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## CHAPTER 5

### COMPLEMENT

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See APPENDIX (8) COMPLEMENT FIXATION ASSAY

The complex of serum proteins known as COMPLEMENT plays key roles in the lytic and inflammatory properties of antibodies. The CLASSICAL pathway is initiated by antigen-antibody complexes (via complement components C1, C4, and C2), while the activation of the ALTERNATE pathway (via components B, D and P), and the MBLLECTIN ("*mannan-binding lectin*") pathway may be initiated by other substances independently of adaptive immune responses; *all three* pathways share those complement components involved in the inflammatory and lytic consequences, namely C3, C5, C6, C7, C8 and C9. The INFLAMMATION which is a consequence of complement fixation is illustrated by the manifestations of SERUM SICKNESS, and complement is also seen to be central to the normal process of clearing immune complexes, which is important in preventing IMMUNE COMPLEX DISEASE.

In the late 19th century, a researcher named Jules Bordet, following the earlier results of Richard Pfeiffer, was investigating the *lysis of the bacterium Cholera vibrio* (the agent which causes cholera) by immune sera, and found that the ability of an immune serum to lyse its targets was *lost upon heating* (e.g., at 56° C for 30 min). This ability to cause lysis was also lost after simple storage of the serum for a few days at room temperature.

Bordet showed that such heating did not destroy the antibodies, however, since the addition of a small amount of normal, non-immune serum, to the heat-inactivated antiserum fully restored its capacity to specifically lyse cholera targets. Thus, the ability of immune serum to lyse bacteria depends not only on antibodies specific for *C. vibrio*, but also on a non-specific *heat-labile substance found in normal serum*. This substance became known as COMPLEMENT, since it "complements" the activity of the antibodies which are still present in heat-inactivated antisera.

COMPLEMENT - A group of serum proteins which can be activated (= "*fixed*") by antigen-antibody complexes or other substances, which may result in lysis of a microbial target, or a variety of other biological effects important in both innate and adaptive immunity. (The majority of these proteins are produced by the liver.)

Before going into the details of the components of the complement cascade and their activation, let's preview the various **biological effects** which can be attributed to the action of complement, and identify those complement components or complexes which are responsible for these effects.

## BIOLOGICAL EFFECTS OF COMPLEMENT

- A) **Cytolysis** [C5b6789] (*Note: the bar identifies an activated complex*)  
Destruction of target cells by lysis of the cell membrane. This is termed **cytotoxicity** in the case of nucleated cells, **hemolysis** for red blood cells, or **bacteriolysis** in the case of bacteria. (NOTE: *Not all bacterial and eukaryotic cells are susceptible to complement-dependent lysis*).
- B) **Anaphylotoxin activity** (= "vasoactive" or "phlogistic") [C3a, C5a]  
Stimulation of mast cells to release histamine and other substances, resulting in increased capillary permeability and local accumulation of fluid in the tissue.
- C) **Chemotaxis** [C5a, C5b67]  
Attraction of polymorphonuclear neutrophils (PMN's) to a local site of inflammation.
- D) **Opsonization** (= "immune adherence") [C3b]  
Facilitation of phagocytosis by macrophages or PMN's via cell-surface receptors specific for complement components ("complement receptors", or "CRs")
- E) **Tissue damage** [C5b6789; PMN's]  
Both the lytic complex and the inflammatory PMN's can cause considerable damage to normal tissues, for instance in an Arthus Reaction or in Immune Complex Disease.

The consequences of complement activation can be categorized into two general classes:

- **Facilitating antibody function**; destruction and removal of foreign material.
  - Target cell *lysis*; the lytic "*membrane attack complex*" ("*MAC*") can be produced by all three of the complement pathways discussed below.
  - Removal of immune complexes* ("*immune clearance*"); this is a critically important function facilitated by the presence of receptors ("CR") for various complement components on the surface of leukocytes and erythrocytes. The special process by which *soluble* (*i.e.* small) immune complexes are normally cleared from serum relies on the presence on erythrocytes of CR1 complement receptors which are capable of binding C4b (this process is discussed later in this chapter).
- **Development of inflammation**; increased circulation and *accumulation of fluids and cells* all contribute to the cardinal signs of inflammation (heat, redness, swelling and pain); these functions are mediated directly and indirectly by proteolytic products of the complement cascade (C3a, C5a).

## THREE PATHWAYS FOR COMPLEMENT FIXATION

The process of complement fixation requires specific *protein/protein interactions*, it involves *proteolytic cleavages* and conformational changes of proteins, and *new biological activities* are generated as a result.

Three distinct (although related) mechanisms are known which can initiate the complement cascade, the **Classical Pathway**, the **Alternate Pathway**, and the more recently recognized **MBLECTIN Pathway**. The central event in all three of these modes of complement activation is the cleavage of component C3. The pathways differ only in the mechanism by which they achieve this cleavage, and we will consider them in turn.



# CLASSICAL COMPLEMENT PATHWAY

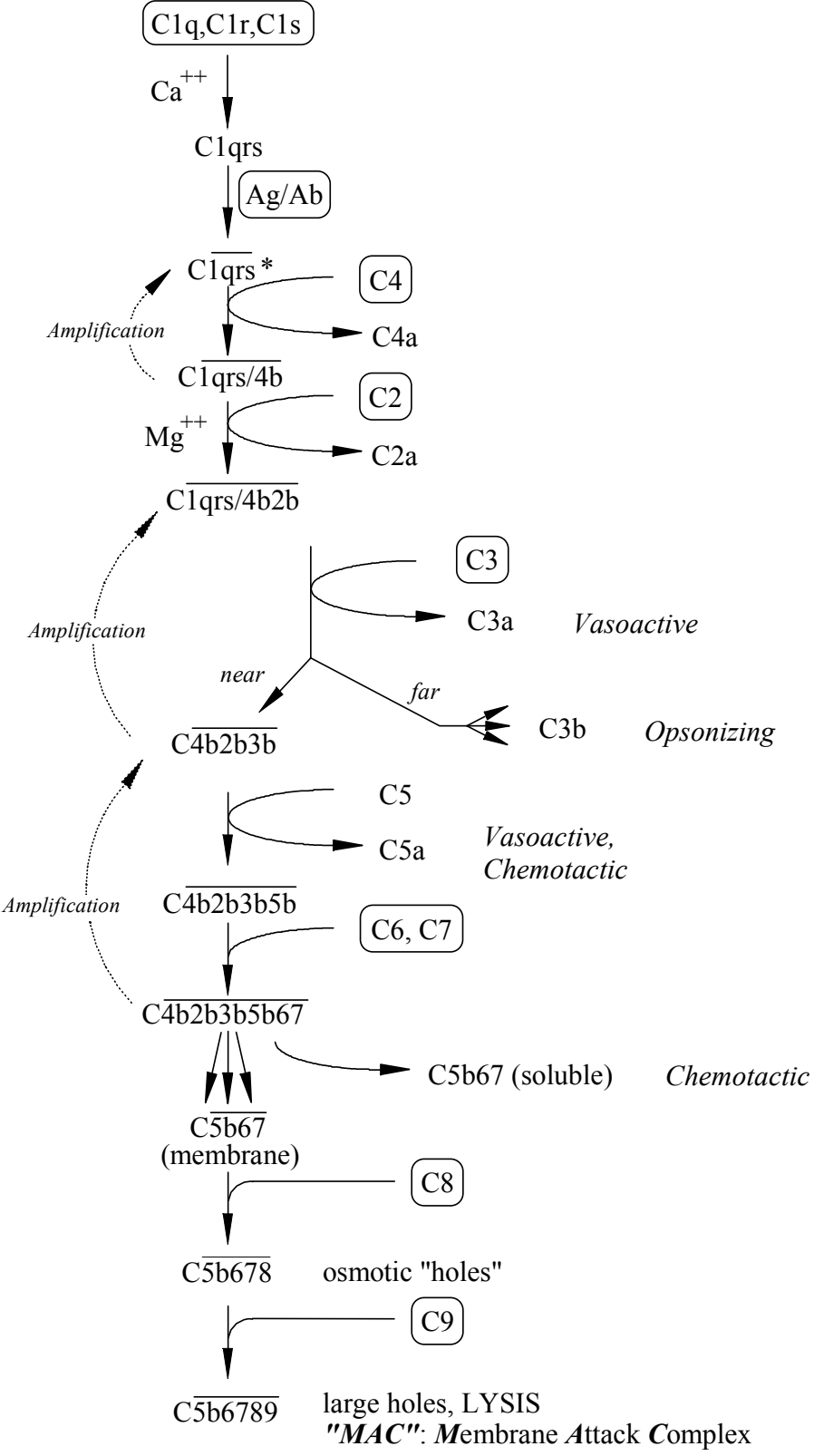
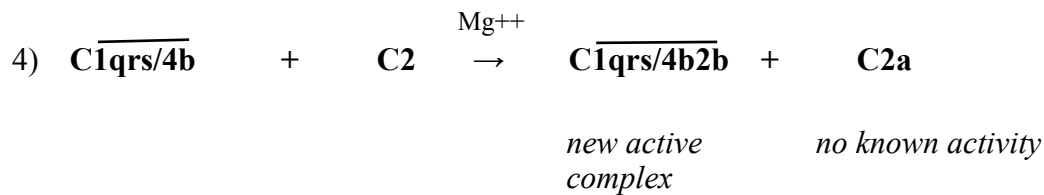
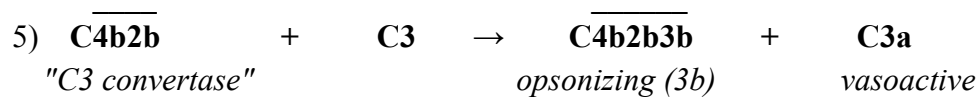


Figure 5-1



The esterase activity of C1s acts again, this time to cleave C2 into C2b (which is bound by 4b in the complex) and C2a (which is released). (NOTE: *The names of 2a and 2b are reversed from the usage you may find in older literature as well as some current sources. Using the present nomenclature, all those fragments which bind to the complex are named "b", while those that are released in soluble form are named "a".*)

At this point the presence of the AgAb $\overline{\text{C1qrs}}$  complex is no longer necessary for the cascade to continue (even though it's still present), and we'll omit it in the diagrams below.

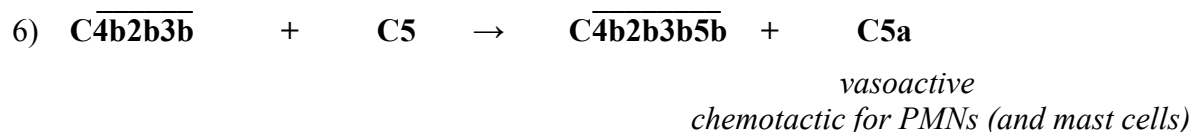


The C4b2b complex has an enzymatic activity called **C3 convertase**, indicating it can cleave C3 into C3a and C3b. This is a *key step* in this pathway, involving *biological amplification* and the generation of molecules with *new biological activities*.

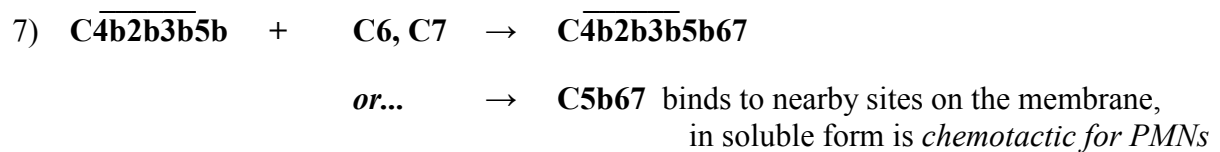
**Many hundreds of C3 molecules can be cleaved** by a single C4b2b complex, resulting in considerable **amplification**. The resulting C3b molecules can either be *bound by the complex* or can be released and *bind elsewhere* on the membrane (C3b molecules which are *not* bound in one place or the other are rapidly destroyed.)

**C3b has powerful opsonizing activity**, whether it is bound in the complex or to some other site on the membrane.

**C3a is strongly vasoactive**. It is also chemotactic for mast cells.



Cleavage of C5 results in a new complex and another biologically active molecule, C5a, which is both **vasoactive and chemotactic**.



The C5b67 complex may be released and bind to a nearby site in the membrane, and in soluble form also exhibits chemotactic activity. The resulting C4b2b3b complex left behind can then sequentially bind additional C5, C6 and C7 molecules, which results in *another important site of amplification in the complement cascade*.

The final reactions of the complement cascade produce the membrane-destroying complexes:

- 8)  $C5b67 + C8 \rightarrow C5b678$   
*osmotic "holes", leakage*
- 9)  $C5b678 + C9 \rightarrow C5b6789$   
*Membrane Attack Complex, "MAC",  
 large "holes", lysis*

The formation of large, open channels in target cell membranes results in the loss of hemoglobin from erythrocytes (visible as HEMOLYSIS) or leakage of cytoplasmic contents and death of nucleated cells (CYTOTOXICITY).

### ALTERNATE PATHWAY OF COMPLEMENT FIXATION

The biochemist Louis Pillemer discovered in the 1950's that complement fixation could be triggered by the yeast polysaccharide Zymosan in the *absence* of antibody, by a process which has become known as the **Alternate Pathway**. The initial steps of this process are quite different from those of the classical pathway and involve several unique serum components, namely factor P (for "properdin"), and factors named B and D. The mechanism is outlined below, and the initial step relies on the fact that *very small amounts of soluble C3b are normally present in serum*, due to low levels of spontaneous C3 cleavage ("tickover"), which may not be C4-dependent.

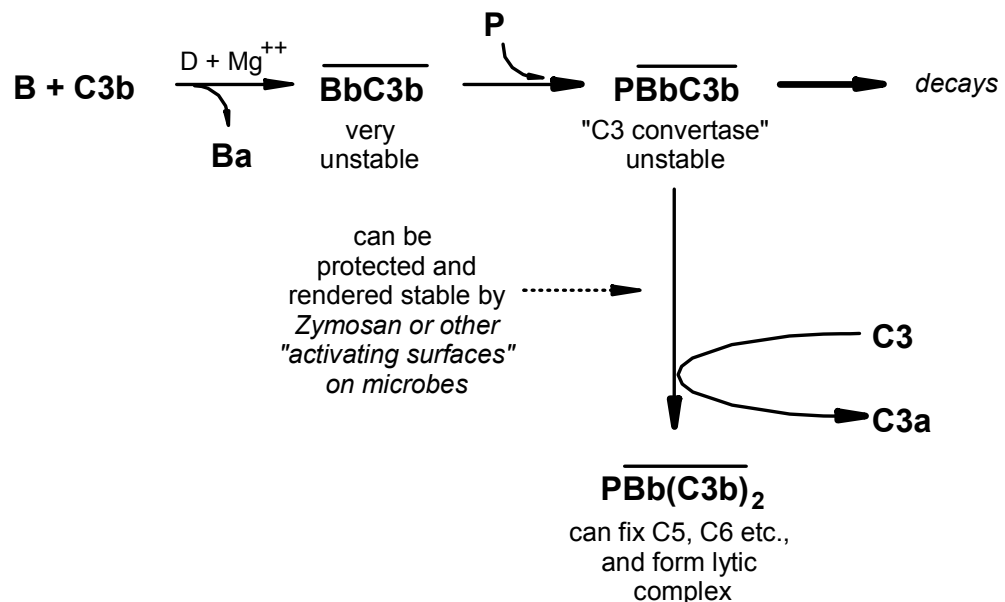


Figure 5-2

The initial steps of the alternate pathway result in the formation of an *unstable* complex which has "C3 convertase" activity (namely PBbC3b). This complex is formed continuously at low levels and rapidly degrades, but it may be bound and stabilized by "activating surfaces" such as *zymosan*-containing yeast cell walls, *LPS*-containing gram-negative bacteria, *teichoic acid*-containing gram-positive bacteria, and others. The result of stabilization is that *these C3-convertase complexes can carry out the remainder of the complement fixation cascade* in a manner identical to what we have outlined above for the classical pathway.

Thus, fixation of complement by the alternate pathway can yield all the variety of biological activities we have already seen -- chemotaxis, opsonization, anaphylotoxic activity and lysis (only if the membrane is susceptible to lysis, of course), in addition to all the inflammatory sequelae.

Initiation of the alternate pathway can be triggered by the presence of a variety of **microorganisms** (as mentioned above) and **parasites**. In addition, **aggregated immunoglobulin** may also trigger the alternate pathway, even isotypes of Ig which are incapable of fixing complement by the classical pathway (such as human IgG4 and IgA).

#### RELATIONSHIP BETWEEN CLASSICAL AND ALTERNATE PATHWAYS

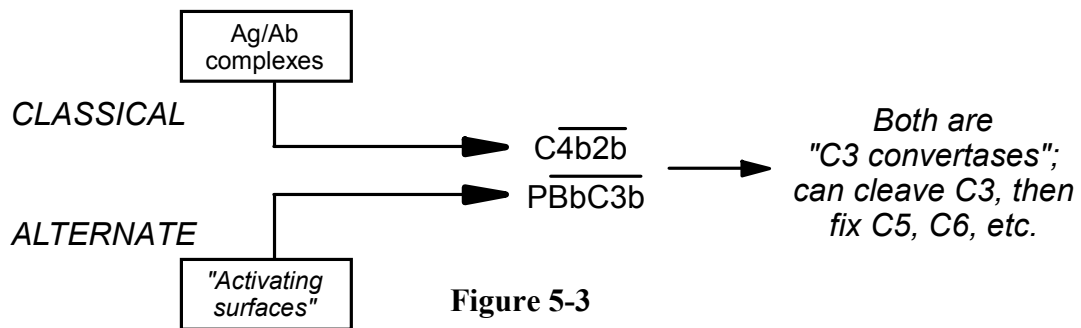


Figure 5-3

Figure 5-3 illustrates the relationship between the two complement pathways which we have just discussed. Both the classical and alternate pathways, using *different* mechanisms, generate complexes which have *C3 convertase activity*. After that point the two pathways are identical - each complex can generate and bind a new C3b molecule, then proceed to fix C5, C6 *etc*. It is worth noting that while the initiation of the alternate pathway is dependent on  $Mg^{++}$  (although this may not be absolute), the early steps of the classical pathway are dependent on *both*  $Mg^{++}$  and  $Ca^{++}$  (see steps 2 and 4 in the classical pathway diagram).

#### MBLECTIN COMPLEMENT PATHWAY

A third mechanism for the initiation of complement fixation has been described which depends on the presence of another normal serum protein known as the *mannan-binding lectin*, or MBLECTIN. This protein is capable of binding to microbial carbohydrates containing terminal mannose residues, and consequently binding two other proteins, MASP-1 and MASP-2 (**m**annan-binding lectin-**a**ssociated serum **p**rotease-1 and -2). The resulting complex has C4-convertase activity (*i.e.* it can bind and cleave C4), and the remainder of the

complement cascade (C2, C3, C5 *etc.*) is activated just as in the case of the classical and alternate pathways. This distinct complement pathway is diagrammed in Figure 5-4.

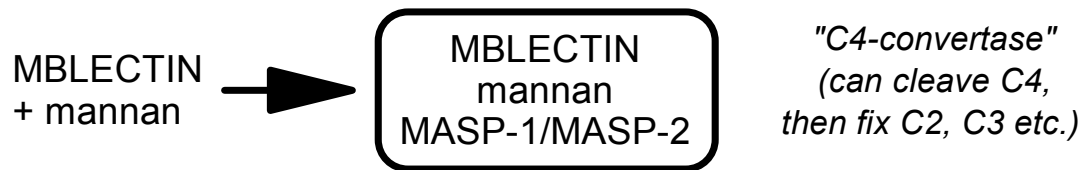


Figure 5-4

### SIGNIFICANCE OF THE NON-CLASSICAL COMPLEMENT PATHWAYS:

#### *RAPID, NONSPECIFIC ACTION*

Since they do not require the presence of antibody, both the ALTERNATE and MBLECTIN pathways can be *initiated much more rapidly* than the classical pathway upon initial exposure to a microorganism. There is no need to wait for several days while antibody formation is initiated, and it is thus a manifestation of "innate" immunity. On the other hand, these pathways do not have the exquisite specificity conferred by antibodies, and they are limited to recognizing only certain kinds of microbial structures for triggering (these are both characteristic features of innate immunity). Nor do they exhibit any form of memory.

Nevertheless, the alternate pathway, at least, can *augment* the biological effectiveness of specific antibodies (produced by *adaptive immunity*) in at least two ways. *First*, as mentioned above, aggregated Ig's (for instance, clustered on the surface of a microorganism) may trigger the alternate pathway, regardless of whether the classical pathway has also been initiated. *Second*, the C3b molecules generated by the classical pathway can promote the formation of the alternate pathway's "C3 convertase", PBbC3b, again resulting in increased complement fixation.

### BIOLOGICAL EFFECTIVENESS OF COMPLEMENT DOES NOT DEPEND SOLELY ON LYSIS

While complement fixation is generally thought of as culminating in lysis, we have already noted that only a limited variety of bacteria (mostly gram-negative organisms) are susceptible to such lysis (and not all nucleated cells, for that matter). This does not mean, however, that such cells are completely resistant to the consequences of complement fixation. A gram-positive bacterium can still be effectively *opsonized* (and consequently phagocytosed and destroyed) as a consequence of complement fixation by either pathway. In addition, the *inflammatory* sequelae of complement fixation (increased blood flow and accumulation of scavenger cells) all tend to increase the effectiveness of microbial destruction.



The final outcome following introduction of a pathogen into an organism will depend on many factors, including its susceptibility to complement dependent lysis and opsonization and its ability to trigger the alternate pathway of complement, as well as on the nature of the *adaptive* immune response which it generates (depending on its degree of immunogenicity and the isotype distribution of the resulting antibodies) and possible previous exposures of the immune system to the same or similar pathogens (immunological memory). Overall, the **opsonizing and inflammatory effects of complement appear to be more significant than lysis** in providing protection against infectious organisms.

## REGULATION OF THE COMPLEMENT CASCADE

We have thus far mentioned only those factors which *activate* complement components and drive the reactions in the direction of biological effectiveness. If these were the *only* relevant factors, then an initial complement fixation event would result in an uncontrolled inflammatory response and the consumption of all the complement available in the system; clearly this does not normally occur.

A variety of factors exist which are responsible for the *inactivation* of the various complement products which are biologically active. Some of these factors are known, including ones which inactivate the C1 complex, the C3a, C3b, C4a and C5a fragments, and the C5b67 complex. These inhibitory factors are critical to the normal balance of the complement system. For example, a hereditary deficiency in C3b-inactivator (called "Factor I"), results in excessive breakdown of C3 by the BbC3b complex and greatly depletes the serum levels of C3, which in turn results in high susceptibility to recurrent bacterial infections. A great deal is known about mechanisms which regulate complement activation, although we will not discuss them further here.

## IMMUNE COMPLEXES: COMPLEMENT-MEDIATED TISSUE DAMAGE

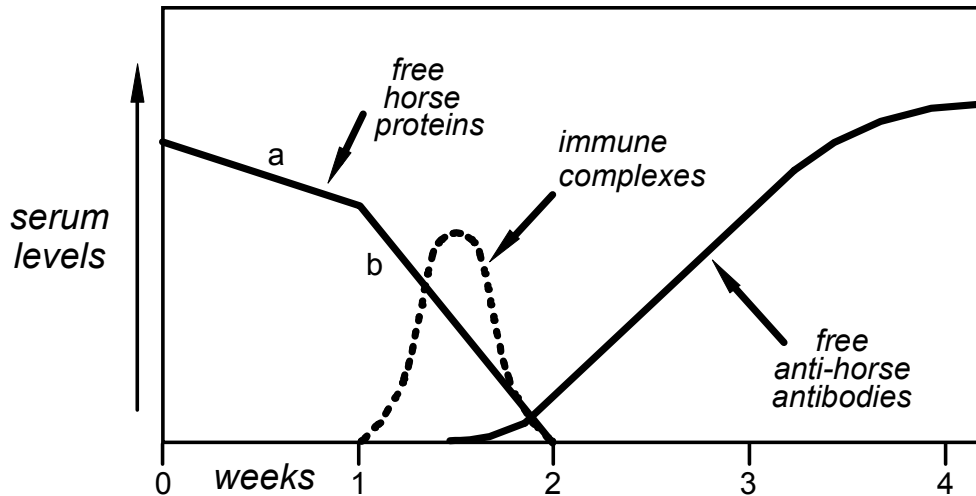
One of the consequences of antibody binding to its antigen is the elimination of the Ag/Ab complex by phagocytic cells; removal of foreign antigens is obviously one of the main goals of the immune response. If antigen/antibody complexes are formed in the circulation, however, they may have other consequences which can be quite harmful to the host.

Antigen/antibody complexes in the bloodstream can bind to the basement membranes of blood vessels and kidney glomeruli; at these sites they can fix complement which results in damage to tissues. This is the basis for IMMUNE COMPLEX DISEASE. The antigen may be associated with a pathogen (a virus, for instance), it may be clinically introduced (with blood transfusions or with drug or antibody therapy), or may be a normal "self" component (in the case of AUTOIMMUNE DISEASES such as RHEUMATOID ARTHRITIS and LUPUS ERYTHEMATOSUS).

**Immune complexes are harmful only if they fall within a limited range of sizes.** If the complexes are very large, they are efficiently removed by phagocytic cells (*e.g.* liver Kupfer cells and lung macrophages); if they are very small, they are effectively soluble and are not trapped in basement membranes (and these small complexes are normally removed by erythrocytes, discussed below). Immune complexes which fall between these two extremes, however, may become trapped in the basement membranes of the vasculature in various organs where they can fix complement and cause tissue damage.

The consequences of deposition of complexes within tissues include local inflammation and tissue necrosis. These are the consequences of the activity of the various complement components which we have already outlined, and there can be more systemic symptoms such as fever and malaise.

Let's illustrate this with a classic example known as **One-Shot Serum Sickness**. A man having suffered a severe tetanus-prone wound is given a single injection of horse serum containing anti-tetanus toxoid. After about a week, he complains of fever, malaise and rashes, which spontaneously subside after another week; and he remains well after that. To understand the progress of this reaction, we will refer to Figure 5-5 below:



### ONE-SHOT SERUM SICKNESS

Figure 5-5

During the first week following the injection, the horse proteins are removed from the circulation at a slow rate determined by their biological properties; this is referred to as **Biological Clearance** ("a"). At the beginning of the second week, they begin disappearing at a more rapid rate; this is due to the production of anti-horse antibodies and a resulting **Immune Clearance** of the complexes ("b"). Later on (in the third week) we can find appreciable levels of free circulating anti-horse antibodies, but only after the horse proteins themselves are gone.

During the period of immune clearance, at a time when *both* horse protein *and* anti-horse antibodies are present, complexes may be formed which are of the appropriate size to be trapped in basement membranes; and the results include the rash (local necrosis) as well as the fever and other systemic manifestations. *In this example the disease is self-limiting*, since there is no continuing source of antigen -- when the horse serum proteins are cleared, the symptoms disappear. However, *if there is a continuing source of antigen*, immune complex disease may present a more serious chronic problem; for example, the antigen may be associated with a chronic viral infection (e.g. hepatitis), or may be a normal tissue component (in the case of AUTOIMMUNITY).

This illustrates the danger of any therapy which makes use of foreign proteins, including anti-toxin therapy, and blood transfusions in general. This example is called "*one shot*" because it involves a single *primary* exposure to the antigen, and the disease symptoms are therefore delayed a week or two while induction of antibody formation takes place; if circulating antibody is already present at the time of injection, the disease will appear much more rapidly. It can be particularly dangerous to treat someone intravenously with a protein to which he has already been exposed, (with a *second* course of horse serum anti-toxin, for instance) due to the presence of pre-existing antibody and the induction of a rapid and powerful secondary antibody response.

#### C1 & C4 COMPLEMENT DEFICIENCIES:

##### INADEQUATE CLEARANCE OF IMMUNE COMPLEXES

We can learn much by examining the clinical *consequences of congenital complement deficiencies*. Deficiencies of components of the membrane attack complex (C5-9), as well as the alternate pathway components B, D and P have the expected results of increasing susceptibility to bacterial infections. More surprising, however, is the fact that deficiencies of C1 and C4 result in increased susceptibility to the development of *autoimmune disease* (SLE, or Systemic Lupus Erythematosus, which will be discussed later), and associated *immune complex disease*.

This reflects the fact that *C1 and C4, unlike C3 and the later components of the classical pathway, can be efficiently "fixed" by soluble Ag/Ab complexes*, and these components are therefore critically important in causing the rapid removal of such complexes from the circulation. This process depends largely on the presence of membrane receptors for C4b on erythrocytes (which express the complement receptor CR1). Soluble immune complexes which contain C4b therefore bind efficiently to RBCs, which carry them to the liver where the complexes are stripped off by macrophages, and the RBCs are returned unharmed to the circulation. When these early complement components are absent, small immune complexes which are normally harmless (because they are rapidly removed) may grow in size and eventually be deposited in tissues, and the alternate pathway components can then promote the inflammatory consequences we have already discussed. The resulting tissue damage can feed a continuing cycle of formation of further immune complexes and induction of autoimmune antibodies (see Chapters 18 and 19 on TOLERANCE and AUTOIMMUNITY).

The importance of this mechanism for clearing immune complexes results from the fact that *normal serum always contains small amounts of immune complexes*, mostly due to the spontaneous formation of autoantibodies to various "self" components. In the presence of the normal complement-dependent clearing mechanism, these autoantibodies and immune complexes are harmless. When this mechanism is defective, these immune complexes may grow and fuel a continuing cycle of tissue damage and autoimmunity (see Chapter 19).

CHAPTER 5, STUDY QUESTIONS:

1. What is required for the initiation of complement fixation by each of the CLASSICAL, ALTERNATE and MBLLECTIN pathways? What do the three pathways have in common?
2. Which proteolytic fragments of complement components have known autonomous biological activities?
3. What are the clinical consequences of the congenital absence of various complement components?
4. What medical situations are likely to result in ONE-SHOT SERUM SICKNESS?
5. What is the process by which soluble immune complexes are normally cleared from the blood?