Therapeutic potential of targeting the complement cascade in critical care medicine

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Caring for the critical care patient involves many different areas of clinical expertise and serves a diverse patient population. Novel therapeutics for the critically ill must be approached with caution, because the underlying molecular mechanisms of the disease process for several commonly seen types of patients (i.e., sepsis, shock, ischemia/reperfusion) are not fully understood. A potentially new and advancing area of therapeutics that may hold promise for the critically ill is inhibition of the complement system. Various novel complement inhibitors are being developed and several are in clinical trials. The advancement of this novel area of therapeutics may one day aid the clinician by providing several different complement inhibitors/antagonists for controlling complement activation or its biologically active mediators. (Crit Care Med 2003; 31[Suppl.]:S97–S104)

The Complement System

The human complement system is part of the immune system; it plays an important role in the elimination of invading foreign cells and initiates inflammation. Three separate pathways can activate complement: the classical, alternative, and lectin pathways (Fig. 1). Similar to the coagulation system, complement system activation occurs in a sequential manner with proteolytic cleavage of complement components yielding enzymatically active molecules to produce biological mediators and further downstream component activation. Thus, there are multiple therapeutic targets within the complement system, which may limit its activation or antagonize biologically active metabolites.

Classical Pathway. Classical complement pathway activation occurs when antibody/antigen complexes interact with the first complement component, C1, leading to the generation of C1q. C1q binds to the Fc portion of immune complexes, resulting in activation of C1r and C1s esterases and cleavage of C2 and C4 and ultimately forming the classical pathway C3 convertase (i.e., C4b2a).

Alternative Pathway. The alternative complement pathway is an antibody-independent pathway and can be activated by a variety of mechanisms including yeast cell walls (zymosan), biomaterials (i.e., cardiopulmonary bypass and hemodialysis tubing), and tissue type plasminogen activator. The alternative pathway is a self-amplifying pathway and is important in the clearance and recognition of pathogens in the absence of antibodies. The alternative pathway also can amplify complement activation following initial complement activation by either the lectin or classical pathways. The rate-limiting step of alternative pathway activation is the enzymatic action of factor D on the cleavage of factor B to form the alternative pathway C3 convertase (i.e., C3bBb).

Lectin Pathway. The lectin pathway is also an antibody-independent pathway activated by binding of mannan-binding lectin (also known as mannan/mannose binding lectin; MBL) to carbohydrate structures present on the surface of bacteria, yeast, parasitic protozoa, and viruses (3). Associated with MBL are two serine proteases, MASp-1 and MASp-2, which cleave C2 and C4 to form the classical complement pathway C3 convertase (4). MBL is structurally related to C1q, whereas MASp-1 and MASp-2 exhibit remarkable homology to the classical pathway serine proteases, C1r and C1s (5). Recent evidence suggests that ficolins also can use MASp1 and 2 to activate the lectin pathway (6).

Terminal Complement Components. All three pathways merge at C3, which is cleaved into C3a and C3b. Addition of C3b to either of the C3 convertases converts its specificity into a C5 convertase, which cleaves C5 to form C5a and C5b. Addition of C6, C7, C8, and multiple C9 units to C5b results in formation of C5b-9 (also known as the membrane attack complex...
or terminal complement complex). Of the complement components that are produced during activation, C3a, iC3b, C5a, and C5b-9 are considered the major complement mediators involved in the inflammatory process. A significant body of experimental data in vivo suggest, however, that the terminal complement components, C5a and C5b-9, mediate a host of proinflammatory responses.

**Direct and Indirect Proinflammatory Actions of Complement**

Complement activation results in the formation of several proinflammatory mediators that can alter vascular homeostasis. Biologically active complement components include C3a/C3a des Arg, C5a/C5a des Arg, iC3b, and C5b-9 (7). C3a and C5a primarily act on white blood cells. C3a interacts predominantly with eosinophils, suggesting that C3a may have a significant role in allergic inflammation (8, 9). In contrast, C5a and its des Arg metabolite interact with a variety of white blood cells and, in particular, neutrophils. C5a is the principle chemotactic factor for circulating neutrophils and increases neutrophil adherence to the endothelium by mobilizing internal stores of neutrophil complement receptor 1, CD11b/CD18, and CD11c (10). C5a also stimulates neutrophils to produce/release reactive oxygen species, proteolytic enzymes, and leukotrienes (11). In addition to directly stimulating white blood cell activation and chemotaxis, C5a can further amplify the inflammatory response indirectly by inducing production of chemokines, cytokines, and other proinflammatory mediators (12, 13). The anaphylatoxins have multiple effects on white blood cells, and these actions could be antagonized directly with appropriate development of anaphylatoxin-specific therapeutics.

Complement components also influence vascular adherence properties. iC3b is formed following C3b cleavage and is a specific ligand for leukocyte adhesion to the vascular endothelium via CD11b/CD18 in vitro (14). However, in multiple animal models, complement inhibition at the level of C5 significantly decreases neutrophil adherence/transmigration, although C3 deposition still occurs (15–18). These data suggest that in vivo conditions do not allow for the adherence of neutrophils to iC3b unless there is subsequent C5 activation or that iC3b-mediated adherence is not potent enough to resist shear stress. Along these same
lines, C5b-9 can activate endothelial nuclear factor-κB to increase leukocyte adhesion molecule transcription and expression (19). Endothelial leukocyte adhesion molecules influenced by complement include vascular cell adhesion molecule-1 (20, 21), intercellular adhesion molecule-1, and E-selectin (22). Data are conflicting regarding complement-mediated P-selectin expression (8, 23). C5b-9 can also promote leukocyte activation and chemotaxis by inducing endothelial interleukin-8 and monocyte chemoattractant protein-1 secretion (13). Further, C5b-9 alters vascular tone by interfering with nitric oxide-mediated relaxation and decreasing endothelial cyclic guanosine monophosphate (21, 24). In vivo, inhibition of C5 significantly decreases polymorphonuclear leukocyte adherence, intercellular adhesion molecule-1 expression, tumor necrosis factor expression, nitric oxide-mediated vasorelaxation, and interleukin-1α and macrophage inflammatory protein-2 expression following ischemia/reperfusion (15–18). Thus, complement alters many aspects of vascular homeostasis and appears to be an early and significant contributor to the inflammatory process.

**Complement Inhibition as a Therapeutic Strategy in Critical Care Medicine**

Physicians deal with a wide variety of diseases within the critical care environment. Not only will the potential use of complement inhibitors in this setting be based on basic science and clinical trial results, but the underlying cause of the disease process must also be evaluated to ensure adequately the safety of the patient. Figure 2 demonstrates the current slate of potential complement inhibitors for clinical use and where they interact. Several specific inhibitors have completed phase II trials and one has entered phase III clinical trials for cardiopulmonary bypass (CPB; pexelizumab). As shown in Table 1 a wide variety of potential complement inhibitors may be available to the critical care physician. In this short review, we will highlight the potential use of complement inhibitors in sepsis and CPB.

**Shock and Sepsis**

Multiple organ dysfunction is a common cause of death in the critically ill patient. The mechanisms leading to organ failure during sepsis and noninfectious inflammation are incompletely understood. In 1990, the Centers for Disease Control estimated that there were...
Table 1. Various complement inhibitors with the potential to be used as a clinically viable therapeutic

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>C1q</td>
<td>Peptides; mAb</td>
<td>77</td>
</tr>
<tr>
<td>MBL</td>
<td>Anti-MBL mAb</td>
<td>78, 79</td>
</tr>
<tr>
<td>Classical; lectin; alternative pathways</td>
<td>C1 inhibitor</td>
<td>80, 81</td>
</tr>
<tr>
<td>Factor D</td>
<td>Anti-factor D mAb</td>
<td>82</td>
</tr>
<tr>
<td>C3/C5 convertase</td>
<td>sCR1 (TP110)</td>
<td>71</td>
</tr>
<tr>
<td>C3</td>
<td>Complementin</td>
<td>83</td>
</tr>
<tr>
<td>C5</td>
<td>Anti-C5 mAb (pexelizumab)</td>
<td>75</td>
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mAb, monoclonal antibody; MBL, mannose-binding lectin; sCR1, soluble complement receptor 1.

450,000 cases of sepsis per year in the United States, resulting in >100,000 deaths (25). Recent data demonstrate that severe sepsis is very common, expensive, and frequently fatal; the number of new sepsis cases per year is increasing, especially in the elderly (26). In a recent review of the treatment of sepsis, multiple therapeutic strategies including inhibitors against endotoxin, reactive oxygen species, cytokines, prostaglandins, adherence molecules, and nitric oxide were mentioned as potential investigational treatments (27). One additional manipulation of host defense that may play a role in the pathogenesis of sepsis is complement activation.

Complement appears to be involved in the pathogenesis of sepsis. Various bacterial products interact with host cells and serum proteins to initiate a multitude of inflammatory events that lead to cell injury and death. The Gram-negative bacteria cell wall component lipopolysaccharide (28, 29) and Gram-positive bacteria cell wall components lipoteichoic acid and peptidoglycan (30, 30) induce inflammatory activity, which leads to the onset of septic shock. Complement is activated by bacterial cell components as evidenced by the increase in plasma concentrations of C3a and C5a upon injection of lipopolysaccharide in rats (31) and humans (32, 33). Furthermore, peptidoglycans from some Gram-positive bacteria also activate complement (34). Thus, once sepsis occurs, complement activation can occur as well, augmenting an already proinflammatory condition.

The role of complement in combating septicemia is paradoxical, since lysis or opsonization of invading microorganisms is an important part of host defense but excessive complement activation can lead to harmful tissue destruction due to severe inflammation (35). Patients and animals that are deficient in C3 are prone to severe bacterial infections and also have increased mortality and morbidity rates (36, 37). The increased severity in these immunocompromised patients/animals is most likely a result of decreased complement amplification and opsonization, resulting in poor pathogen killing and lysis. However, specific inhibition of the C5a receptor has been demonstrated to increase survival during sepsis (38). Thus, it may not be therapeutically advantageous to inhibit the complement system entirely during sepsis. Selective inhibition of specific molecules or pathways may be more advantageous.

Complement is activated in sepsis mainly via the classical pathway. Effective antimicrobial defense against polymicrobial peritonitis (the cecal ligation puncture model; CLP) appears to be complement-dependent and requires the presence of C1q and immunoglobulin M (39, 40). Elimination of C2 and factor B results increased mortality rates compared with C1q-deficient or wild-type (129/Sv) mice following CLP (40). However, the 129/Sv mouse is known to have an impaired inflammatory cell recruitment response, which may influence the outcome during sepsis (41). Regardless, the data clearly demonstrate that impairment of host defense and innate immunity significantly decrease survival during sepsis.

The lectin pathway and MBL were initially discovered in children with opsonic defects (42). Subsequently, it was shown that MBL deficiency increases the susceptibility of individuals to infectious disease (43). A recent study in MBL-A-deficient mice has raised some interesting questions about MBL’s involvement in sepsis (44). MBL-A-deficient mice subjected to CLP showed increased survival times compared with C3-deficient or wild-type mice. Reconstitution of MBL-A-deficient mice with human MBL restored the lethal effects of CLP. Furthermore, serum concentrations of tumor necrosis factor and interleukin-6 were significantly lower in the MBL-A-deficient compared with wild-type mice following CLP. These data demonstrate that MBL-A, complement activation via the lectin pathway, or both decrease the survival of mice subjected to sepsis. This somewhat unexpected finding suggests that more interesting data about this novel complement pathway and MBL in human disease remain to be discovered.

Because of the major biological roles of C5a and its actions on neutrophils, this anaphylatoxin has been studied extensively in sepsis. In a porcine model of severe sepsis, treatment with anti-C5a monoclonal antibodies (mAbs) leads to better blood oxygenation (45). Anti-C5a antibodies alleviate symptoms of adult respiratory distress syndrome in septic animals (46). Czermak et al. (47), using a rat model of CLP, have developed a large body of information on the role of C5a in sepsis. In rats, treatment with polyclonal immunoglobulin G antibody against C5a significantly improves survival rates and preserves the H2O2 response of blood neutrophils (47). Anti-C5a antibody treatment also appears to enable the rat’s neutrophils to retain function (48). Even delayed administration of anti-C5a antibodies following induction of sepsis demonstrated protective actions, suggesting potential clinical usefulness of C5a inhibitors in sepsis (48).

The molecular mechanisms underlying the protective actions of anti-C5a in sepsis are not completely understood. Excessive production of C5a leads to the deactivation of neutrophil function, decreased production of H2O2, and failure of the host bactericidal defense system (47). Anti-C5a reverses these adverse actions (48). Sepsis is also known to increase the incidence of apoptosis (49, 50). Recent data show that during sepsis the expression of C5a receptor is up-regulated in thymocytes, which in turn leads to C5a-dependent apoptosis (51, 52). Apoptosis may be inhibited following anti-C5a treatment during sepsis. Recent data suggest that C5a receptor expression increases not only on neutrophil but is also on parenchymal cells (53). Furthermore, inhibition of the C5a receptor has been demonstrated to improve outcome in models of sepsis (53, 54). Specific neutralizing mAbs against human C5a have been developed, but none have been taken into commercial development (55). Recently, a small orally active molecule, C5a receptor antagonist, was announced by Neurogen. Small molecular weight C5a receptor antagonists or anti-C5a an-
Therapeutic inhibition of C5 (i.e., inhibition of C5a and C5b-9) in sepsis has not been examined to date. However, C5-deficient mice demonstrate increased survival compared with congenic C5 sufficient mice (36). Increased pulmonary function as well as decreased pulmonary neutrophil sequestration was observed in C5-deficient mice compared with controls during sepsis (56). Gastrointestinal ischemia and reperfusion are known to induce bacterial translocation (57). Anti-C5 antibodies protect against gastrointestinal ischemia/reperfusion-induced local and remote tissue injury (15, 18). C5 inhibition decreased multiple proinflammatory events associated with this model, including tumor necrosis factor expression, intercellular adhesion molecule-1 expression, chemokine expression, and neutrophil adherence within local and remote tissues (15, 18). These data, plus data from studies that inhibited C5a or its receptor, suggest that C5a (and perhaps C5) is an important therapeutic target in sepsis. However, the role of C5b-9 and its actions in sepsis are not currently understood and are an area that needs investigation. Therefore, selective targeting of complement might be an effective therapeutic approach in sepsis and septic shock.

Cardiopulmonary Bypass

CPB induces a systemic inflammatory response that contributes to clinical morbidity (58). A therapeutic approach to limit inflammation associated with CPB has been an area of active clinical investigation. Several humoral inflammatory pathways (e.g., complement, coagulation, cytokines) and products from these pathways induce proinflammatory effects, especially on white blood cells. CPB during cardiac surgery activates the complement cascade due to contact of blood with biomaterials and as a result of tissue injury associated with surgery (59–61). Early in vitro studies demonstrated the production of anaphylatoxins following contact of blood with biomaterials and have resulted in the manufacturing of different types of biomaterials to decrease complement activation (60, 62–68). However, even with heparin-bonded tubing and biocompatible circuits, systemic inflammation and complement activation still occur during CPB (69). Inhibition of complement activation or complement components may reduce morbidity and mortality rates associated with CPB. Two complement inhibitors have advanced into clinical trials; TP10 (Avant Immunotherapeutics) and pexelizumab (Alexion Pharmaceuticals).

TP10, which is a recombinant form of soluble complement receptor type 1 (sCR1), acts as a C3/C5 convertase inhibitor and is probably the most studied complement inhibitor to date (70). Soluble CR1 has been demonstrated to limit tissue injury in a multitude of animal models related to ischemia/reperfusion injury and transplantation and various other clinically meaningful models (71). TP10 has been shown to limit tissue injury and improve myocardial dysfunction during and following experimental CPB (72, 73). These initial positive in vitro studies in CPB led to two different clinical trials. In September 1999, a phase I/II trial involving 15 infants undergoing congenital heart defect surgery commenced. Results from this trial demonstrated that the drug was well tolerated and showed clinical improvement by decreasing time on the ventilator and in the intensive care unit by 30% and inotropic use by 70% (71). A placebo-controlled phase IIb trial in 30 infants was started in September 2000. In November 2000, a placebo-controlled phase II trial of TP10 in 600 adult patients undergoing cardiac surgery utilizing cardiopulmonary bypass was also initiated. Results from this adult trial were announced in February 2002. A total of 564 patients were randomized to receive one of four doses (1, 3, 5, or 10 mg/kg) of TP10 or placebo and were followed for 28 days after surgery. The primary end point was a composite consisting of death, myocardial infarction, prolonged intubation, or requirement for intra-arterial balloon pump therapy. Although the data from this study have not yet been made public, it was stated in a press release and conference call that TP-10 failed to meet its primary end point and that TP-10 would not be further advanced in clinical development. During the conference call, Dr. Una Ryan (CEO, Avant Immunotherapeutics) stated that a “ray of hope” in the data were observed but did not comment further. Thus, it is difficult to comment on these negative clinical findings without knowing the full extent of the data collected, but the little information that has been realized raises the questions whether the study was properly powered or if the drug was given in high enough doses to inhibit adequately complement activation.

The other anticomplement inhibitor that is currently in clinical trials is an anti-C5 mAb developed by Alexion Pharmaceuticals. This mAb binds to complement C5 and inhibits the formation of C5a and C5b-9. A recombinant single chain of the variable region (scFv) of this mAb (h5G1.1-scFv) was made and humanized and is called pexelizumab. Initial basic science studies demonstrated that anti-C5 significantly inhibited C5 cleavage and neutrophil and platelet activation in an in vitro CPB circuit (74). A phase Ia trial was initiated in December 1996 and enrolled 35 adult patients into one of four doses (0.2, 0.5, 1, or 2 mg/kg) of pexelizumab or placebo. Results from this trial demonstrated that the drug was well tolerated and dose-dependently inhibited C5 cleavage. Dosing at 1 or 2 mg/kg pexelizumab decreased CD11b expression on polymorphonuclear leukocytes or monocytes. In addition, a significant reduction in cumulative CK-MB release and new visuospatial deficits was observed in this small trial (75).

In January 1999, Alexion initiated a multiple-center, double-blinded, placebo-controlled phase IIb clinical study in 1,000 adult patients. Results from this phase IIb trial were given in a January 2001 press release. The trial enrolled 914 patients who were stratified into two groups: those undergoing only coronary artery bypass grafting (CABG) with CPB and those undergoing CABG with concomitant valve surgery during CPB. The
initial primary composite end point, which included a non-Q-wave myocardial infarction, neurologic deficits, and left ventricular dysfunction, was not achieved. Subgroup analysis of the data presented by Alexion, however, yielded interesting results.

Approximately 90% of the patients were in the CABG-only group (n = 796). Patients were treated with placebo, pexelizumab 2.0 mg/kg bolus, or pexelsizumab 2.0 mg/kg bolus followed by a 24-hr infusion of pexelizumab at 0.05 mg·kg\(^{-1}\)·hr\(^{-1}\) and then were followed for safety and efficacy for 30 days. The drug was safe and well tolerated and inhibited complement activation at 4 and 24 hrs. Non-Q-wave myocardial infarctions (defined as the isoenzyme of creatine kinase with muscle and brain subunits >100 ng/mL) were observed in 2.7% of pexelizumab-treated patients and 8.0% of placebo patients at 30 days. Furthermore, at 30 days, the death rate was 0.4% in pexelizumab-treated patients and 1.9% in the placebo group. The composite incidence of death or myocardial infarction (Q-wave or non-Q-wave) was observed in 7.8% of pexelizumab-treated patients and 13.2% of placebo patients at 30 days. These data are similar to those recently observed by others using C1 inhibitor during thrombolytic therapy for coronary artery occlusion (76). Thus, it appears that the beneficial actions of complement inhibition in the CABG group are more readily observed in those patients at risk for large infarcts. In January 2002, Alexion initiated a 3,000-patient phase III clinical trial called “Pexelizumab for Reduction in Infarction and Mortality in Coronary Artery Bypass Graft Surgery,” or “PRIMO-CABG.” End of enrollment and results are anticipated during 2003.

**Complement Inhibition in the Critically Ill**

Although complement clearly plays an important role in animal models of human disease, the impact of complement inhibition in human disease is in its infancy. Clearly other strategies to inhibit complement exist, but most of these are not specific and lend themselves to theoretical and potential serious side effects, if not adequately controlled (i.e., heparin, nonspecific serine protease inhibitors, cobra venom factor, etc.). Specific, selective complement pathway inhibitors or individual complement component inhibitors (i.e., C5a, C3a, C5, C2, factor D, MBL, C1q, C6) offer the potential promise of being able to inhibit selectively that aspect of the complement system that is involved in a particular disease state. For example, it may be more advantageous to inhibit selectively C5a in sepsis, while leaving the opsonization (C3b), lysing (C5b-9), phagocytosis, and killing actions of the complement system available for pathogen clearance. Novel complement inhibitors in clinical development (i.e., anti-C5, anti-factor D, anti-MBL, anti-C2) and others still being developed may offer the critical care physician a host of new therapeutic approaches to manage the critically ill patient (77–83).

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