CHAPTER 87

Complement and Its Consequences in Sepsis

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OBJECTIVES

This chapter will:

1. Describe products of complement activation during sepsis.
2. Demonstrate protective effects of blocking antibodies to C5a, together with its structural details, in the setting of sepsis.
3. Explain pathophysiologic events related to complement activation that develop during sepsis and acute lung injury, and protection against tissue damage and lethality in C5aR knockout mice or blockage of C5a receptors.

Robust activation of complement occurs in sepsis in humans and animals.[1] Most information in septic animals is related to the use of cecal ligation and puncture (CLP), which induce polymicrobial sepsis in mice and rats. Complement activation occurs via the classical and the alternative pathways of activation, together with activation of the lectin pathway, although very little is known about how and why complement activation is occurring in the setting of sepsis. Complement activation includes products derived from C3 (e.g., C3b, iC3b) and C5 (C5a, C5b, and C5b-9, the membrane attack complex). All of these products have proinflammatory properties.[1] Obviously, the appearance of complement

activation products is not specific for sepsis, because similar products can appear in plasma after endotoxemia, burn injury, autoimmune diseases (such as systemic lupus erythematosus); after ischemia-reperfusion injury; and in many other conditions. Other biomarkers of sepsis include a long list of factors (e.g., precalcitonin, cytokines and chemokines, histones, lactate), but these are nonspecific for sepsis, although their plasma levels often correlate with the intensity of the sepsis condition.

COMPLEMENT ACTIVATION PRODUCTS IN PLASMA DURING SEPSIS

As indicated above, complement activation products usually appear in the plasma of humans and animals with sepsis. Using C3 or C4 KO mice, RAG2 KO mice (which lack T and B cells) or mice infused with the C1 esterase inhibitor, involvement of the classical pathway has been demonstrated in sepsis. Engagement of the alternative pathway in sepsis has been suggested by the presence of activated factors B and D in plasma, which are critical factors that promote complement activation. Finally, activation of the mannose-binding lectin (MBL) pathway has been suggested by the plasma presence of activated MBL-associated serine protease-2 (MASP-2) in septic mice.[1] Although lipopolysaccharide frequently has been incriminated in sepsis, there is no consensus about its role in sepsis, either in mice or in humans. Furthermore, "sterile sepsis" (which develops after nonpenetrating polytrauma, hemorrhagic shock, or after ischemia-reperfusion injury and after chemical injury) shows similar patterns of complement activation in the absence of an identifiable infectious pathogen. Infectious sepsis features the appearance of pathogen-associated molecular patterns (PAMPs) with Toll-like receptors (TLRs) present on surfaces of cells (e.g., phagocytes, endothelial cells). Such interactions trigger production of proinflammatory mediators (IL-1β, TNF, IL-6). In contrast, "sterile" sepsis (described above) often mimics infectious sepsis but in the absence of infectious agents. These pathways also lead to engagement of danger-associated molecular patterns (DAMPs), which are endogenous to cells and engage intracellular nucleotide oligomerization domain (NOD)-like receptors (NLRs). Examples of DAMPs that engage NLRs are heat shock proteins, uric acid crystals, hyaluronan, mitochondrial DNA, and defensins. Complement activation and proinflammatory mediator appearance usually occur together, similar to outcomes that develop when the TLR system is activated by agents such as bacterial lipopolysaccharide (LPS).

NEUTRALIZING ANTIBODIES TO RAT AND MOUSE C5a

When we started our studies of CLP-induced sepsis in rats and mice in the late 1990s, we were obliged to develop recombinant mouse and rat C5a that was essentially endotoxin free. Then we developed neutralizing antibodies to C5a for use in rodents, in which sepsis was induced by CLP, inducing polymicrobial sepsis. "Polymicrobial" refers to the presence of both gram-positive and gram-negative bacteria. The CLP model was becoming a standard for the study of sepsis in rodents. The first major problem was that there were no commercially available

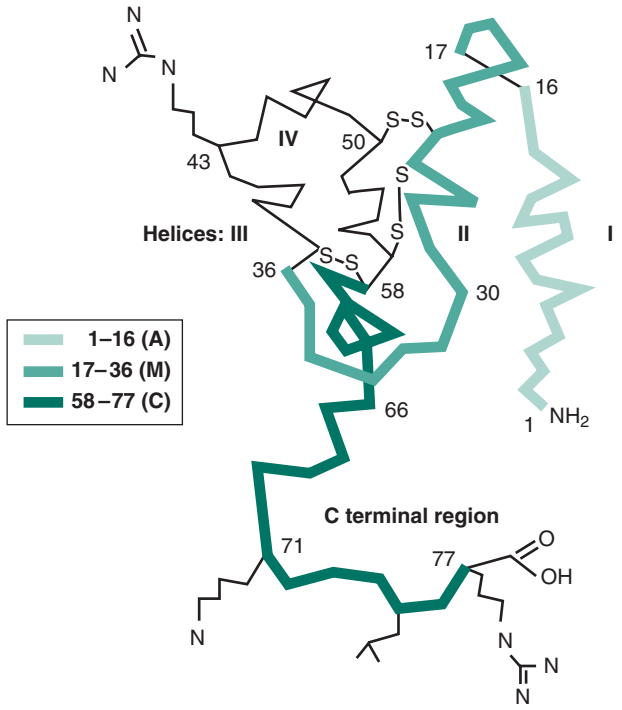

FIGURE 87.1 Location of selected peptide regions, based on the antigenic index and predicted molecular structure of rat C5a. *A,* Amino-terminal; *M,* middle peptide region; *C,* carboxyterminal region.

recombinant rat or mouse C5a preparations. The same was true for availability of neutralizing antibodies to rat or mouse C5a. This resulted in slow progress for the first few years, because we had to develop all of our own reagents.

Fig. 87.1 shows the structure rat C5a, which is very similar to that for human and mouse C5a. There are four helical domains, I through IV. Our studies focused on three peptide antigenic regions of C5a: the A region, which is in the N terminal region of C5a, containing amino acid residues 1 through 16; the M region (middle) containing residues 17 through 36; and the C terminal region containing residues 58 through 77. Fig. 87.1 also shows the numerous disulfide bridges, which cause C5a to be a very stable molecule in oxidizing environments. We developed rabbit antibodies to the A, M, and C peptide regions of C5a and demonstrated that the antibodies were reactive with the appropriate peptide regions (based on western blots) and did not overlap in their reactivities.[2] Using rat polymorphonuclear cells (PMNs) as a readout for effects of the antibodies for in vitro chemotactic responses to C5a, we used antibodies to the A, M, and C regions of C5a. The presence of these antibodies caused the following reductions in chemotactic responses to C5a (100 nM): 18% (P, not significant) for antibody to region A; 55% ($P < .01$) for antibody to region M; and 80% ($P < .01$) for antibody to region C.

These data suggested that blockade of the C terminal region was the most effective intervention for blockade of biologic activity of C5a, followed by blockade of the M region of C5a. When survival was determined in CLP rats infused intravenously with 400 μg of IgG just before CLP, 10-day survival rates were: preimmune IgG, 24%; IgG to A peptide, 30%; IgG to M peptide 90%; IgG to C peptide 84%. Like in chemotaxis, the most protective antibodies in the setting of sepsis were to the C and M regions of rat

C5a.[2] When we delayed antibody infusion until 12 hours after CLP, survival with the pre-IgG was only 10%, whereas IgG to the M regions gave 38% survival, and antibody to the C region provided 45% survival. These data suggest that antibodies to the C and M regions of rat C5a were still protective when infusion was delayed for 12 hours after onset of CLP-induced sepsis. Although clinical use in humans of C5a involving neutralizing antibody to C5a would likely involve "humanized" mAbs in clinical trials, these data suggested that the C5a target for antibodies should be for the M or the C regions of C5a.

PATHOPHYSIOLOGIC EVENTS INVOLVING COMPLEMENT DURING SEPSIS

Fig. 87.2 summarizes pathophysiologic events related to complement activation that develop during sepsis. Events after CLP include a complex of pathways after CLP. Robust activation of complement occurs, as defined by the presence in plasma of C5a although, as described above, it is not clear what triggers activation of all three pathways of complement. In most cases, endotoxemia does not appear to be the cause of septic shock. Plasma C5a was detectable for several hours after onset of sepsis (data not shown). C5a interacts with its receptors, C5aR1 and C5aR2, which are present on a variety of cells, especially PMNs and macrophages and vascular endothelial cells. Activation of the MAP kinase (MAPK) pathways appears likely as a result of ligation of C5a with its receptors. Activation of phagocytes causes release of a variety of proinflammatory mediators, whereas the chief outcome in endothelial cells is probably endothelial cell dysfunction, related to adherence of platelets as well as thrombus formation, causing reduced electrical resistance of the cells, reduced barrier function, and increased vascular permeability. On the basis of KO of C5aR or antibody-induced blockade of C5a receptors, the result is protection against tissue damage, reduced levels in blood of proinflammatory mediators, reduced organ dysfunction, and improved survival after onset of sepsis.[3] C5a interaction with C5aRs on PMNs also results in the formation of neutrophil extracellular traps (NETs), which feature extrusion of long filaments of DNA from cells and the presence of granule products of PMNs (e.g., myeloperoxidase, proteases) and the appearance of extracellular histones. It appears that NETs can capture and kill a variety of bacteria in the vicinity of the PMNs. However, their protective role in sepsis has not been proven. NETs also release extracellular histones, which are strongly proinflammatory and prothrombotic, causing extensive organ injury. Simultaneously, activated PMNs and macrophages release chemokines and cytokines, oxidants, and proteases, all of which establish a cell-toxic environment. The distal region of the complement system generates the membrane attack complex (MAC), which appears to contribute to cell injury and activates the NLRP3 inflammasome. The possible role of MAC in sepsis is supported by studies with C6 KO rats or mice that demonstrated protection against polymicrobial sepsis (and lethality).

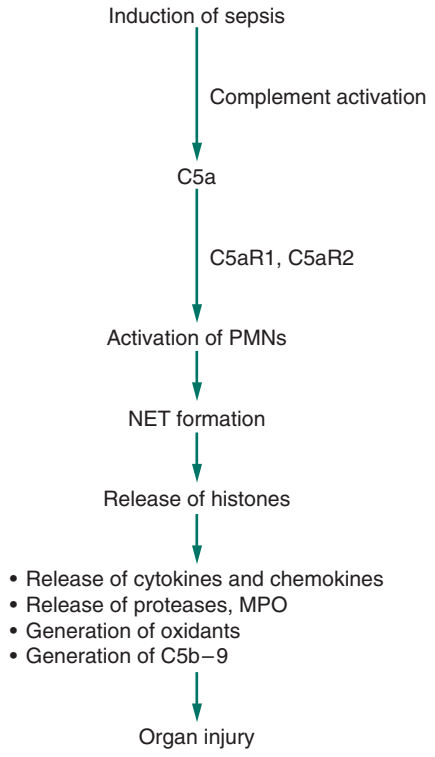

FIGURE 87.2 Pathophysiologic pathways involving complement in sepsis. *MPO,* Myeloperoxidase; *NET,* neutrophil extracellular trap; *PMNs,* polymorphonuclear cells.

ROLE OF COMPLEMENT IN ACUTE LUNG INJURY; SIMILARITIES TO EVENTS IN SEPSIS

Over the past several years, our laboratory has employed a model of acute lung injury (ALI) in rats and mice. This model has distinctive advantages of being able to sample the "external environment" of the lung (the distal airway) for cells and soluble products that can be retrieved in the bronchoalveolar lavage fluids (BALF). Another advantage of using the lung as the target of inflammation is the ability to deliver materials via the airways in a manner that precludes whole body dissemination of the drug, which, if this occurred, could cause responses of other organs that would confound analysis of the lung responses. ALI can be induced by airway instillation of IgG immune complexes, C5a or LPS, each of which induces acute disruption of the capillary endothelial cell and alveolar epithelial cell barrier, resulting in albumin leak into the lung, intraalveolar hemorrhage, and rapid buildup of PMNs in BALF. In these cases, both C5a receptors are required for development of full injury of the lung.[4] Inflammatory cells and products, such as PMNs and cytokines, can be measured in BALF, along with C5a, histones, and other relevant factors. All of these events peak in 6 to 8 hours, in contrast to CLP-induced sepsis, with some of the end points, such as survival, not being complete until 7 days after the induction of CLP. The ALI model also allows the instillation of such products into the lung as neutralizing antibodies that can neutralize critical factors (e.g., cytokines, histones). Accordingly, the ALI model permits us to obtain extensive information about the pathophysiology of ALI and compare the results with those obtained in the setting of sepsis. In general, except for the time frame of events, the pathophysiology of ALI and sepsis are similar, being complement and histone-dependent.

Our model of ALI induced by airway deposition of LPS or C5a or IgG immune complexes established that full lung injury was dependent on availability of both C5a receptors.

Extracellular histones were found in BALF of these mice. Neutralization of histones with antibody sharply reduced the inflammatory damage in lung. Intratracheal instillation of a histone mix (containing H1, H2A, H2B, H3, and H4) was highly lung damaging, causing a strong buildup of albumin in lung. The data indicated that histones were very damaging to the blood vascular and alveolar epithelial barrier. In addition, the histone mix (containing all five histones) was prothrombotic, causing the widespread appearance of thrombi in lungs.[4]

All of these data suggest various strategies as to how this sequence of events in ALI and sepsis can be blocked. Such strategies may include blockade of C5a with neutralizing antibodies to C5a or to histones, use of compounds that block either C5 or C5a, or blockade of signaling pathways linked to NF-$_k$B activation or the MAPK pathway, although the available data on the most effective interventions in the signaling pathways do not currently permit definitive predictions. In addition, other approaches could involve interventions to block the complement activation pathways. Compstatin is a potent inhibitor of the C3 convertase and appears to be relatively nontoxic in humans and animals. The potential concern about compstatin is its ability to reliably regulate generation of C3b, the chief opsonic product of the complement that promotes phagocytosis of bacteria, viruses, and protozoa. Systemic use of compstatin would require reliable dose-responses and a wide margin of safety for the drug.

NOVEL FORM OF COMPLEMENT ACTIVATION

The bulk of complement activation has been documented to occur in the extracellular or plasma compartments, resulting in generation of anaphylatoxins (C3a, C5a), which are powerful phlogistic agents. C3b is a key opsonic-promoting protein that interacts with the innate immune system, facilitating more efficient phagocytosis and destruction of invading bacteria, viruses, and protozoa. C5b interacts with C6-C9 to generate the membrane attack complex (MAC), which also causes lysis of infectious organisms as well as cells of the host and has additional biologic functions such as activation of the NLRP3 inflammasome in phagocytes.

Recent evidence[5] now compels us to revise our understanding of the biologic compartment in which complement activation occurs. The recent report demonstrates that T cells contain endosomal stores of C3 and the T cell–expressed protease, cathepsin L, which functions as an intracellular C3 convertase, generating C3a and C3b within T cells. It appears that the C3 activation products then are shuttled to the T cell surface, coincident with activation of the T cell. It has been suggested that "tonic" intracellular presence of C3 and formation of C3a are required for T cell survival and function, and that transposition of the C3-cleaving program and its products to the T cell surface promotes autoimmune T cell production of proinflammatory cytokines. In patients with autoimmune arthritis, T cells demonstrated hyperactive intracellular complement activation together with interferon γ production. This T cell phenotype was diminished greatly in the presence of an inhibitor of the cathepsin L enzyme.

These observations represent an important change in our understanding of the intracellular complement system and raise several critical questions:

1. Is there a parallel story related to intracellular C5?
2. Beyond T cells, do other cell types possess intracellular C3 and the C3 convertase machinery, and to what extent is this pathway important for physiologic functioning of other cell types beyond T cells?
3. What other T cell subtypes also possess a similar complement-dependent intracellular environment that is required for cell function?

It is clear that the findings of intracellular activation of complement, which has functional implications for T cells, opens up a whole new area of complement discovery that could well change our understanding of the biology of the complement system.

Key Points

1. Complement activation products derived from C3 and C5 appear in the extracellular or plasma compartments of humans and mice after sepsis.
2. Blockade of C terminal and M (middle) regions of C5a was the most protective intervention using this antibody in the setting of sepsis.
3. Following sepsis, generation of C5a, which interacts with its receptors (C5aR1, C5aR2) on a variety of cells, especially neutrophils, causes release of proinflammatory mediators, thrombosis formation, activation of MAPK pathways, and other signaling pathways.

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A complete reference list can be found online at ExpertConsult.com.

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