Role of the complement in experimental sepsis

Peter A. Ward[1]

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Abstract: At the Trauma, Shock, Inflammation and Sepsis 2007 conference, the roles of complement activation products and relevant receptors were stressed in the setting of experimental sepsis [cecal ligation and puncture (CLP)] in mice and rats. In addition, some limited evidence was presented related to humans with septic shock (requiring vasopressor support). Collectively, the data suggested that events found in CLP also occur in human sepsis. Experimental sepsis (CLP) in rodents is associated with robust complement consumption and appearance of activation products (C3a, C5a) in plasma. During sepsis, there is up-regulation of C5a receptors (C5aR, C5L2) on blood polymorphonuclear neutrophils (PMNs) and in lungs, liver, kidneys, and heart. CLP also leads to dramatic reductions of C5aRs on blood PMNs, the intensity of which correlates with lethality. Interception in vivo of C5a or C5aR dramatically improves survival after CLP, preserves innate immune functions of blood PMNs, and greatly attenuates the intensity of consumptive coagulopathy and activation of the fibrinolytic system after CLP. In humans with septic shock, there is evidence of complement activation products in plasma along with loss of C5aRs on blood PMNs. These data suggest that in septic humans, interception of C5a or C5aR might be clinically efficacious. *J. Leukoc. Biol.* 83: 467–470; 2008.

Key Words: *mediators · C5a · C5aR · inflammation*

INTRODUCTION

It is well known that humans with sepsis have evidence of systemic activation of complement, as demonstrated by the presence in plasma/serum of complement activation products (C3a, C5a, C5b-9) [1]. The complement anaphylatoxins, C3a and C5a, are known to have a spectrum of proinflammatory activities [2], suggesting that systematic formation of C3a and C5a is highly undesirable, as these complement products can function at low nM concentrations and are intensely proinflammatory. Plasma contains carboxypeptidases, which rapidly convert C3a and C5a to *des arg* forms, that are less biologically reactive. However, the situation is confused by the fact that plasma complement proteins, which originate predominately from hepatocytes, can also be synthesized locally {in the relative absence of carboxypeptidases, as in lung (reviewed in refs. [3, 4]}. In addition, phagocytic cells, such as neutrophils and tissue macrophages, contain a C5 convertase, which can generate C5a from C5 in the absence of other complement proteins [4]. In addition, thrombin can generate C5a from C5 [5], indicating a linkage between the coagulation and complement systems [5].

THE C5a RECEPTOR (C5aR) AND ROLE IN SEPSIS

The C5aR (CD88) is described in **Table 1**. C5aR is a 7 transmembrane-spanning receptor, which binds C5a (and C5a *des arg*) with high affinity (low nM range), and is linked with intracellular G proteins, necessary for cellular signaling. Interaction of C5aR with C5a results in a rapid increase in cytosolic Ca^{2+} as a result of release of Ca^{2+} from intracellular stores. The cytosolic Ca^{2+} increase is transient but is one of the earliest responses of cells to C5a. Although it used to be thought that the C5aR presence was restricted to myelopoietic cells, it is now clear that C5aR is expressed on a variety of other cell types, such as endothelial cells, alveolar epithelial cells, neurons, and cardiomyocytes (CMs), to name just a few examples. The expression of C5aR is constitutive and inducible. In the setting of experimental sepsis induced by cecal ligation and puncture (CLP), C5aR is up-regulated substantially in a variety of organs [6]. In thymocytes, up-regulation occurs within a few hours after CLP [7]. Up-regulation of C5aR after CLP has also been found on CMs [8], as well as in liver, lung, and kidney [6]. Such up-regulation infers that a variety of tissues may develop highly undesirable effects after encounters with C5a. In the heart, profound CM contractile defects develop after CLP [8]. In the thymus and perhaps in other lymphoid tissues, apoptosis develops, via the intrinsic pathway (featuring mitochondrial release of cytochrome c and caspase activation) [9]. Apoptosis is known to occur in lymphoid tissues of septic humans and has been postulated to be the cause of the immunosuppression of sepsis [10].

In vitro, it is known that C5aR can be up-regulated by cell contact with LPS, TNF-α, or IL-6. In vivo, blockade of IL-6 prevents up-regulation of C5aR in CLP rodents and improves survival [11]. In the setting of CLP, organ up-regulation of C5aR can be blocked in mice by administration of neutralizing antibody to IL-6 [2]. Finally, interaction of C5a with C5aR on

[1] Correspondence: Department of Pathology, University of Michigan Medical School, 1301 Catherine Rd., Room 7520 MSRB I, Ann Arbor, MI 48109-0602, USA. E-mail: pward@umich.edu

Received June 9, 2007; revised August 2, 2007; accepted August 28, 2007.
doi: 10.1189/jlb.0607376

TABLE 1. C5aR

1.	7 Transmembrane-spanning receptor.
2.	Linked with cytosolic G-protein signaling.
3.	Specifically reactive with C5a and C5a *des arg*.
4.	Present on phagocytic cells as well as nonmyelopoietic cells (endothelial cells, Kupfer cells, alveolar epithelial cells, etc.).
5.	C5aR is inducible by LPS, IL-6, TNF-α.
6.	Facilitates a variety of functional responses (intracellular Ca^{2+} signaling, secretory granule release, activation of NADPH oxidase, etc.).
7.	C5a binding to C5aR induces intracellular translocation and subsequent recycling of C5aR.

blood polymorphonuclear neutrophils (PMNs) results in internalization of the C5a•C5aR complex, with translocation to the Golgi region, where in an acidic environment, the ligand (C5a) is hydrolyzed, and at least some of the C5aR is recycled to the cell membrane. In CLP rats, the greater the loss of C5aR on the PMN surfaces, the higher the lethality [12]. This relationship may reflect the intensity of in vivo complement activation during sepsis or the ability of PMNs to replete C5aR rapidly on cell surfaces. Currently, there are ongoing studies in human sepsis to determine if the loss of C5aR on PMNs is predictive of a lethal outcome.

THE C5L2R FOR C5a AND CHANGES IN SEPSIS

The recently described second C5aR on leukocytes, C5L2, is rather enigmatic [13–18]. C5L2 was described several years ago [13–18]. C5L2 is reviewed in **Table 2**. It was quickly determined that, although C5L2 binds C5a and C5a *des arg* with high affinity, no intracellular Ca^{2+} signal developed, and no functional response occurred (e.g., enzyme release, respiratory burst, MAPK signaling, etc.). Accordingly, C5L2 was judged to be a default or "scavenger" receptor, in which it would bind C5a and compete with C5aR for C5a binding. Although it has been suggested that C5L2 is a "promiscuous" receptor, which can also bind C3a and C3a *des arg* on myeloid cells and adipocytes, such claims have been questioned recently [15]. Although no cell signaling was thought to occur upon engagement of C5L2, this matter has been confused in light of two recent reports, which suggest signaling in adipocytes exposed to C3a or C3a *des arg* [16] and mouse bone marrow-derived phagocytes incubated with C5a [18]. In the case of bone marrow-derived macrophages, it has been suggested that in the absence of C5aR or C5L2, ERK1/2 phosphorylation is diminished. It is difficult to reconcile these contrasting claims at the present time.

In vivo experiments suggest that C5L2 may alter the balance of the inflammatory response. When C5L2 was blocked in CLP mice, there was a dramatic increase in serum IL-6, whereas blockade of C5aR with antibody had the opposite effect, virtually abolishing serum IL-6 after CLP [19]. When IgG immune complex-induced injury was evaluated, the intensity of lung injury was amplified in $C5L2^{-/-}$ mice compared with wild-type mice [20]. These in vivo observations suggest that C5L2 functions to negatively regulate the level of the inflammatory response in vivo, but it is clear that there is inadequate information to be certain about the function of C5L2. Finally, studies to date suggest that C5a ligation of C5L2 does not result in internalization of the receptor, which remains on the cell surface. It is possible that this could be explained by the inability of C5a to initiate cell signaling via C5L2. Evidence for changes in C5L2 in the setting of CLP-induced sepsis or in human sepsis is described below.

TABLE 2. C5L2

1.	7 Transmembrane-spanning receptor which is not linked to G-protein signaling because of a substitution in the Asp-Arg-Tyr (DRY) region.
2.	Reaction with C5a and C5a *des arg;* questionable binding of C3a and C3a *des arg*.
3.	A "default" receptor.
4.	Present on a variety of myelopoietic and nonmyelopoietic cells.
5.	Inducible on a variety of cells.
6.	Ligation of C5L2 does not appear to result in internalization.

EVIDENCE FOR THE ROLE OF C5a IN EXPERIMENTAL SEPSIS

The data described in **Table 3** are the foundations for concluding that in CLP rodents, the septic syndrome is linked to generation of C5a. As in humans with sepsis, complement activation after CLP is associated with consumptive depletion of complement and the appearance in plasma/serum of C5a [21]. When C5a was blocked in vivo (at the time of CLP), there was a dramatic improvement in survival [21, 22]. Paralysis of the ERK1/2 signaling pathway was found in blood PMNs within 12–18 h after CLP [23], resulting in marked defects in phagocytosis, chemotaxis, and assembly of NADPH oxidase. These functional defects in PMNs were prevented when C5a was intercepted with anti-C5a immediately after CLP. Similar restrictions in innate immune responses were found in human blood PMNs exposed in vitro to C5a. The basis for the defect in the respiratory burst was the inability of stimulated PMNs to translocate one of the critical subunits ($p47^{phox}$) from the cytosol to the cell membrane, where assembly of NADPH oxidase occurs.

When C5a was blocked in CLP rats, this also resulted in preservation of CM contractility as measured in vitro, sharply contrasting with the 50–60% loss of contractility when CLP

TABLE 3. Role of C5a in CLP-Induced Sepsis

1.	Increased C5a levels in plasma after CLP.
2.	Protective effects of C5a blockade.
3.	Ability of C5a *in vitro* and *in vivo* to induce signaling paralysis of ERK1/2 signaling.
4.	Ability of C5a blockade *in vivo* to preserve contractility of cardiomyocytes.
5.	Ability of C5a to cause defective cardiomyocyte contractility *in vitro*.
6.	Ability of anti-C5a to attenuate the consumptive coagulopathy of experimental sepsis.

rodents were treated with preimmune serum at the time of CLP [8]. It was also shown that addition of C5a in vitro to sham CMs in a dose-dependent manner caused progressive reductions in CM contractility. This could be explained by the constitutive presence of C5aR on CMs. Finally, in CLP rats, the expected evidence of consumptive coagulopathy was demonstrated readily together with evidence for activation of the fibrinolytic system. All such abnormalities were greatly attenuated when C5a blockade was initiated at the start of CLP [24]. Thus, the abnormalities developing in the innate immune pathways during experimental sepsis in CMs and in the clotting/coagulation systems are linked to the generation of C5a after CLP.

ROLE FOR C5aR IN EXPERIMENTAL SEPSIS

As indicated above, C5aR is up-regulated after CLP in rats and in mice. There is considerable evidence for the participation of C5aR in the harmful consequences of experimental sepsis. Blockade of C5aR has been accomplished by the use of antibody to C5aR or by the use of a cyclic peptide, which prevents interaction of C5a with C5aR [25]. In vivo blockade of C5aR resulted in greatly improved survival after sepsis. In addition, interception of IL-6 (which is a potent inducer of C5aR) significantly improved survival after CLP in mice [11]. Collectively, these data provide direct evidence for the harmful role of C5aR in the setting of experimental sepsis. The harmful effects of C5a interaction with C5aR in the setting of experimental sepsis are described in **Figure 1**, in which sepsis-induced activation of complement results in two harmful outcomes: loss of innate immune functions of blood neutrophils (PMNs) and reduced CM contractility. The appearance of both types of defects is C5a- and C5aR-dependent. In the case of PMNs, signaling paralysis results in loss of three key functions

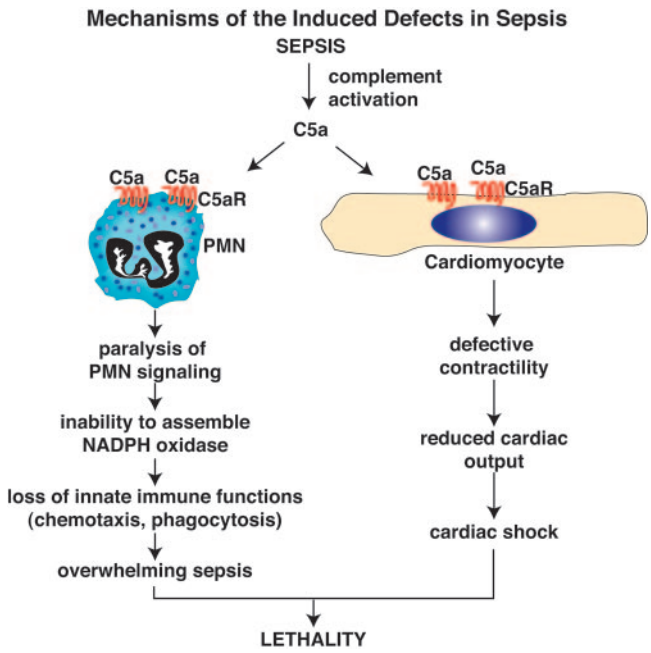

Fig. 1. Summary of C5a-C5aR interactions in sepsis, leading to loss of innate immune functions of PMNs and contractile dysfunction of CMs.

(phagocytosis, chemotaxis, assembly of NADPH oxidase), all of which results in the loss of protective functions of PMNs. In the case of CM, sepsis results in increased cell surface expression of C5aR. Upon interaction with C5a, CM contractility is reduced greatly, resulting in falling left ventricular pressures and reduced cardiac output. Development of these dysfunctions can be blocked by in vivo interception (with antibody) of C5a.

CLINICAL CORRELATES IN HUMAN SEPSIS

There is developing evidence that humans with septic shock (requiring vasopressors to maintain adequate blood pressure) show several of the features observed in septic rodents after CLP. In both situations, there is evidence of consumptive depletion of complement in serum/plasma and the presence of complement activation products (C3a, C5a). Another set of observations relates to C5L2 content on blood PMN lysates (defined by Western blots) and on blood PMNs (as determined by flow cytometric analysis) from humans with septic shock. In the case of rats, lysates from blood PMNs, 24 h after CLP, showed a dramatic reduction in C5L2 content, as defined by Western blot analysis [26]. The loss of C5L2 occurred as a function of time after CLP, and substantial reductions occurred at 3 h and 4 h. In addition, it was shown that the loss of PMN C5L2 after CLP was dependent on C5a, as in vivo blockade of C5a prevented the loss of C5L2 from blood PMNs. In the case of humans with septic shock, C5L2 levels fell >95% on PMNs in the aggregate group of septic patients, by 84% in septic patients without multiorgan failure (MOF), and by nearly 90% in septic patients with MOF. The loss of C5L2 on normal human PMNs could be reproduced when cells were exposed in vitro to C5a, indicating a loss of receptor by internalization or by receptor shedding. These data indicate that sepsis causes dramatic reductions in PMN surface content of C5L2 and that the same occurs on blood PMNs taken from CLP rats. The loss of C5L2 has been shown to be C5a-dependent. To what extent similar changes occur in C5aR in blood PMNs in septic humans is a matter of current study.

SUMMARY

There is accumulating evidence that complement activation occurs during experimental sepsis (CLP) and also in human sepsis. Interaction of the anaphylatoxin, C5a, with its receptor, C5aR, has highly adverse consequences. When C5a interacts with blood PMNs, signaling pathways are paralyzed, associated with internalization of C5a-C5aRs complexes. Signaling paralysis results in loss of innate immune functions of PMNs (phagocytosis, chemotaxis, assembly of NADPH oxidase), which has ominous implications for defenses against bacteremia. With respect to the well-known cardiomyopathy of sepsis, the loss of cardiac function can be linked to C5a interaction with C5aR on CMs. In vivo interception of C5a or C5aR is highly protective in the setting of experimental sepsis, greatly improving survival after CLP, preserving the innate immune functions of PMNs, and normalizing cardiac function. The data suggest that in vivo

blockade of C5a or C5aR may be protective in the setting of sepsis in humans.

ACKNOWLEDGMENT

This work was based on presentations at the 7th World Congress on Trauma, Shock, Inflammation and Sepsis (Munich, March 2007).

REFERENCES

1. Riedemann, N. C., Guo, R. F., Ward, P. A. (2003) Novel strategies for the treatment of sepsis. *Nat. Med.* **9,** 517–524.
2. Rabiet, M. J., Huet, E., Boulay, F. (2007) The N-formyl peptide receptors and the anaphylatoxin C5a receptors: an overview. *Biochimie* **89,** 1089–1106–.
3. Guo, R. F., Ward, P. A. (2005) Role of C5a in inflammatory responses. *Annu. Rev. Immunol.* **23,** 821–852.
4. Huber-Lang, M., Younkin, E. M., Sarma, J. V., Riedemann, N., McGuire, S. R., Lu, K. T., Kunkel, R., Younger, J. G., Zetoune, F. S., Ward, P. A. (2002) Generation of C5a by phagocytic cells. *Am. J. Pathol.* **161,** 1849–1859.
5. Huber-Lang, M., Sarma, J. V., Zetoune, F. S., Rittirsch, D., Neff, T. A., McGuire, S. R., Lambris, J. D., Warner, R. L., Flierl, M. A., Hoesel, L. M., et al. (2006) Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* **12,** 682–687.
6. Riedemann, N. C., Guo, R. F., Neff, T. A., Laudes, I. J., Keller, K. A., Sarma, J. V., Markiewski, M. M., Mastellos, D., Strey, C. W., Pierson, C. L., Lambris, J. D., Zetoune, F. S., Ward, P. A. (2002) Increased C5a receptor expression in sepsis. *J. Clin. Invest.* **110,** 101–108.
7. Riedemann, N. C., Guo, R. F., Laudes, I. J., Keller, K., Sarma, V. J., Padgaonkar, V., Ward, P. A. (2002) C5a receptor and thymocyte apoptosis in sepsis. *FASEB J.* **16,** 887–888.
8. Niederbichler, A. D., Hoesel, L. M., Westfall, M. V., Gao, H., Ipaktchi, K. R., Sun, L., Zetoune, F. S., Su, G. L., Arbabi, S., Sarma, J. V., et al. (2005) An essential role for complement C5a in the pathogenesis of septic cardiac dysfunction. *J. Exp. Med.* **203,** 53–61.
9. Riedemann, N. C., Guo, R. F., Laudes, I. J., Keller, K., Sarma, V. J., Padgaonkar, V., Ward, P. A. (2002) C5a receptor and thymocyte apoptosis in sepsis. *FASEB J.* **16,** 887–888.
10. Hotchkiss, R. S., Nicholson, D. W. (2006) Apoptosis and caspases regulate death and inflammation in sepsis. *Nat. Rev. Immunol.* **6,** 813–822.
11. Riedemann, N. C., Neff, T. A., Guo, R. F., Bernacki, K. D., Laudes, I. J., Sarma, J. V., Lambris, J. D., Ward, P. A. (2003) Protective effects of IL-6 blockade in sepsis are linked to reduced C5a receptor expression. *J. Immunol.* **170,** 503–507.
12. Guo, R. F., Riedemann, N. C., Bernacki, K. D., Sarma, J. V., Laudes, I. J., Reuben, J. S., Younkin, E. M., Neff, T. A., Paulauskis, J. D., Zetoune, F. S., Ward, P. A. (2003) Neutrophil C5a receptor and the outcome in a rat model of sepsis. *FASEB J.* **17,** 1889–1891.
13. Cain, S. A., Monk, P. N. (2002) The orphan receptor C5L2 has high affinity binding sites for complement fragments C5a and C5a des-Arg(74). *J. Biol. Chem.* **277,** 7165–7169.
14. Okinaga, S., Slattery, D., Humbles, A., Zsengeller, Z., Morteau, O., Kinrade, M. B., Brodbeck, R. M., Krause, J. E., Choe, H. R., Gerard, N. P., Gerard, C. (2003) C5L2, a nonsignaling C5A binding protein. *Biochemistry* **42,** 9406–9415.
15. Johswich, K., Martin, M., Thalmann, J., Rheinheimer, C., Monk, P. N., Klos, A. (2006) Ligand specificity of the anaphylatoxin C5L2 receptor and its regulation on myeloid and epithelial cell lines. *J. Biol. Chem.* **281,** 39088–39095.
16. Kalant, D., MacLaren, R., Cui, W., Samanta, R., Monk, P. N., Laporte, S. A., Cianflone, K. (2005) C5L2 is a functional receptor for acylation-stimulating protein. *J. Biol. Chem.* **280,** 23936–23944.
17. Kalant, D., Cain, S. A., Maslowska, M., Sniderman, A. D., Cianflone, K., Monk, P. N. (2003) The chemoattractant receptor-like protein C5L2 binds the C3a des-Arg77/acylation-stimulating protein. *J. Biol. Chem.* **278,** 11123–11129.
18. Chen, N. J., Mirtsos, C., Suh, D., Lu, Y. C., Lin, W. J., McKerlie, C., Lee, T., Baribault, H., Tian, H., Yeh, W. C. (2007) C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* **446,** 203–207.
19. Gao, H., Neff, T. A., Guo, R. F., Speyer, C. L., Sarma, J. V., Tomlins, S., Man, Y., Riedemann, N. C., Hoesel, L. M., Younkin, E. M., Zetoune, F. S., Ward, P. A. (2005) Evidence for a functional role of the second C5a receptor, C5L2. *FASEB J.* **19,** 1003–1005.
20. Gerard, N. P., Lu, B., Liu, P., Craig, S., Fujiwara, Y., Okinaga, S., Gerard, C. (2005) An anti-inflammatory function for the complement anaphylatoxin C5a-binding protein, C5L2. *J. Biol. Chem.* **280,** 39677–39680.
21. Czermak, B. J., Sarma, V., Pierson, C. L., Warner, R. L., Huber-Lang, M., Bless, N. M., Schmal, H., Friedl, H. P., Ward, P. A. (1999) Protective effects of C5a blockade in sepsis. *Nat. Med.* **5,** 788–792.
22. Huber-Lang, M. S., Sarma, J. V., McGuire, S. R., Lu, K. T., Guo, R. F., Padgaonkar, V. A., Younkin, E. M., Laudes, I. J., Riedemann, N. C., Younger, J. G., Ward, P. A. (2001) Protective effects of anti-C5a peptide antibodies in experimental sepsis. *FASEB J.* **15,** 568–570.
23. Huber-Lang, M. S., Younkin, E. M., Sarma, J. V., McGuire, S. R., Lu, K. T., Guo, R. F., Padgaonkar, V. A., Curnutte, J. T., Erickson, R., Ward, P. A. (2002) Complement-induced impairment of innate immunity during sepsis. *J. Immunol.* **169,** 3223–3231.
24. Laudes, I. J., Chu, J. C., Sikranth, S., Huber-Lang, M., Guo, R. F., Riedemann, N., Sarma, J. V., Schmaier, A. H., Ward, P. A. (2002) Anti-C5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. *Am. J. Pathol.* **160,** 1867–1875.
25. Huber-Lang, M. S., Riedemann, N. C., Sarma, J. V., Younkin, E. M., McGuire, S. R., Laudes, I. J., Lu, K. T., Guo, R., Neff, T. A., Padgaonkar, V. A., et al. (2002) Protection of innate immunity by C5aR antagonist in septic mice. *FASEB J.* **16,** 1567–1574.
26. Huber-Lang, M., Sarma, J. V., Rittirsch, D., Schreiber, H., Weiss, M., Flierl, M., Younkin, E., Schneider, M., Suger-Wiedeck, H., Gebhard, F., et al. (2005) Changes in the novel orphan, C5a receptor (C5L2), during experimental sepsis and sepsis in humans. *J. Immunol.* **174,** 1104–1110.