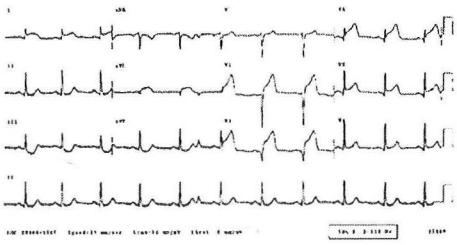
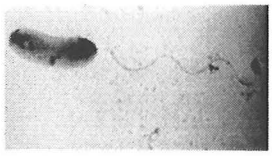
Angina, Arthritis, and Asthma – Can Complement Inhibition Cure Them All?





Jules Bordet
1870-1961

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This is to acknowledge that David Karp, M.D., PhD., has disclosed no financial interestes or other relationships with commercial concerns related directly or indirectly to this program. Dr. Karp will be discussing investigational therapeutic trials and will discuss off-label uses in his presentation.

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Dr. Karp's research interests include:

- The role of complement proteins and their receptors in lymphocyte physiology
- Control of oxidative stress by mononuclear cells
- Biological control of inflammation in rheumatic diseases

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Introduction

In 1919, the Nobel Prize in Physiology or Medicine was awarded to the Belgian microbiologist Jules Bordet for his "discoveries concerning immunity" (1). This followed over a quarter century of work by Bordet and others that defined the existence and function of what is now referred to as the complement system. The bactericidal nature of immune serum had been demonstrated in the 1880's and 1890's. The term, "alexin," was used by Buchner for this protective substance found in cell-free serum (2). Alexin was thought to be a heat-labile enzyme that destroyed bacteria.

In 1894, Pfeiffer reported that cholera vibrios injected into the peritoneum of immune guinea pigs were rapidly killed and that immune serum could transfer this effect to normal animals (3). Bordet and Metschnikoff showed that this could occur *in vitro*, and developed a novel, non-infectious, method to investigate this phenomenon by immunizing guinea pigs with rabbit erythrocytes and then monitoring the release of hemoglobin when the cells were lysed (4, 5). Based on these studies, Bordet was clearly able to identify the heat-stabile specific antibody fraction of immune serum, and the heat-labile" bactericidal fraction that "complemented" the antibody.

From the 1920's to the 1970's, the study of complement was largely biochemical. The eleven proteins of the classical pathway of complement activation their interactions were described. In 1954, Pillemer reported the existence of an alternative pathway that activates complement in the absence of specific antibody (6). Although the controversy over this finding contributed to Pillemer's suicide, he was ultimately vindicated. The alternative pathway is not only recognized as older evolutionarily than the "classical" pathway, but it is also responsible for complement activation by a diverse set of compounds such as bacterial endotoxin and biomaterials.

The last twenty-five years have focused on the identification of the regulatory proteins of the complement cascades. Almost as many serum and cell-surface proteins are involved in the regulation of complement function as are involved in its activation. In addition, an entirely new pathway of complement activation, the mannan-binding lectin pathway has been described. This pathway combines features of the classical and alternative pathways.

It is clear that the complement system serves a number of protective functions ascribed to the innate immune system. As originally described, it helps to maintain blood sterility by depositing the membrane attack complex in bacterial cell walls and lysing them. It also participates in the opsonization of pathogens for phagocytic removal. The peptide "anaphylatoxins" produced during complement activation promote inflammatory responses with microbicidal effects. The deposition of complement on immune complexes helps to keep them soluble and remove them from the circulation.

There is also increasing evidence that complement can shape the adaptive immune response. Antigens decorated by complement proteins are taken up by B cells and other antigen presenting cells resulting in T cell activation (7-10). Studies from mice deficient in various complement proteins have shown that complement activation is needed for optimal antibody production by B cells (11, 12). Lastly, it is well known that both humans and experimental animals that are deficient in early complement components are often predisposed to autoimmune diseases,

particularly systemic lupus erythematosus (13-15). This observation suggests that complement is required in some way to identify soluble self-antigens and eliminate self-reactive B cells.

In order for complement to become activated immediately upon exposure to immune complexes or other targets, it lacks the immunologic memory of T or B cells with clonotypic receptors that discriminate between self and non-self. Thus, activated complement can be deposited on host as well as pathogenic surfaces. This potentially dangerous situation is controlled by a series of genetically, structurally, and functionally similar proteins termed the Regulators of Complement Activation (described below). These proteins provide species-specific down-regulation of complement activation on host tissues. This fact is taken advantage of experimentally, where sheep erythrocytes coated with rabbit antibody are the typical targets for the measurement of complement in human serum. It is also one of the major obstacles to xenotransplantation.

Inappropriate complement action occurs when the non-discriminating activating proteins function in excess of the regulatory proteins that limit damage on self-tissues. This can be seen in almost any inflammatory disease. Some conditions are obvious, such as autoimmune hemolytic anemia, lupus nephritis and immune complex vasculitis. In others, the role of complement may be contributory, but less clear. These include myocardial infarction, stroke, cardiopulmonary bypass, and hemodialysis. Table 1 lists of conditions where complement activation is associated with pathology rather than protection.

Table 1. Pathologic conditions associated with complement activation

Alzheimer s disease	Allotransplantation
Asthma	ARDS
Arthus Reaction	Bullous Pemphigoid
Burns	Crohn s disease
Glomerulonephritis (many causes)	Hemolytic anemia
Hemodialysis	Hereditary angioedema
Ischemia/reperfusion injury	Immune complex vasculitis
Multi-system organ failure	Multiple sclerosis
Myasthenia gravis	Post-cardiopulmonary bypass
Psoriasis	Rheumatoid arthritis
Septic shock	Systemic lupus erythematosus
Stroke	Xenotransplantation

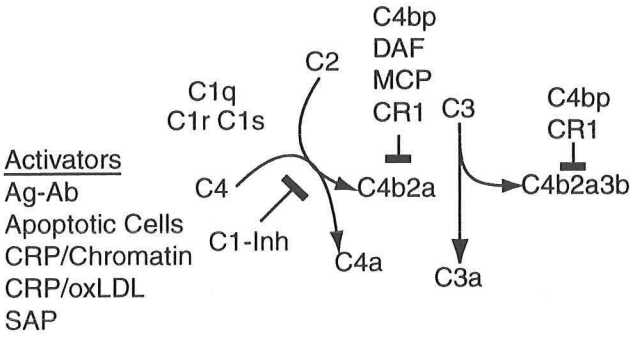
Adapted from (16)

In each of these conditions, inhibition of complement activation would limit tissue damage. A number of strategies have been developed to discover inhibitors that can work at different parts of the complement activation cascades. These include both small molecules designed like traditional drugs, as well as newer, biologic agents. The latter are both antibodies that inhibit complement activation as well as versions of human complement regulatory proteins. None of these compounds is available yet, although several are in advanced clinical trials.

Activation of Complement

As an essential component of the innate immune system, complement is endowed with redundant, yet carefully controlled, activation pathways. The molecular events that occur during activation are not only responsible for the pathology of complement-associated disease states, but also offer opportunities for the rational design of inhibitors. For simplicity, it is convenient to think of the different parts of the complement activation pathways as **recognition**, **convertase/amplification**, and **effector**.

Classical C3 convertase



The **recognition** step of the classical pathway requires the interaction of C1 with antigen antibody complexes. This is mediated by the binding of the globular heads of the C1q subunit to the Fc portion of IgM or certain IgG isotypes. This binding results in the autocatalytic cleavage of C1r, which in turn cleaves C1s. C1s is a serine protease that sequentially cleaves C4 and C2 to form C4b2a, a multi-protein complex that is the classical C3 convertase (cleaving enzyme).

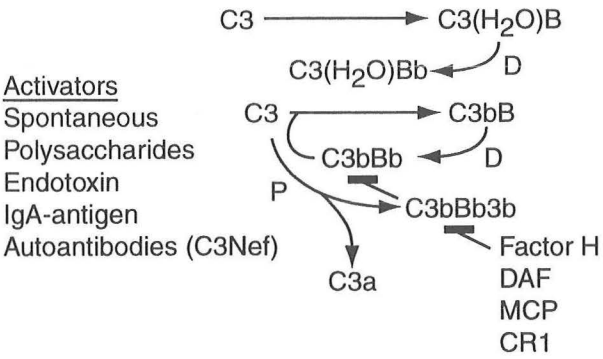
It has recently been shown that targets other than immune complexes can also activate the classical pathway. Notably, apoptotic cells bind C1q and activate the C1 proteases (17, 18). C1 is also activated by the accumulated Aβ protein found in the neuritic plaques of Alzheimer's disease (19). In addition, C-reactive protein and serum amyloid protein bind to chromatin and other ribonucleoprotein complexes released from apoptotic cells. The CRP-nuclear antigen complexes bind and activate C1 (20). Thus, C1q and the classical pathway appear to play a role in the opsonization and removal of nuclear materials that are frequently autoantigens. The few patients who have hereditary C1q deficiency all have systemic lupus erythematosus (21). Likewise, mice that have been engineered to lack C1q develop a lupus-like illness and have deposition of apoptotic bodies in their glomeruli (18, 22). The addition of CRP and enzymatically modified LDL to human serum causes the activation of complement as determined by nearly quantitative conversion of C3 to C3b (23). Finally, deposits of CRP and activated C1 have also been demonstrated in infarcted human myocardium. Together, these observations suggest that the antibody-independent classical pathway activation is important in the control of both protective immune responses and pathogenic inflammatory reactions.

Regulation of classical pathway activation occurs at several levels. First is the serine protease inhibitor (serpin), C1-Inh. C1-Inh inhibits the activity of a number of proteases, including Facto XIIa, kallikrein, and Factor XIa of the clotting system, and C1r and C1s of the complement system. The importance of C1-Inh is seen in the disease hereditary angioedema (HAE). In this instance, the heterozygous deficiency of C1-Inh allows uncontrolled proteolysis of C2 and C4 following minor trauma. A vasoactive peptide is released from C2 leading to painless (but

occasionally life-threatening) soft-tissue swelling. Treatment of acute attacks of HAE includes purified C1-Inh and anti-fibrinolytic drugs such as ε-aminocaproic acid.

Classical pathway activation is also regulated by a series of proteins termed Regulators of Complement Activation (RCA). These proteins form the basis for the ability of the complement system to discriminate self from non-self targets. They are discussed in depth below. The RCA proteins C4-binding protein (C4-bp) and Complement Receptor 1 (CR1) are specific to classical pathway regulation.

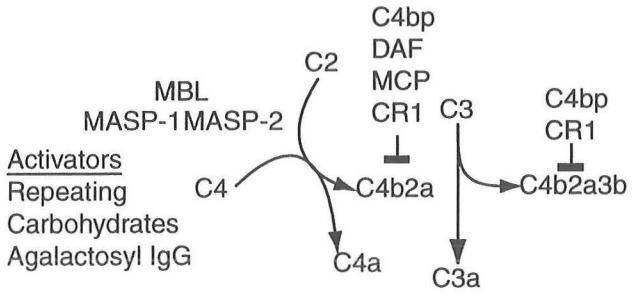
Alternative C3 convertase



The alternative pathway is much less stringent in its **recognition** requirements. It takes advantage of the fact that C3 undergoes spontaneous low-grade activation in the fluid-phase. Based on estimates of the glomerular filtration of C3a and its steady-state serum concentration, it has been suggested that 1 to 2% of serum C3 is activated in serum each hour. This allows the covalent attachment of C3 to the polysaccharides of fungi and bacteria as well other appropriately charged targets such as endotoxin and virally infected cells. Other alternative pathway activators include IgA immune complexes and biomaterials such as cardiopulmonary bypass and hemodialysis membranes.

Once bound to a surface, C3 acquires a C3b-like conformation and binds Factor B. B is cleaved by the serine protease, Factor D to form the alternative pathway C3 convertase C3bBb. This complex has a short half-life. It is stabilized by Properdin (Factor P) during physiological complement activation. It can also be stabilized by the autoantibody, C3-nephritic factor, which is associated with Type I membranoproliferative glomerulonephritis (MPGN). The alternative pathway C3 convertase is negatively regulated by the RCA proteins Factor H, DAF, and CR1 (see below).

Lectin activation pathway



The latest complement recognition and activation pathway to be described is the lectin pathway. The protein mannose binding lectin (MBL) is a member of the collectin family that includes pulmonary surfactants A and D (24). It has a structure similar to C1q, in that it consists of several subunits each having a globular recognition domain and a collagen-like portion that interacts with serine proteases. In the case of MBL, the globular domain is a lectin that binds to repeating carbohydrates (mannose and *N*-acetylglucosamine) on the surface of

pathogens. Many microorganisms are recognized by MBL, including gram-positive and –negative bacteria, mycobacteria, fungi, parasites, and viruses (25). In general, mammalian glycoproteins and glycolipids are not recognized by MBL. One notable exception is agalactosyl-IgG (26). The levels of this modified immunoglobulin are increased in inflammatory conditions such as rheumatoid arthritis, raising the possibility that excessive activation of the lectin pathway is clinically relevant.

Two serine proteases MASP-1 and MASP-2 associate with MBL, presumably through the collagen-like domain. This has not been formally proved, but is analogous to C1q. Activation of MASP-1 and –2 results in cleavage of C2 and C4, with the subsequent formation of the classical pathway C3 convertase (C4b2a).

Variation in the structural and regulatory portions of the MBL gene leads to wide individual differences in serum levels (27). Low levels have been associated with recurrent infections in both children and adults, and have been shown to be a minor risk factor for the development of SLE (28-31). More striking is the association of low levels of MBL with infection in SLE. In a recent study of Danish lupus patients, heterozygous MBL deficiency was associated with a 4-fold increase in the risk of bacterial pneumonia, while homozygous deficiency carried a greater than 100-fold risk (32).

Amplification of the C3 and C5 Convertases

The three activation pathways converge at C3. C3 (and C4) contains a reactive glutamic acid residue buried within the three-dimensional structure of the protein. Normally, the γ-carboxy group of that amino acid is linked to a nearby cysteine in an "internal thiolester." Upon activation, the thiolester is exposed to the surface of the protein where it can react with amino or hydroxyl groups. Most of the thiolesters are hydrolyzed by water to form inactive C3 or C4. Some form amide or ester bonds to proteins or carbohydrates, thus covalently attaching C3b (and C4b) to target surfaces. This enables cells bearing CR1 to bind these targets and opsonize them, representing one of the effector mechanisms of complement.

The covalently bound C3b associates with C4b2a (classical or lectin pathways) or C3bBb (alternative pathway) to form a convertase for C5. The fact that C3b is both part of the alternative pathway C3 convertase and its product generates an amplification loop that can deposit thousands of C3b molecules on a target, regardless of the initial activation step.

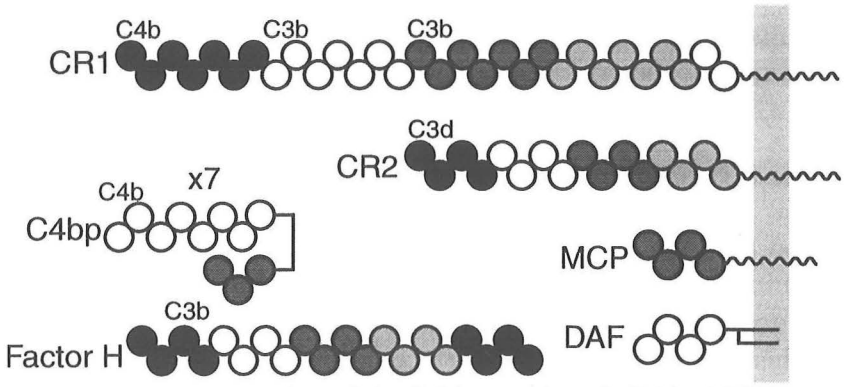
Regulators of Complement Activation

The most significant recent advance in the area of complement research has been the description of a series of related proteins, termed regulators of complement activation (RCA) (33). The major function of these proteins as a group is to limit the production of C3b by either the classical or alternative C3 convertases. Since the addition of C3b to a C3 convertase makes it a C5 convertase, regulation of the two enzyme complexes is linked. Modulation of their activity on host cells is a limitation to tissue destruction and the production of inflammatory mediators.

There are six RCA proteins that control the C3/C5 convertases. They are Factor H, C4 binding protein (C4bp), membrane cofactor protein (MCP; CD46), decay accelerating factor (DAF;

CD55), complement receptor 1 (CR1; CD35), and complement receptor 2 (CR2; CD21). The genes for all of these proteins are found in a cluster on human chromosome 1.q32. Structurally, they are composed of repeating subunits termed short consensus repeats (SCR) sometimes referred to as complement control protein (CCP) modules. Each SCR has approximately 60 amino acids with four invariant cysteine residues. The pairing of the disulfides leads to a 4-5 β pleated sheet structure causing the SCRs to appear like beads on a string.

Although the SCRs are structurally related, the individual RCA proteins may recognize different parts of the C3 molecule. They do so using specific combinations of SCRs. The RCA proteins function to control complement activation by two processes. First is decay acceleration. This refers to the process where the RCA protein binds to C3b in the convertase and dissociates the other members of the complex, rendering it enzymatically inactive. The second effect is co-factor activity. Some of the RCA proteins facilitate the recognition of C3b or C4b by a serum protease, factor I. Cleavage of C3b or C4b by factor I also render the convertase inactive.



Schematic representation of the RCA proteins. Individual SCRs are indicated by circles. The relative binding sites for C3 and C4 fragments are shown.

Table 2. Distribution and Function of RCA Proteins

RCA Protein	Distribution	Function
C4 binding protein	Serum	Cofactor for C4b; decay of classical C3/C5 convertases
Factor H	Serum	Cofactor for C3b; decay of alternative C3/C5 convertases
Decay Accelerating Factor	Epithelial, endothelial, and most blood cells	Decay of classical and alternative C3/C5 convertases
Membrane Cofactor Protein	Epithelial, endothelial, and most blood cells (not RBC)	Cofactor for C3b and C4b
Complement Receptor 1	Most blood cells; mast cells	Cofactor for C3b and C4b; decay of C3/C5 convertases; receptor for C3b/C4b
Complement Receptor 2	B cells; Follicular dendritic cells	Receptor for C3b fragments; regulation of B cells

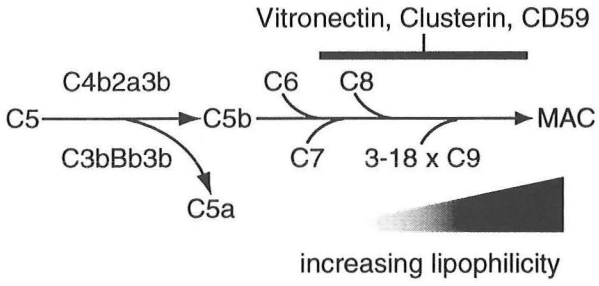
Adapted from (34)

Despite their relatedness, the RCA proteins have differences in their overall structure, distribution and function. All of the RCA proteins except MCP and CR2 have decay accelera-

tion activity. It is the only function of DAF. This glycosylphosphatidylinositol-linked protein is widely expressed. It causes the removal of C2a or Bb from the C3 and C5 convertases. DAF lacks the co-factor activity seen by the other RCA proteins (again, except CR2). Factor H and C4bp are serum proteins. MCP and DAF are ubiquitously expressed membrane proteins. CR1 and CR2 are membrane proteins expressed primarily on hematopoetic cells.

RCA proteins have been linked to several disease states. DAF is missing from the abnormal erythrocytes of patients with paroxysmal nocturnal hemoglobinuria (35). Although the hemolysis of these cells is ultimately due to the fact that the cells also lack CD59 (see below), the DAF deficiency promotes complement activation on these cells. Genetic factor H deficiency has been associated with Type II membranoproliferative glomerulonephritis in both humans and a strain of Yorkshire pigs (36, 37). Renal biopsies in both cases show evidence of robust alternative pathway activation. There is also evidence that mutations in factor H are responsible for some of the pathology seen in either sporadic or familial hemolytic uremic syndrome (38). Lastly, low levels of CR1 and/or CR2 have been seen in patients with systemic lupus erythematosus. CR1 does have cofactor and decay activity. Its major role is in the removal of immune complexes from the circulation, while CR2 is necessary for optimal B cell regulation, including the down modulation of autoreactive B cells.

The Membrane Attack Complex



The cleavage of C5 by either convertase generates C5a, the most potent of the complement anaphylatoxins, and C5b. C5b associates with C6 and C7 to create a lipophilic trimer. On the surface of a target cell, <1% of the C5b67 trimers formed will insert into the lipid bilayer and serves as binding sites for C8. This attracts C9 to the membrane. C9 has the capacity to self-polymerize. 12-18 C9 molecules form a ring structure, completing the membrane attack complex (MAC). In its complete form, the MAC appears like a doughnut with a 10 nm pore running through the center. This can allow water and ions to enter the cells, ultimately leading to cell lysis. However, a MAC with only one or two C9 molecules can also cause lysis, suggesting the MAC disrupts the lipid integrity in its general vicinity, rather than creating holes in the membrane (39).

The MAC itself appears to be largely redundant in terms of protection against infection. It only appears to be essential for efficient elimination of *Neisseria* species (40). Individuals who are homozygous deficient for C6, C7, C8, or C9 are at risk for meningococcal and gonococcal infection. However, C9 deficiency is the most common immunodeficiency in Japan, with a heterozygote frequency of 3-5% (41). Clearly, absence of an efficient membrane attack complex is not deleterious to the population in general and may have some selective advantage.

Extensive complement activation during an inflammatory response can result in sufficient MAC deposition to cause host cell lysis. However, most nucleated cells have mechanisms to resist the osmotic changes caused by the MAC, and may actually "disassemble" the MAC as it is formed. Rather, the non-lethal effects of sub-lytic MAC deposition are more likely to contribute to

pathology. In most cells, this occurs by a general activation of multiple cell signaling pathways. Calcium enters the cell activating protein kinases, phospholipase C, and up-regulates cAMP production (42-44). G-proteins and their associated factors are concentrated at the cell membrane, perhaps localized to C9 directly (45). The mitogen activated protein kinase pathway (ERK, JNK, and p38) are activated, resulting in the induction of transcription factors such as c-*jun* and *fos*, cell proliferation, and inhibition of apoptosis (46-48).

The response to MAC deposition depends on the cell type (Table 3). In phagocytic cells such as PMN or macrophages, sub-lytic MAC activation leads to the production of reactive oxygen species such as superoxide and hydrogen peroxide, as well as prostaglandins and leukotrienes (49, 50). Platelets undergo the exposure of phosphatidylserine on their outer membrane, resulting in greater formation of blood coagulation enzyme complexes (51). This has a potentially procoagulant effect. On endothelial cells, MAC deposition leads to a number of important events. It induces the synthesis of IL-1α, which leads to further autocrine and paracrine endothelial cell activation (52). It stimulates a procoagulant state by altering the phospholipid composition of the endothelial membrane, inducing the synthesis of tissue factor, and up-regulating the synthesis of plasminogen activator inhibitor. MAC treatment of endothelial cells causes an increase in the expression of adhesion molecules including ICAM-1 and E-selectin (53). Finally, the MAC stimulates endothelial cells to proliferate through growth factor production (54). Thus, despite the fact that cell lysis does not occur, deposition of the MAC leads to a potentially more dangerous situation with increased inflammation, coagulation, and cellular proliferation.

Table 3. Responses to Sub-Lytic Membrane Attack Complex Activation

Cell Type	Effects
Most Cells	• Increased intracellular calcium flux • G protein activation • Activation of protein kinases • Activation of transcription factors • Proliferation
Neutrophils and Macrophages	• Release of reactive oxygen species • Activation of phospholipase A2 • Release of prostaglandins, thromboxane, and leukotrienes
Platelets	• ATP release • Increased P-selectin expression • Procoagulant membrane changes
Endothelial cells	• Increased IL-1α synthesis • Increased tissue factor release • Increased von Willebrand factor release • Increased basic fibroblast and platelet derived growth factor synthesis
Synoviocytes	• Increased prostaglandin synthesis • Increased IL-6 synthesis • Increased matrix metalloproteinase production
Glomerular epithelium	• Phospholipase A2 activation • Prostaglandin synthesis • Increased collagen and fibronectin synthesis
Oligodendrocytes	• Increased myelin basic protein and proteolipid synthesis • Increased proliferation

Regulation of MAC formation is important clinically and has become an area of therapeutic research (55). Two fluid-phase proteins, clusterin and S-protein (vitronectin) bind the C5b-7 complex and prevent its association with the lipid membrane. C8 and usually 2-4 C9 molecules bind to this soluble complex termed sC5b-9. The sC5b-9 is lytically inactive. CD59 is a membrane-bound inhibitor of MAC formation. This small glycoprotein is attached to the cell membrane through a glycosylphosphotidylinositol (GPI) tail. It binds tightly to C5b-8, preventing the binding and polymerization of C9. CD59 shows strong species restriction. That is, it is most effective in the inhibition of MAC formation by the same or closely related species. Lastly, the expression of CD59 is defective in patients with paroxysmal nocturnal hemoglobinuria (PNH). This is due to failure to synthesize the GPI tail on this and many other cell-surface proteins, including decay accelerating factor. The clinical features of PNH are protean. The hemolysis, however, is felt to be due to low-grade complement activation on red cells. Without CD59, MAC formation proceeds and allows hemolysis.

Anaphylatoxins

In addition to the membrane attack complex, the other major source of pathological damage due to complement activation comes from the action of the anaphylatoxins. These are the peptides C3a, C4a, and C5a cleaved from their respective proteins during activation. They were named by Friedberger in 1910 to describe the toxic effects following the transfer of complement activated serum into laboratory animals (56). They are 77 (C3a and C4a) or 74 (C5a) amino acids long and contain a carboxy terminal arginine. The structures of C3a and C5a have been determined by x-ray crystallography and NMR (57, 58). They have a compact amino terminal region that is held together by conserved disulfide bonds. This part of the molecule contains cationic amino acids that are felt to interact with the anaphylatoxin receptors. The carboxy terminal regions of the anaphylatoxins are extended sequences. Only the last five amino acids are required for activity. In plasma, the C-terminal arginine is rapidly removed by Carboxypeptidase N from anaphylatoxins not bound to their receptors. Depending on the response studied, this totally inactivates the anaphylatoxin, or reduces its potency by up to 1000-fold.

The most important advance in the understanding of anaphylatoxin effects has been the identification of their receptors. This has been done by a combination of molecular cloning and immunochemical techniques. The C5a receptor (C5aR, CD88) was the first anaphylatoxin receptor characterized (59, 60). It is a seven-transmembrane spanning protein that couples ligand binding to G-protein signaling. Traditionally, it was thought to be expressed only on myeloid cells, particularly neutrophils and eosinophils. It mediates the potent chemoattractant property of C5a for both these cell types. Signaling through CD88 leads to rapid secretion of all granule contents. These include proteases, peroxidases, and lactoferrin from neutrophils and peroxidase, major basic protein, and eosinophil cationic protein from eosinophils. C5a also induces the release of cytokines such as TNF, IL-1, IL-6, and IL-8 as well as adhesion molecules, thus promoting the inflammatory response (61-64).

The C5aR has also been found on numerous other tissues (Table 4). These include hepatocytes, bronchial and alveolar epithelium, vascular endothelium, renal mesangial and tubular epithelial cells, and brain astrocytes, microglia, and neurons (see (65) for discussion). The function of C5a

in these tissues is not entirely clear. *In vitro*, these cells are activated by exposure to the anaphylatoxins, producing cytokines, chemokines, prostaglandins, and proliferating.

The C3a receptor has recently been identified. It is also a seven-transmembrane domain protein (66). Like the C5aR, the tissue distribution of the C3aR is much greater than previously thought. It is expressed on nearly all myeloid cells, including mast cells, where it mediates allergic mediator release. The C3aR has also been detected on many tissues, including the brain by Northern blot for RNA expression. Detailed functional studies have not been carried out.

The anaphylatoxins have many biological effects. In general, they cause smooth muscle contraction, and recruitment of granulocytes, monocytes, and mast cells. In theory, they can contribute to the pathophysiology of any inflammatory condition. Examples where C3a and C5a have been shown to play a role in disease include acute respiratory distress syndrome (ARDS) (67, 68), multisystem organ failure (MSOF) (69), septic shock (70), myocardial ischemia/reperfusion injury (71-73), rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease. The anaphylatoxin peptides are also responsible for the "post-pump" syndrome seen in patients undergoing cardiopulmonary bypass or hemodialysis (74-77). Exposure of blood to dialysis or perfusion membranes leads to complement activation. Within minutes of starting bypass, there is a sharp increase in the level of C3a and C5a in the extracorporeal circuit being returned to the patient. This can be associated with respiratory distress, pulmonary hypertension, and pulmonary edema. It has been shown that the length of time that patients stay on the ventilator after bypass surgery depends on the level of C3a generated during reperfusion (77).

Table 4. Distribution of Anaphylatoxin Receptors and Their Cellular Responses

	Cell Type	Response
C5aR (CD88)	Neutrophils Eosinophils Basophils Mast cells Monocytes Hepatocytes Pulmonary epithelium Neuronal cells Endothelial cells Renal epithelial/mesangial cells	• Chemotaxis • Enzyme release • Generation of reactive oxygen species • Upregulation of adhesion molecules • Increased IL-1, IL-6, and IL-8 synthesis • Prostaglandin/leukotriene synthesis • Increased synthesis of acute phase reactants • Increased IL-8 ? • Increased expression of P-selectin • Proliferation • Synthesis of growth factors
C3aR	Eosinophils Mast cells Platelets CNS (multiple cells)	• Chemotaxis • Enzyme release • Generation of reactive oxygen species • Upregulation of adhesion molecules ?

Adapted from (65, 78).

C3a and C5a have been implicated in both the initiation and prolongation of ARDS and MSOF. After severe trauma, levels of C3a have been measured that suggest activation of the entire circulating C3 pool. This leads to bronchoconstriction, increased vascular permeability, and vascular plugging with leukocytes. The activation of white blood cells then continues the cycle

of tissue damage and further complement activation. Continued elevation of C3a in shock or ARDS is a poor prognostic sign (67).

The role of C5a in pulmonary pathology has also been shown using C5aR "knock-out" mice. A model of immune complex damage to the lung was used (79). The wild-type mice had expected increases in lung permeability and leukocyte infiltration after induction of intra-pulmonary ovalbumin/anti-ovalbumin complexes. These effects were not seen in the C5aR-deficient animals. In other animal models, antibodies to C5a have been shown to limit infarct size due to myocardial ischemia-reperfusion and tissue damage in experimental septic shock.

Complement Inhibitors

Given the large number of disease states where complement is one of the central mediators of pathology, it is no surprise that several complement inhibitors are in pre-clinical or clinical development. These take several different forms. Some are variations of physiological inhibitors, while others are the products of molecular biological searches for novel compounds.

It is important to consider where in the complement pathway to design an inhibitor. Inhibition of the activation pathways will limit the production of biologically active peptides. However, all three pathways will need to be inhibited for this to be effective. Inhibiting the activation of C3 will prevent the generation of the C3a anaphylatoxin, but may also leave the patient susceptible to infection by limiting the deposition of C3b on targets as an opsonin. Inhibition of C3b deposition would also theoretically decrease the patient's ability to clear immune complexes, resulting in renal, pulmonary, and vascular damage. It may also promote the development of antibodies to self-antigens.

Inhibition of the C5 convertases is an attractive goal, as it would prevent the generation of the C5a anaphylatoxin as well as the membrane attack complex. This strategy would inhibit complement activation from any cause without the potentially immunosuppressive effects of limiting C3b deposition. Inhibitors based on this concept are the farthest along in clinical trials.

Other concerns about complement inhibition include the question of whether it is short or long-term, and whether it is systemic or localized. Long-term inhibition of complement, particularly at early steps, is likely to predispose the patient to infection. Short-term (hours to days) inhibition at any step is unlikely to cause problems. Given that inflammation is usually a local phenomenon, there are several mechanisms being tested to target complement inhibitors to these sites. In this way, higher levels of inhibition can be achieved where needed with lower doses of inhibitor.

Natural Complement Inhibitors

There is a large literature on naturally occurring compounds that control complement activation (80). These include products or extracts of plants, fungi, insects, venoms, and cell lines. The mechanism of complement inhibition by some of these natural products is known and of either clinical or experimental importance. For example, cobra venom factor (CVF) isolated from *Naja naja* is a 144,000 dalton glycoprotein that forms an alternative pathway convertase in association with Bb. This leads to massive activation of complement that causes pulmonary microvascular

injury in experimental animals. However, this is usually not fatal and the end result is a total depletion of complement that lasts 4-6 days. During this window, it has been possible to demonstrate the importance of the complement system in animal models of immune complex vasculitis, glomerulonephritis, multiple sclerosis, and graft rejection.

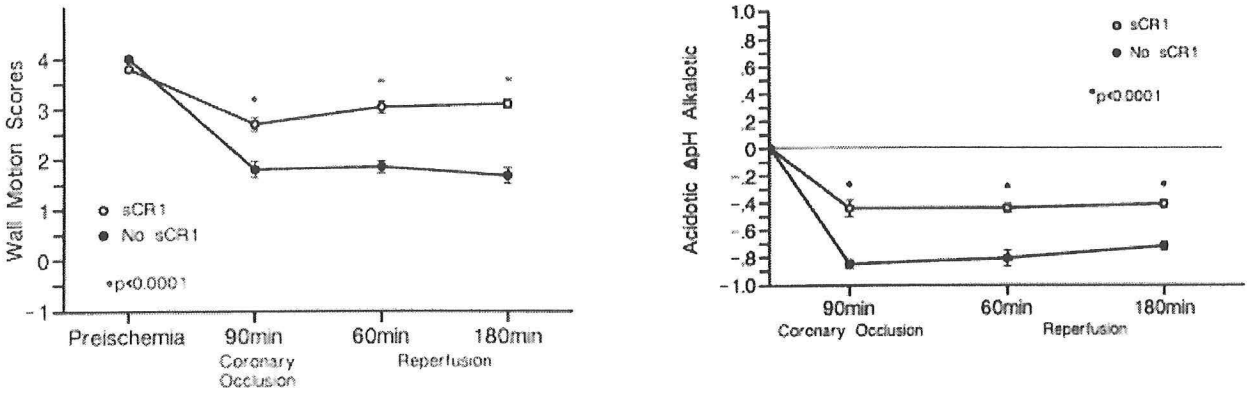
Perhaps the most widely used natural inhibitor of complement activation is heparin. Although heparin has been known to inhibit complement since 1929, the mechanism is not entirely clear (81). It decreases activation of both the classical and alternative pathways. It has been reported to block association of C3b with Bb, inhibit binding of C4 to C1s and C2, and inactivate C1q (80). In clinical practice, the anti-complementary effect of heparin has been used to prevent complement activation during cardiopulmonary bypass. Measurement of complement activation products such as C3a or soluble C5b-9 following bypass demonstrated decreases of 35-70% for both adult and pediatric patients when heparin coated extracorporeal circuits (e.g., Duraflo II) were used. Although numerous studies have looked at the decrease in complement activation by heparin-coated bypass circuits, there have been few attempts to correlate this with clinical outcome (82-87). In one study of 120 patients undergoing coronary artery bypass surgery, the use of heparin-bonded biomaterials was associated with a significantly shorter time on the ventilator (9.2 vs 12.4 hr) and earlier hospital discharge (88). There was also significantly less postoperative fever, leukocytosis, and creatinine elevation seen in the patients perfused with heparin-coated bypass circuits.

Soluble CR1

Soluble CR1 (sCR1) was the first rationally designed complement inhibitor to undergo extensive testing. The idea behind the use of this RCA protein was that it had multiple mechanisms of action. It has two separate binding sites for C3b and one for C4b. Therefore it not only serves as a cofactor for the enzymatic degradation of C3b and C4b, but it also can dissociate both the classical (C3b4b) and alternative ($C3b_2$) C5 convertases. It is produced by recombinant methodology in animal cells. It consists of the entire extracellular portion of CR1 (30 SCRs) and has been termed TP10 by its manufacturer, Avant Immunotherapeutics. A modified version, TP20, is produced in a manner that decorates the protein with the carbohydrate sialyl Lewis^x, the ligand for P- and E-selectin. This modification targets sCR1 directly to activated (inflamed) endothelium. Another modified form of sCR1 has been developed by AdProTech, Ltd. Rather than the entire 30 SCR protein, they have produced just the first three SCRs that retain complement inactivating capacity. A cationic peptide is added to the carboxy terminus of the protein followed by a myristyl group. This targets the protein to the lipid membrane of cells. This technique has been shown to be effective in situations where sCR1 can be delivered locally, such as intra-articular injections, or the perfusion of donor organs prior to transplantation.

Like its membrane-bound counterpart, sCR1 binds C3b and C4b and blocks human classical and alternative pathway activation. It also blocks activation of complement in a number of experimental animals, leading to almost 100 publications of its utility in a wide number of disease models.

The first disease model that was tested with sCR1 was myocardial ischemia/reperfusion injury in rats (89). This has been confirmed in several other species (90-93). Human studies have been limited to a few Phase I trials reported in abstract form. In one, 24 patients who had myocardial



Effect of Soluble CR1 on Myocardial Wall Motion and Acidosis in Pigs Following Coronary Artery Ligation and Reperfusion (91)

infarctions received sCR1 and either thrombolytic therapy or angioplasty. There was a dose-dependent decrease in the CH50 and blunting of the usual rise in C3a seen after MI. In the nine patients who received thrombolysis and larger doses of sCR1, there was a trend toward lower levels of peak CK-MB, although this did not reach statistical significance.

Other models of ischemia/reperfusion injury have also been investigated. These include intestinal ischemia in mice and rats (94), and middle cerebral artery ligation in mice. In each case, the administration of sCR1 was associated with decreased tissue injury, less neutrophil accumulation, and lower concentrations of inflammatory mediators such as leukotriene B4. In allograft transplantation, the donor organ undergoes significant ischemia/reperfusion injury. Animal models of allogeneic renal and lung transplantation have shown that sCR1 prolongs graft survival, which may prevent early rejection episodes. In pig to primate cardiac xenotransplantation, sCR1 prolongs graft survival remarkably (95). Untreated cynomolgus monkey recipients reject pig hearts in one hour or less. A single bolus of sCR1 prolongs graft survival 48 to 90 hr. Continuous infusion of sCR1 (40 mg/kg/d) resulted in survival of the graft (and the monkeys) for up to 168 hr. Thus, it appears feasible to use sCR1 as one method to overcome hyperacute rejection.

In one study of human lung transplantation, 59 patients were randomized to receive a single infusion of 10 mg/kg of sCR1 before restoration of blood flow in the graft (96). Complement activation was suppressed for up to two days after surgery. There were trends toward decreased time on the ventilator and in the ICU that did not reach statistical significance. For patients on who had been on cardiopulmonary bypass during there was a significant (9.5 vs. 21.5 hr) decrease in time on the ventilator.

Finally, sCR1 has been tested in models of autoimmune disease. Given intravenously, it delays the onset of collagen-induced arthritis in rats (97), as well as blunting the progression of established disease. In experimental autoimmune neuritis, a model for Guillain-Barré syndrome, daily sCR1 administration prevented the development of paresis and sciatic nerve damage (98). Similar, beneficial results were seen in models of myasthenia gravis, multiple sclerosis, and glomerulonephritis (99-101).

CD55-CD46

Another soluble RCA protein under development as a therapeutic complement inhibitor is based on DAF and MCP. As stated above, DAF acts as only as a decay accelerator of C3 and C5 convertases, while MCP acts as a cofactor of for the degradation of C3. A recombinant fusion protein has been made that combines the four SCRs of MCP followed by the four SCRs of DAF. This protein now has both decay accelerating and cofactor activity, much like sCR1. It is under preclinical development by Millennium Pharmaceuticals under the name CAB-2. Its indicated use will be the control of complement activation during cardiopulmonary bypass procedures. Published data in this circumstance are lacking. However, it has been shown to prolong the survival of pig hearts exposed to human blood from a control value of 17.3 minutes to over three hours. It prevented the release of C3a and soluble membrane attack complex as indicators of ongoing complement activation (102).

Transgenic Animals

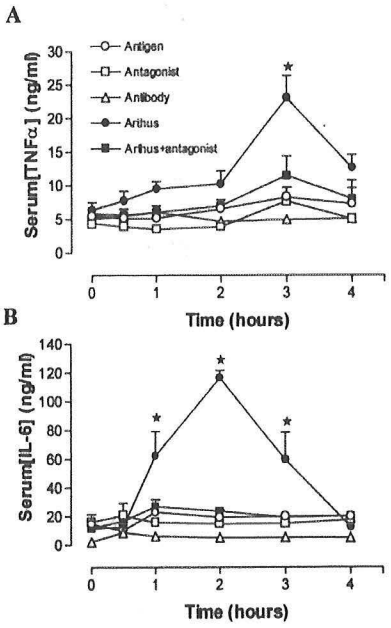
Xenotrasplantation offers a solution to the chronic lack of solid organs for transplantation. The most studied donor animal is the pig, as swine have a number of desirable experimental and practical characteristics such as size and ease of production. While immunosuppression and other strategies may be able to overcome cellular immune barriers, the immediate problem facing xenotransplantation is hyperacute rejection (HAR). This is the immediate (within minutes) cessation of graft function due to natural IgM antibodies that react with the vascular endothelium of the xenograft. The target of these antibodies is mainly the carbohydrate moiety, Galactose-(α1,3)-Galactose present on the graft. These antibodies quickly activate complement, leading to intravascular coagulation, tissue edema, hemorrhage, and endothelial activation. Prevention of HAR would require the reduction of the antibodies, their antigen, complement activation, or a combination of all three.

Since complement regulatory proteins display species specificity, the approach to limit complement activation in xenografts has been to make transgenic pigs expressing one or more membrane proteins of human origin. In one recent study, pigs were generated that were transgenic for human CD55 (DAF), CD59, and the enzyme α1,3-fucosyltransferase (HT). This enzyme modifies the carbohydrate antigen and had successfully been shown to prolong orthotopic heart transplants in mice (103). Kidneys from the triple transgenic pigs were transplanted into bilaterally nephrectomized baboons (104). No immunosuppression or pretreatment of the recipients was given. Under these circumstances, the function of a non-transgenic pig kidney ceases within three minutes and the graft rapidly become non-viable. The function of transgenic kidneys was maintained with good urine output for 3-5 days in the six baboons that received these grafts. The experiment was not perfect however. The production of the HT enzyme was sub-optimal and xenogeneic IgM still bound to the graft, although complement activation was reduced. The presence of the IgM in the graft was sufficient to cause delayed xenograft rejection with systemic disseminated intravascular coagulation, vascular infarction within the graft, and pronounced perivascular cellular infiltration. There is also evidence for direct activation of human monocytes and natural killer cells by the Gal (α1,3)-Gal epitope (105, 106).

While the technical barriers to preventing HAR can theoretically be overcome, another problem is the potential for the transmission of porcine endogenous retroviruses (PERVS). All contain several copies of these viruses in their genomes, and they have been shown to infect human cell lines *in vitro*. They have also been shown to be transcribed and form infectious virus in an animal model of an immunosuppressed host (107). On the other hand, no evidence of PERV infection has been found in patients who were exposed to porcine tissues or received xenografts (108). Thus, while transgenic pigs appear to be a promising resource for xenotransplantation, both technical issues relating to hyperacute rejection and safety issues relating to transmission of disease need to be addressed.

C3a and C5a Receptor Antagonists

The profound biological effects of the anaphylatoxins and the great number of conditions where C3a and/or C5a are felt to play a pathological role make the development of specific inhibitors attractive. Since the active portion of the anaphylatoxins is contained in the very carboxyl terminal part of the protein, it is possible that small molecule antagonists could be developed that may be orally available, easy to synthesize, and inexpensive. These are all advantages over the use of biologics such as monoclonal antibodies or recombinant RCA proteins.



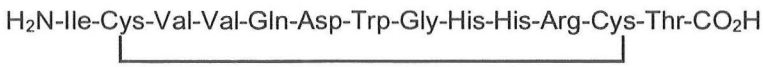
Inhibition of the Arthus reaction by a cyclic C5a analog

To-date, there have been several synthetic C5a antagonists described. One is a cyclic analog of C5a, acetyl-Phe[L-ornithine-Pro-D-cyclohexylalanine-Trp-Arg]. This compound inhibits C5a binding to CD88 with an IC_{50} of 20 nM. *In vivo*, it inhibits an Arthus-type reaction, completely blocking vascular permeability, cellular efflux, and systemic IL-6 and TNF production (109). The other C5a antagonist that has been studied is actually a mutant version of C5a itself, selected using random phage methodology. This compound was also effective in the inhibition of Arthus reactions in mice as well as preventing ischemia reperfusion injury (110). Neither of these C5a inhibitors is in human clinical trials. Finally, a potent small molecule inhibitor of C3a has been reported in an abstract. This compound is active *in vitro* as an inhibitor of C3a-mediated cellular activation, chemotaxis, and smooth muscle contraction.

Compstatin

Since the proteolytic cleavage of C3 and its subsequent attachment to target surfaces is the central event of complement activation, it seems rational to look for inhibitors of this step. The laboratory of John Lambris at Pennsylvania used the technique of random peptide libraries to address this question. A library of 2 x 10^8 unique bacteriophage clones expressing random 27 amino acid peptides was constructed. Phage that bound specifically to C3b were selected and amplified. From the 200 million different phage, a single clone was identified. It contained a

cyclic peptide, by virtue of two cysteines that formed a disulfide bond. The core sequence that retained C3 binding was found in a 13 amino acid cyclic peptide, termed Compstatin (111).



Structure of the Cyclic Peptide, Compstatin

Compstatin binds intact C3, as well as the activation products C3b and C3c. It inhibits the classical and alternative pathways of complement activation with IC_{50} values of 63 and 12 μM, respectively. The mechanism of Compstatin action is not clear. It appears to bind to native C3 at a site distinct from the C3a/C3b cleavage site and alters the protein so that it is not recognized by either C3 convertase. Compstatin has been tested in several disease models. First, pig kidneys were perfused *ex vivo* with human serum (112). The addition of Compstatin led to a 4-fold increase in graft survival time, from 90 minutes to 360 minutes. Second, Compstatin was shown to inhibit the activation of complement in loops of plastic tubing. This is a model for the generation of complement activation products during extracorporeal circulation (113). Compstatin effectively prevented the generation of C3a, soluble C5b-9 (membrane attack complex) and the attachment of C3 fragments to the biopolymer. The ability of Compstatin to block C3 activation prevented the activation of polymorphonuclear cells and their attachment to the plastic. Lastly, Compstatin administered to baboons at dose of 21 mg/kg (bolus plus infusion) completely inhibited the activation of C3 following the administration of protamine-heparin complexes (114). There were no adverse effects on heart rate, arterial or venous pressures, red cell counts or indices, platelet counts, or white blood cell counts.

These data indicate that Compstatin is an effective and apparently safe inhibitor of C3 activation. Its major drawback is the fact that it must be given as an intravenous infusion. This is not a problem during times when complement activation is anticipated, i.e., cardiac bypass surgery. The three-dimensional structure of the Compstatin peptide is under investigation. It should be possible to use that to design more potent; orally bioavailable compounds for conditions such as organ transplantation or lupus nephritis.

Anti-C5

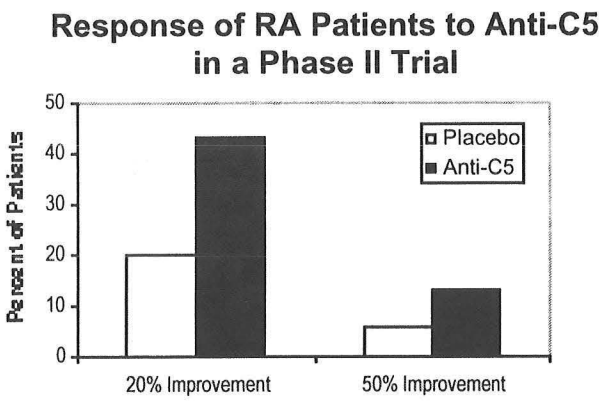
The complement inhibitor that has achieved the widest attention as a potential therapeutic is a monoclonal antibody to C5. The advantage to this strategy is that it prevents the generation of C5a, the most potent of the anaphylatoxins, as well as the membrane attack complex. The generation of C3b and C4b opsonins would still occur. This would allow proper clearance of pathogens and immune complexes even if C5 conversion were chronically inhibited. Since there is evidence that activation of early complement components is important for the maintenance of tolerance to self-antigens, inhibition of C5 activation would be less worrisome than inhibiting C3 activation. Lastly, there seems to be little detrimental effect of genetic C5 deficiency. Certain inbred mouse strains are C5 deficient with no apparent increase in infections or decrease in lifespan. The only consequence of C5 deficiency in humans seems to be an increased risk of *Neisseria* infection (115).

Several groups have reported the use of an anti-C5 monoclonal antibody in either complement activation assays or animal model systems. All of these antibodies block the formation of the

membrane attack complex, while a subset of them also blocks the generation of C5a. These are the proteins that have been selected for clinical development.

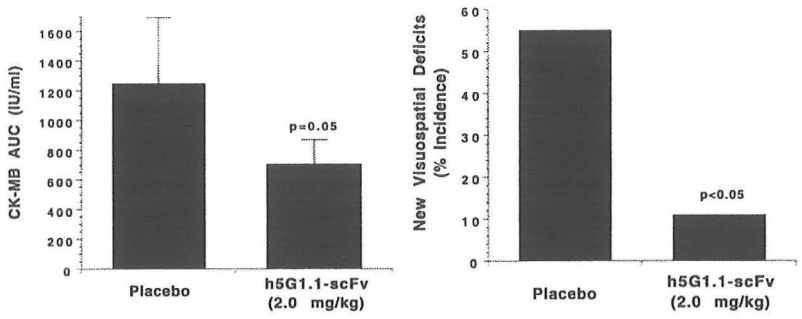
Arthritis:

The availability of naturally C5-deficient mice has enabled the generation of monoclonal mouse anti-mouse C5 to test some of these therapies. For example, collagen induced arthritis is a model for human rheumatoid arthritis (RA). Mice are immunized with bovine Type II collagen in complete Freund's adjuvant resulting in an inflammatory peripheral arthritis with histopathology similar to RA. Treatment of the mice with anti-C5 for three weeks prior to the first immunization with collagen decreased the level of total hemolytic complement by about 60% (116). Moreover, it totally prevented the appearance of arthritis in the mice. Histology of mice with arthritis that received a control antibody demonstrated the typical proliferative synovitis with neutrophil and monocyte infiltration along with cartilage loss and bone erosion. In contrast, the anti-C5 treated mice had normal joints with smooth articular surfaces. The investigators then looked at the ability of anti-C5 to ameliorate established disease. Either anti-C5 or control antibody was not given until arthritis was evident clinically. The mice that received control antibody had continued increases in paw thickness, clinical score, and number of joints involved. The mice treated with anti-C5 had a halt in the progression of the arthritis and some indication of regression of disease with a significant decrease in the number of joint erosions.



These results have led to a recent Phase II clinical trial of anti-C5 in human RA. The biological agent is a chimeric monoclonal antibody consisting of human constant region and framework regions into which the complementarity determining regions (CDR) of a high-affinity mouse anti-human C5 were inserted. This inhibitor is made by Alexion Pharmaceuticals and is termed hu5G1.1. It is IgG4 (non-complement activating), and blocks the formation of both C5a and the membrane attack complex when bound to C5 in a 1:1 stoichiometry. 208 adult patients with established RA were studied in a double-blind randomized, placebo-controlled study. They all had active disease despite taking methotrexate. They received one of four treatments: placebo, 5G1.1 at 8 mg/kg (IV) each week for 4 weeks then once a month thereafter, 5G1.1 once a week for 4 weeks then every other week, and 5G1.1 every other week. The interim results of three months of therapy are available. 43% of the induction/monthly patients had a significant improvement in a composite measure of their disease (the ACR20 score). Only 20% of placebo patients had a similar response. In addition, the induction/monthly and induction/biweekly groups had decreases in their C-reactive protein levels of 0.4 and 0.2 mg/dl, respectively, while the placebo group had a 0.4 mg/dl increase in CRP. These preliminary data are encouraging and suggest that larger, longer Phase III trials are warranted. The patients in this study are still being monitored for safety, but no drug-related adverse events were seen in the first three months.

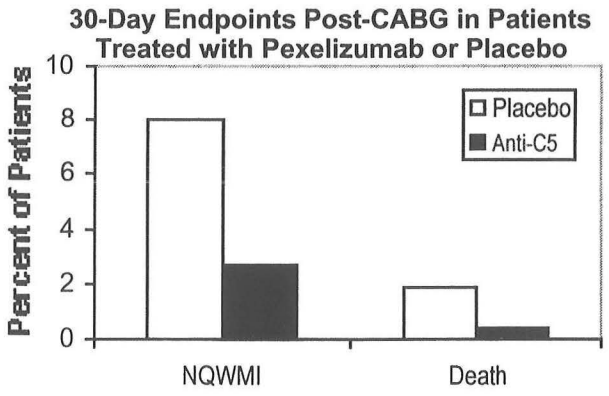
Cardiopulmonary Bypass and Myocardial Ischemia:



Total CK-MB and Visuospatial Defects in a Phase I Trial of Pexelizumab in Patients Undergoing Cardiopulmonary Bypass

Alexion has also produced smaller version of the 5G1.1 antibody. This is a "single-chain Fv" that is an ~30,000 mw recombinant protein with characteristics of the Fab portion of the original monoclonal antibody (117). It binds to C5 and inhibits the C5 convertases with nearly identical effectiveness as the intact antibody. It is being developed in collaboration with Procter and Gamble under the name pexelizumab for unstable angina, thrombolysis/angioplasty following myocardial infarction, and myocardial damage following cardiopulmonary bypass. In 1999, they published the results of a Phase I trial of pexelizumab in 35 patients undergoing primary, non-emergent cardiopulmonary bypass for coronary artery disease (118). Patients received a single bolus dose of antibody ranging from 0.2 to 2.0 mg/kg. Ten patients received placebo infusions. There was a dose-dependent decrease in total hemolytic complement and inhibition of the serum C5b-9 generation seen with bypass. The higher doses of drug prevented the activation of neutrophils and monocytes as determined by expression of the adhesion molecule CD11b. Myocardial injury appeared to be lowered. Total CK-MB for the 24 hr. after surgery was 1,245 ± 449 IU in the placebo group and 704 ± 166 in the group receiving 2 mg/kg of pexelizumab.

They also looked at neurocognitive function in these patients. It has long been recognized that cognitive decline is a complication cardiopulmonary bypass. A recent study at Duke University found that 53 percent of patients undergoing bypass had a measurable deficit on one or more tests of cognitive function (119). Forty-two percent of patients had a deficit at five years after surgery. A cognitive decline immediately after surgery was the strongest predictor of long-term dysfunction. In the nine patients who received the highest dose of anti-C5 prior to cardiopulmonary bypass, none developed deficits in the language portion of the Mini Mental Status Exam, while four of nine in the placebo group did. There was also a significant decrease in the number of visuospatial deficits in the anti-C5 treated group. Whether the results of this very small trial can be generalized is not clear. Given the high percentage of patients who develop cognitive deficits after bypass, any improvement would be clinically important.



Recently, the results of a Phase II trial of pexelizumab in patients undergoing coronary artery bypass grafting were announced. 914 patients at 62 centers were studied in a placebo-controlled, randomized double blind trial. Patients received placebo, pexelizumab as a 2 mg/kg bolus just prior to surgery, or as a bolus plus 0.05

mg/kg infusion for 24 hr. after surgery. The bolus of pexelizumab inhibited complement activation for 4 hr., while the bolus plus infusion suppressed complement activation for the full 24 hr. Patients were followed for the incidence of MI (as defined by CK-MB) and death for 30 days. CK-MB elevations in the range of 20-40 ng/ml were seen equally in both the placebo and pexelizumab groups. The investigators stated that CK-MB at this level is "not unusual" following coronary artery bypass grafting and likely to reflect surgical trauma. However, CK-MB elevations > 100 ng/ml, felt to represent non-Q wave myocardial infarctions were seen 8.0% of the placebo patients but only 2.7% of the anti-C5 treated patients. In addition, the death rate at 30 days was 1.9% for the placebo treated patients and 0.4% for the pexelizumab treated patients. A composite end point of death or MI (both Q wave and non-Q wave) at 30 days was seen in 13.2% of placebo and 7.8% of anti-C5 patients, respectively. Caution is advised, as these are preliminary results, and the full efficacy and safety data from this trial have not been published. A larger, Phase III trial is being organized. As pointed out by Dr. Meidell in his Grand Rounds March 1, 2001, these elevations in cardiac enzymes have previously been shown to have an impact on long-term morbidity and mortality. Whether anti-C5 treatment in the perioperative period will lower long-term death rates remains to be seen.

Finally, researchers from Alexion have investigated the effect of anti-C5 therapy on myocardial damage caused by ischemia reperfusion. In a rat model, the left anterior descending coronary artery was completely occluded for 30 minutes followed by four hours of reperfusion (120). Pre-treatment of the animals with an inhibitory anti-C5 monoclonal antibody caused a near total abrogation of myocardial CK release and neutrophil accumulation. A control anti-C5 that lacks convertase inhibition did not have these effects. Infarct size was assessed histopathologically. When anti-C5 was given prior to ischemia, the size of the infarct was reduced by approximately two-thirds. Even when anti-C5 was not administered until after 25 minutes of total coronary occlusion, the size of the resulting necrotic myocardium was reduced by 50%. Seven days after reperfusion, there was still a 42% decrease in infarcted myocardium seen in the anti-C5 treated rats. Based on this and other animal models of myocardial ischemia, Alexion has two 1,000 patient trials underway to assess the safety and efficacy of pexelizumab in coronary thrombolysis and angioplasty following myocardial infarction.

Graft Rejection:

Like soluble CR1 and the other complement inhibitors described, anti-C5 treatment has also been used to prolong xenograft survival (121). BALB/c mice that receive hearts from Lewis rats reject them within 10-15 minutes if they have been pre-sensitized with donor spleen cells. This was not improved by immunosuppression with Cyclosporin A and cyclophosphamide alone, as would be expected for hyperacute rejection. Treatment of the mice with anti-C5 increased the graft survival to over 5 days by itself, and to nearly 25 days with immunosuppression. These results suggest that anti-C5 could play a role in managing acute graft rejection in humans.

Other inflammatory conditions:

Anti-C5 has several other therapeutic uses. It has been shown to delay the onset of proteinuria and prolong survival in the (NZB × NZW)F_1 mouse model of systemic lupus erythematosus (122). A trial of hu5G1.1 in idiopathic membranous nephritis is underway, and a trial in lupus

nephritis will begin soon. Alexion also has trial underway or planned in psoriasis, dermatomyositis and pemphigoid.

Summary

Our understanding of the complement system continues to evolve. It is no longer a simple antimicrobial effector of the humoral immune system. Although it has important roles in infection and inflammatory responses, it also has many deleterious effects that must be controlled in conditions ranging from immune complex injury to reproduction. Over half of the proteins associated with the complement system are dedicated to the control of activation or effector functions.

Knowledge of how complement is activated and how it can be controlled offers new opportunities for the development of therapeutic agents. Currently, even the most thoroughly tested of these agents is still in clinical trials and is likely to be one to two years away from FDA approval. Most are still in preclinical stages of development. The knowledge gained from the current large biomolecules being tested will likely lead to agents with greater bioavailability and simpler routes of administration.

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