound C3b molecules not involved in the C4b2a3b complex form an opsonic macromolecular coat on the erythrocyte or other target, which renders it susceptible to immune adherence by C3b receptors on phagocytic cells.
- When fully assembled in the correct proportions, C7, C6,C5b, and C8 form the MAC (see Fig. 5-2, *inset*). The C5bC6 complex is **hydrophilic** but, with the addition of C7, it has additional detergent and phospholipid-binding properties as well. The presence of **hydrophobic** and hydrophilic groups within the same complex may account for its tendency to **polymerize** and form small protein micelles (a packet of chain molecules in parallel arrangement). It can attach to any lipid bilayer within its effective diffusion radius, which produces the phenomenon of reactive lysis on innocent so-called bystander cells. Once membrane bound, C5bC6C7 is relatively stable and can interact with C8 and C9.

ALTERNATIVE PATHWAY

- Microbial and mammalian cell surfaces can activate the alternative pathway in the absence of specific antigen-antibody complexes. Factors capable of activating the alternative pathway include inulin, **zymosan** (polysaccharide complex from surface of yeast cells), bacterial polysaccharides and endotoxins, and the aggregated IgG2, IgA, and IgE
- Polysaccharides are called activator surfaces and favor the uptake of factor B on the chain of C3b, with the corresponding displacement of factor H. In this situation, binding of factor H is inhibited, and consequently factor B will replace H at the common binding site. When factor H is excluded, C3b is thought to be formed continuously in small amounts.
- Another controlling point in the amplification loop depends on the stability of the C3b,Bb convertase. Ordinarily, C3b,Bb decays because of the loss of Bb, with a half-life of approximately 5 minutes. However, if properdin (P) binds toC3b,Bb, forming C3b,BbP, the half-life is extended to30 minutes.

Table 5-1 Three Main Physiologic Activities of the Complement System

Activity	Responsible Complement Protein
Host Defense Against Infections	
Opsonization	Covalently bonded fragments of C3 and C4
Chemotaxis and leukocyte activation	C5a, C3a, and C4a; anaphylatoxin leukocyte receptors
Lysis of bacterial and mammalian cells	C5-C9 membrane attack complex
Interface Between Innate and Adaptive Immunity	
Augmentation of antibody	C3b and C4b bound to immune complexes and to antigen
Responses	C3 receptors on B cells and antigen-presenting cells
Enhancement of immunologic memory	C3b and C4b bound to immune complexes and to antigen; C3 receptors on follicular dendritic cells
Disposal of Waste	
Clearance of immune complexes from tissues	C1q; covalently bonded fragments of C3 and C4
Clearance of apoptotic cells	

- A deficiency of mannose-binding lectin is caused by one of three point mutations in its gene, each of which reduces levels of the lectin. After the discovery that the binding of mannose binding lectin to mannose residues can initiate complement activation, the mannose-binding lectin–associated serine protease (MASP) enzymes were discovered.
- MASP activates complement by interacting with two serine proteases called MASP1 and MASP2. These components make up the mannose- binding lectin pathway.

- The association of numerous C3b units, factor Bb, and properdin on the surface of an aggregate of protein or the surface of a microorganism has potent activity as a C5 convertase. With the cleavage of C5, the remainder of the complement cascade continues as in the classic pathway.

MANNOSE-BINDING LECTIN PATHWAY

- Mannose-binding lectin is a member of a family of calcium dependent lectins, the collectins (collagenous lectins), and is homologous in structure to C1q. Mannose-binding lectin, a pattern recognition molecule of the innate immune system, binds to arrays of terminal mannose groups on a variety of bacteria.