# Protective Effects of IL-6 Blockade in Sepsis Are Linked to Reduced C5a Receptor Expression[1]

**Niels C. Riedemann,[2]* Thomas A. Neff,[2]* Ren-Feng Guo,* Kurt D. Bernacki,* Ines J. Laudes,* J. Vidya Sarma,* John D. Lambris,† and Peter A. Ward[3]***

**IL-6 is known to be an important pro- and anti-inflammatory cytokine, which is up-regulated during sepsis. Our previous work has suggested a role for IL-6 in the up-regulation of C5aR in sepsis. We reported earlier that interception of C5a or C5aR results in improved outcomes in experimental sepsis. Using the cecal ligation/puncture (CLP) model in mice, we now demonstrate that treatment with anti-IL-6 Ab (anti-IL-6) results in significantly improved survival, dependent on the amount of Ab infused. CLP animals showed significantly increased binding of $^{125}$I-labeled anti-C5aR to organs when compared to either control mice at 0 h or CLP animals infused with normal rabbit $^{125}$I-labeled IgG. Binding of $^{125}$I-labeled anti-C5aR to lung, liver, kidney, and heart was significantly decreased in anti-IL-6-treated animals 6 h after CLP. RT-PCR experiments with mRNA isolated from various organs obtained 3, 6, and 12 h after CLP demonstrated increased C5aR mRNA expression during the onset of sepsis, which was greatly suppressed in CLP mice treated with anti-IL-6. These data suggest that IL-6 plays an important role in the increased expression of C5aR in lung, liver, kidney, and heart during the development of sepsis in mice and that interception of IL-6 leads to reduced expression of C5aR and improved survival.** ***The Journal of Immunology*****, 2003, 170: 503–507.**

Interleukin-6 is a 212-aa polypeptide (1) known to have a variety of biological effects. Initially, IL-6 was named according to its ability to induce IFN-$\beta$2, 26-kDa protein, B cell stimulatory factor-2, hybridoma/plasmocytoma growth factor, hepatocyte stimulating factor, monocyte granulocyte inducer-2, cytotoxic T cell differentiation factor, and thrombopoietin (2). IL-6 facilitates its effects through a specific 80-kDa receptor (3) together with the membrane gp130, which is required for intracellular signal transduction (4, 5). IL-6 has been described as a cytokine with proinflammatory and anti-inflammatory effects, one important feature being the ability to induce production of acute phase proteins in the liver (6, 7). Although IL-6 does not appear to significantly influence serum levels of TNF-$\alpha$ and IL-8 or induce neutrophil degranulation in a model of endotoxemia in chimpanzees (8), its serum levels are reported to be influenced by TNF-$\alpha$ and IL-1 in humans (9, 10). It has been known since 1989 that IL-6 is greatly up-regulated in the serum of patients with bacterial infection or sepsis (11–13). Many studies have reported a positive correlation between IL-6 serum levels and outcomes in septic patients (14–17). The impact of IL-6 on the outcome in experimental sepsis is controversial. It has been reported that injections of recombinant IL-6, even in high doses, had no harmful effects in otherwise normal dogs (18). Several studies have demonstrated beneficial effects of blocking Abs to IL-6 when used in models of TNF-$\alpha$ or *Escherichia coli* challenge in mice (19). The underlying mechanism of such beneficial effects has not been defined. It is known that other serum cytokine levels do not seem to be altered during IL-6 blockade.

There is strong evidence that, in the early phases of sepsis, the complement activation product, C5a, plays a harmful role in rodents following cecal ligation/puncture (CLP)[4] or infusion of LPS (20–24). This adverse outcome had been linked to a C5a-dependent loss of the respiratory burst ($H_2O_2$ production) in blood neutrophils from septic rats. The responses to C5a are mediated by a pertussis toxin-sensitive G protein-linked seven transmembrane-spanning C5aR, which belongs to the superfamily of rhodopsin-type receptors (25, 26). We recently found that this receptor is strongly up-regulated in various organs during the onset of sepsis in mice and that its blockade results in greatly improved survival in CLP mice (27). In liver, IL-6 has been reported to up-regulate mRNA expression for C5aR (28). We have found that IL-6 strongly up-regulates C5aR on rat lung epithelial cells, thymocytes, and endothelial cells (29, 30).

The goal of the current study was to investigate effects of IL-6 blockade during the onset of sepsis in mice and to assess the linkage between IL-6 and regulation of C5aR during CLP-induced sepsis. We demonstrate for the first time that IL-6 blockade during CLP-induced sepsis in mice results in a significantly reduced induction of C5aR in lung, liver, kidney, and heart, leading to greatly improved survival.

## Materials and Methods

### *Reagents and anti-IL-6 Ab (anti-IL-6)*

Unless otherwise specified, reagents were obtained from Sigma-Aldrich (St. Louis, MO). Specific monoclonal rat anti-mouse IL-6 Ab (anti-IL-6) was obtained from BD PharMingen (San Diego, CA). This Ab was characterized by the manufacturer as specifically neutralizing of recombinant mouse IL-6 (references provided by BD PharMingen), with a neutralizing activity of >95% when used in a concentration between 0.5 and 2.0 $\mu$g/ml.

*Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109 and †Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

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[2] N.C.R. and T.A.N. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Peter A. Ward, Department of Pathology, University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, MI 48109-0602. E-mail address: pward@umich.edu

[4] Abbreviations used in this paper: CLP, cecal ligation/puncture; DPBS, Dulbecco's PBS.



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*Peptide synthesis and production of anti-mouse C5aR Abs*

A 37-aa peptide spanning the N terminus of the mouse C5aR (MDP IDNSSFEINYDHYGTMDPNIPADGIHLPKRQPGDC) was synthesized using an Applied Biosystem 430A peptide synthesizer (Foster City, CA), as described elsewhere (31). The peptide was then coupled to keyhole limpet hemocyanin by the glutaraldehyde method and used for the immunization of rabbits. The anti-peptide specific Ab was purified by affinity chromatography using the synthetic peptide coupled to cyanogen bromide-activated Sepharose 4B (Amersham Pharmacia Biotech, Piscataway, NJ).

*Quantitation of IL-6 by ELISA*

IL-6 serum levels were determined using ELISA kits (BioSource International, Camarillo, CA) according to the manufacturer's instructions. Approximately 500–800 $\mu$l blood were drawn from healthy control animals and from animals at various time points after CLP. The blood samples were placed on ice and allowed to clot before centrifugation at 3000 $\times$ *g* for 10 min. Serum samples were then used in various dilutions for ELISA performance.

*Experimental sepsis induced by CLP and organ preparation*

Seven- to 8-wk-old specific pathogen-free male B10/D2 nsnJ mice (The Jackson Laboratory, Bar Harbor, ME) were used in all studies. Anesthesia was achieved by i.p. injection of a ketamine/xylozine/Dulbecco's PBS (DPBS) solution (11 $\mu$l/g body weight; 1 ml of ketamine containing 9% xylozine was diluted with 7 ml of DPBS). In the CLP model, approximately two-thirds of the cecum was ligated, using a 1.5-cm abdominal midline surgical incision. The ligated part of the cecum was punctured through and through with a 21-gauge needle. After repositioning of the bowel, the abdomen was closed in layers using a 4.0 surgical suture (Ethicon, Somerville, NJ) and metallic clips. Sham animals underwent the same procedure without ligation or puncture of the cecum. For animal sacrifice, the inferior vena cava was incised and ~600 $\mu$l of blood withdrawn. The chest was then opened and the pulmonary artery was slowly perfused with 40 ml of DPBS, with perfusion liquid leaking out of the open caval vein after perfusion of the arterial system. Perfusion quality was optically controlled by observing the organ color change (to white) as the blood was completely removed. Immunohistochemical staining experiments for mouse neutrophils were conducted for organs of CLP animals to assure the complete removal of neutrophils from the vessels and endothelium with the above-mentioned flushing method. Organs were removed for radioactivity analysis or snap frozen for RT-PCR experiments and immunohistochemical staining. In lungs, bronchoalveolar lavage was also performed with a total volume of 3 ml.

*In vivo binding studies*

Polyclonal rabbit anti-mouse C5aR IgG (affinity purified using the N-terminal peptide) or normal rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was labeled with $^{125}$I, using the chloramine method, as described elsewhere (32). This protocol involves gentle oxidation. Immediately after CLP, animals received anti-IL-6 (1.33 mg/kg body weight) or control IgG in 200 $\mu$l of DPBS via injection into the penile vein. For the binding studies animals were sacrificed 6 and 12 h thereafter. Organ radioactivity was compared with that in CLP animals sacrificed at 0 h. $^{125}$I-labeled anti-mouse C5aR IgG (100 ng) together with 2 $\mu$g nonlabeled Ab as carrier in a total volume of 200 $\mu$l of DPBS was administered i.v. 15 min before sacrificing the animals. Blood was withdrawn from the abdominal caval vein to determine the amount of radioactivity in the blood 15 min after injection of $^{125}$I-labeled anti-C5aR IgG. Organs were then thoroughly flushed with DPBS and then harvested (as described above) and weighed. Radioactivity was measured in a gamma counter (1261 Multi; Wallac, Gaithersburg, MD). Data were expressed as cpm per gram of tissue, divided by the cpm in 100-$\mu$l blood sample for each individual animal.

*RNA isolation and detection of C5aR mRNA by semiquantitative RT-PCR*

Organs from mice were obtained 0, 3, 6, and 12 h after induction of CLP and prepared as described above. Total RNA was isolated with the TRIzol method (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Digestion of any contaminating DNA was achieved by treatment of samples with RQ1 RNase-free DNase (Promega, Madison, WI).

Reverse transcription was performed with 5 $\mu$g of RNA using the Superscript II RNase H$^-$ Reverse Transcriptase (Life Technologies) according to the manufacturer's protocol. PCR was then performed with the following primers for C5aR: 5′ primer, 5′-TAT AGT CCT GCC CTC GCT CAT-3′, and 3′ primer, 5′-TCA CCA CTT TGA GCG TCT TGG-3′. The primers were designed for a 409-bp cDNA amplification in the middle region of the rat C5aR cDNA (position 373–781). The primers for the "housekeeping" gene *GAPDH* were as follows: 5′ primer, 5′-GCC TCG TCT CAT AGA CAA GAT G-3′, and 3′ primer, 5′-CAG TAG ACT CCA CGA CAT AC-3′. Thirty-five cycles were used for amplification. The RT-PCR product was confirmed by electrophoresis of samples in 1.2% agarose gel. Control experiments were performed with the samples in which reverse transcriptase was not added to rule out contaminating DNA being responsible for any results. PCR was performed using different cycle numbers for C5aR and GAPDH primers, to assure that DNA was detected within the linear part of the amplifying curves for both primers. Results are presented in a semiquantitative manner.

*Statistics*

Data sets were analyzed using one-way ANOVA, and individual group means were then compared with the Student-Newman-Keuls multiple comparison test. Statistical analyzes for survival studies were performed using proportional hazards modeling. In both tests, significance was considered for values of $p < 0.05$.

## Results

*Suppression of serum IL-6 levels in CLP mice by Abs*

To determine the efficiency of monoclonal neutralizing rat IgG Ab (anti-IL-6) to suppress IL-6 in vivo, we investigated by ELISA serum IL-6 levels during the onset of CLP-induced sepsis in mice. Fig. 1 demonstrates the ability of anti-IL-6 to greatly suppress IL-6 in the serum of mice 6 h after induction of sepsis by CLP. Reduction of detectable IL-6 was dose dependent when compared with animals receiving irrelevant rat IgG (IgG). Healthy control animals showed no detectable IL-6 serum levels (<3 pg/ml). The most protective dose of anti-IL-6 (1.33 mg/kg) in sepsis (see Fig. 2) resulted in serum IL-6 levels of 184 pg/ml on average, which presents a significant increase when compared with healthy control animals and a significant decrease when compared with control IgG-injected CLP mice. The doses of 2.66 and 0.33 mg anti-IL-6/kg body weight resulted in serum IL-6 levels of 15 and 629 pg/ml, respectively. The intermediate dose of (1.33 mg/kg) of anti-IL-6 was used for subsequent in vivo experiments.

*Improved survival in anti-IL-6-treated CLP mice*

To determine potential beneficial effects of anti-IL-6 treatment in sepsis, we injected various concentrations (0.33, 1.33, and 2.66 mg/kg body weight) of anti-IL-6 i.v. in 200 $\mu$l of DPBS at the start of CLP (time 0 h). Companion mice received normal rat IgG (1.33 mg/kg body weight) at the time of CLP. The results of these experiments are presented in Fig. 2*A*. Anti-IL-6 treatment resulted in significantly improved survival that was dose dependent. A dose of

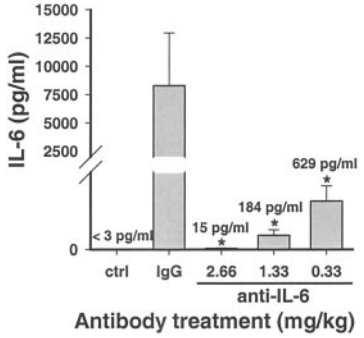


**FIGURE 1.** Reduction of serum IL-6 levels by anti-IL-6 treatment. Serum IL-6 levels from healthy control mice and from mice 6 h after CLP, as measured by ELISA. IL-6 levels from animals treated with anti-IL-6 IgG (1.33 mg/kg body weight) were significantly reduced (*, $p < 0.05$) when compared with IL-6 levels from mice treated with irrelevant rat IgG. Data are representative for four animals per group.

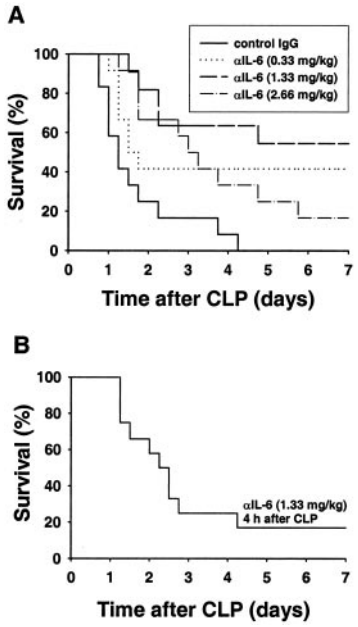


**FIGURE 2.** Survival in CLP-induced sepsis; effect of IL-6 blockade. *A*, Dose-dependency of anti-IL-6 treatment on survival in CLP mice. All treated groups showed statistically significantly improved survival ($p < 0.05$) when compared with the CLP control group treated with irrelevant IgG. Data represent 12 animals per treatment group. *B*, Delayed infusion of anti-IL-6 at 4 h after CLP did not result in significantly improved survival when compared with the control group.

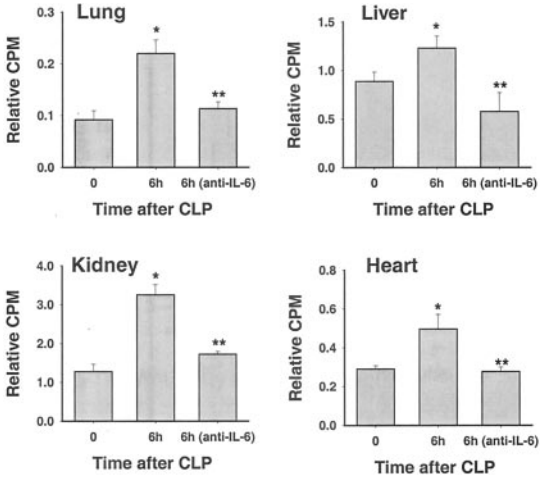


**FIGURE 3.** Effect of anti-IL-6 treatment on $^{125}$I-labeled anti-C5aR binding in vivo during sepsis. In vivo binding of $^{125}$I-labeled anti-C5aR to various organs in mice 0 and 6 h after CLP. Binding is expressed as the ratio of cpm per gram organ to cpm in 100 $\mu$l of blood from each animal obtained 15 min after i.v. injection of $^{125}$I-labeled anti-C5aR. ∗, Statistical significance ($p < 0.05$) from CLP mice sacrificed at 0 h. ∗∗, Statistically significant reduction of binding in organs of anti-IL-6-treated (1.33 mg/kg) CLP animals compared with those of CLP animals (sacrificed at 0 h). Data are representative of four to six animals per group.

1.33 mg/kg body weight resulted in a 7-day survival of 56%, whereas 0.33 mg/kg body weight showed a survival of 42%. Surprisingly, the highest dose of anti-IL-6 (2.66 mg/kg body weight) resulted in a much less improved survival, with a 7-day survival of only 18%. Each of the anti-IL-6-treated groups was statistically significantly different from the control group receiving an irrelevant rat IgG at time 0. This group had no survivors by the fifth day. In the anti-IL-6-treated groups, the survival curves showed additional drop-offs from days 5 to 7. These results suggest that sepsis-induced IL-6 is harmful and that there is a certain "threshold" of IL-6, below which host defenses are compromised. Delayed infusion of the most potent dose of anti-IL-6 (1.33 mg/kg body weight) at 4 h after CLP resulted in no significant beneficial effects on survival, even though 16% of the mice survived at day 7 (Fig. 2*B*). Because, as pointed out in the introduction, we found that in vitro exposure to IL-6 can induce C5aR in various tissues and cells, and we have reported before that blockade of C5a or C5aR resulted in greatly improved survival, we sought to investigate whether the observed beneficial effects of anti-IL-6 treatment could be linked to altered C5aR expression during sepsis.

*Reduced binding of anti-C5aR Ab in anti-IL-6-treated CLP mice*

We recently found that binding of $^{125}$I-labeled IgG Ab to mouse C5aR is significantly increased in lung, liver, kidney, and heart during the onset of sepsis in mice at 3, 6, and 12 h after CLP (27). In these studies, an irrelevant $^{125}$I-labeled control IgG showed no evidence of increased organ binding. In the current studies, we investigated the effects of anti-IL-6 treatment on the induction of C5aR in CLP mice. Fig. 3 summarizes the results of these studies. Six hours after CLP, binding of $^{125}$I-labeled anti-C5aR IgG to lung, liver, kidney, and heart was significantly increased when compared with results from CLP mice at time 0. Treatment with 1.33 mg anti-IL-6/kg body weight resulted in a significant suppression in binding of Ab to C5aR in all four organs, as defined by reduced binding of $^{125}$I-labeled anti-C5aR IgG. The observed binding in the four anti-IL-6-treated groups 6 h after CLP was not statistically different from the binding to organs from 0-h CLP animals. Interestingly, by 12 h after CLP, the increase in binding of $^{125}$I-labeled anti-C5aR to the various organs in the control IgG-treated group had fallen back to the levels found at 0 h following CLP (data not shown). Therefore, IL-6 appears to have a time-limited effect in CLP mice in terms of increased levels of C5aR. The binding data suggest that C5aR induction early after the onset of sepsis (6 h after CLP) is greatly suppressed in lung, liver, kidney, and heart when animals were first treated with anti-IL-6.

*Suppression by anti-IL-6 of C5aR mRNA in organs after CLP*

To extend the results from the binding studies, we isolated total RNA at various time points (0, 3, 6, and 12 h) after CLP from lung, liver, kidney and heart from mice that had been treated with irrelevant IgG Ab or with anti-IL-6 IgG and performed RT-PCR for C5aR mRNA expression. The results of these experiments are demonstrated in Fig. 4. Healthy control animals showed little or no detectable expression (ctrl column) of mRNA for C5aR in each of the organs. Three, 6, and 12 h after induction of sepsis by CLP, a significant increase in C5aR mRNA expression was observed in each of the four organs, peaking at 6 h after CLP. In animals treated with anti-IL-6, increased mRNA expression for C5aR was significantly suppressed at 3, 6, and 12 h after CLP in all four organs when compared with CLP mice treated with normal (ctrl) IgG. Interestingly, in liver from CLP mice treated with anti-IL-6, there was a late increase (12 h after CLP) in C5aR mRNA expression. The results of the RT-PCR experiments suggest that IL-6 blockade reduces C5aR mRNA expression during the early onset of sepsis, which is consistent with the organ binding data using $^{125}$I-labeled anti-C5aR (Fig. 3).

## Discussion

As pointed out above, IL-6 levels in serum are well known to be up-regulated in septic humans and can be used to predict outcome. IL-6 levels in experimental models of sepsis in animals have also

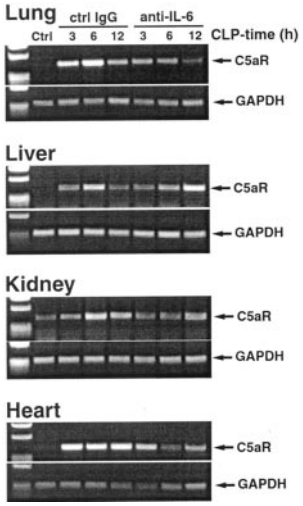


**FIGURE 4.** Effect of anti-IL-6 treatment on C5aR mRNA expression during sepsis. Semiquantitative RT-PCR results for C5aR mRNA in lung, liver, kidney, and heart from anti-IL-6-treated animals and nontreated animals. RT-PCR was performed using RNA isolated 0, 3, 6, and 12 h after CLP. Approximately equal loading of the DNA product is demonstrated by expression of GAPDH mRNA. Results are representative of two independent and separate experiments for each group and time point.

been described to be significantly higher in serum, especially in the early onset of sepsis (33, 34). However, beneficial effects of IL-6 blockade have been controversial. For example, blockade of IL-6 with mAbs in endotoxic shock models in mice (after LPS or TNF-$\alpha$ infusion) has been reported to have no protective effects in terms of improved survival (35). Other groups have reported in rodents the beneficial effects of anti-IL-6 in models of *Escherichia coli* challenge, TNF-$\alpha$ infusion (19), endotoxic shock (36), and gut-derived sepsis with *Escherichia coli* gavage and thermal injury (37). In our study, we determined the effects of blockade of mouse IL-6 with a neutralizing rat mAb in CLP mice. This CLP model of sepsis is believed to closely simulate clinical sepsis in humans because of the polymicrobial-driven inflammatory process. We have demonstrated a dose-dependent beneficial effect of IL-6 blockade in this sepsis model, using anti-IL-6 in mice. Interestingly, high doses of anti-IL-6 (2.66 mg/kg), as well as low doses (0.3 mg/kg), appeared to be less beneficial for the outcome in sepsis, correlating with lower or higher detectable IL-6 levels in the serum at 6 h after CLP (Fig. 1). This suggests that, in CLP-induced sepsis, high levels of IL-6 are harmful and that, if Ab-induced blockade of IL-6 is excessive, increased deaths occur, perhaps because low levels of IL-6 are beneficial for host defenses, in a manner similar to what has been described for Ab-induced blockade of TNF-$\alpha$ in murine sepsis (38).

As pointed out above, IL-6 is responsible for proinflammatory as well as for anti-inflammatory effects in addition to its numerous other functions, such as stimulation of cell growth. This variety of effects may be the reason why IL-6 blockade is useful in one inflammatory setting but harmful or nonbeneficial in other settings. This may be the reason for the difficulties in previously published studies that have attempted to define the biology of IL-6. We were especially interested in finding an explanation for the improved survival by investigating the influence of IL-6 blockade on the expression of C5aR during sepsis. Our recent studies have indicated that C5aR is strongly up-regulated during the onset of CLP-induced sepsis in mice in lung, liver, kidney, and heart, and that Ab-induced blockade of C5aR in CLP mice results in dramatically improved survival. In the current studies, we demonstrated that blockade of IL-6 during CLP-induced sepsis resulted in decreased elevation of C5aR mRNA and significantly reduced in vivo binding of $^{125}$I-labeled anti-C5aR IgG in lung, liver, kidney, and heart when compared with the strong increase occurring in control CLP mice 6 h after CLP and treated with normal IgG. Interestingly, in the liver we observed a late increase in C5aR mRNA 12 h after CLP in the anti-IL-6-treated animals, which was not reflected on the protein level with the binding data. This phenomenon is currently not understood, but it is possible that the regulation of C5aR mRNA production in the liver may differ from that of other organs at late stages of sepsis when IL-6 has initially been blocked.

We also investigated the possible influence of IL-6 blockade on TNF-$\alpha$ and MIP-2 serum levels during CLP-induced sepsis in mice, but we were unable to detect significant differences in CLP animals treated with either anti-IL-6 or irrelevant IgG (data not shown). It has been reported that IL-6 does not alter serum levels of TNF-$\alpha$ or IL-8 in an endotoxic shock model in vivo in chimpanzees (8). Because there is a broad variety of cytokines that may play a role during IL-6 blockade, additional mechanisms may also be involved in the improved survival of CLP mice treated with anti-IL-6.

It has been reported that IL-6 knockout mice do not show improved survival in CLP-induced sepsis (39) and that they suffer from impaired immune and acute-phase responses (40). These reports suggest that a certain amount of IL-6 production in acute inflammation may be required for an appropriate immune response or innate immunity. In fact, we demonstrated that the most potent dose of anti-IL-6 (1.33 $\mu$g/kg body weight) for optimal survival was associated with measurable IL-6 serum levels 6 h after CLP as compared with serum from healthy control animals (Fig. 1). Although IL-6 levels in the sera of CLP mice treated with anti-IL-6 (1.33 mg/kg) were suppressed by ~90% (Fig. 1), there still remained detectable IL-6. It is possible that this intermediate dose of anti-IL-6 permitted a small but significant amount of IL-6 to remain, resulting in much less up-regulation of C5aR but adequate to facilitate other protective host-defense responses. The lower dose of IL-6 (0.33 mg/kg) may have been inadequate to reduce IL-6 levels sufficient to suppress up-regulation of C5aR. The paradoxical finding that a higher dose of anti-IL-6 resulted in reduced survival (Fig. 2) may reflect that the total blockade of IL-6 results in the loss of some beneficial effects of IL-6. The latter finding is supported by the finding that IL-6 knockout mice also fail to show improved survival in CLP-induced sepsis (39). The dose dependency of anti-IL-6 for the beneficial outcome (Fig. 2) provides a possible explanation for inconsistent findings dealing with blockade of IL-6, as reported above. Delayed infusion of anti-IL-6 at 4 h after CLP resulted in no significant improvement of survival, similar to the findings with delayed anti-C5aR treatment (27), suggesting that an early interception of C5aR up-regulation is necessary to counteract a harmful compromising of innate immunity.

We have recently found that blockade of C5aR during CLP-induced sepsis in mice resulted in reduced serum IL-6 levels 6 h after CLP (27). In the current study, we present evidence that blockade of IL-6 during sepsis leads to a diminished increase in C5aR expression in various organs. These results suggest a complex feedback mechanism between C5a/C5aR interaction and serum IL-6 levels. Blockade of one of the two mediators ultimately leads to a reduced production of the other one. In experimental sepsis, there is evidence that both, C5a and IL-6, are produced excessively, maybe due to a positive feedback between both parameters. This could explain why interception of either one of them has been shown to be beneficial for the outcome in sepsis while simultaneously the increase of the other mediator or its receptor was diminished.

The question arises as to why increased expression of C5aR in lung, kidney, heart, and liver may be linked to worsened survival in CLP. There is no direct evidence to resolve this question. It is possible that heightened C5aR expression in these organs in CLP mice is concomitant with C5a generation in blood. This combination might result in diminished organ performance, resulting in multiorgan failure.

Taken together, our data suggest that the appropriate degree of IL-6 blockade is beneficial for the outcome in CLP-induced sepsis in mice, and this is linked to inhibition of the early increases of C5aR, in a variety of organs from CLP mice. The results of the current study suggest a functional mechanism for beneficial IL-6 blockade during the onset of sepsis. In addition, our findings suggest a potential preventive treatment, targeting IL-6 and C5a/C5aR in patients at high risk for development of sepsis. A balanced amount of IL-6 serum content during the onset of sepsis may thereby be a key for successful intervention.

## References


1. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, et al. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature 324:73.*
2. Akira, S., T. Taga, and T. Kishimoto. 1993. Interleukin-6 in biology and medicine. *Adv. Immunol. 54:1.*
3. Yamasaki, K., T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, B. Seed, T. Taniguchi, T. Hirano, and T. Kishimoto. 1988. Cloning and expression of the human interleukin-6 (BSF-2/IFN-$\beta$2) receptor. *Science 241:825.*
4. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell 58:573.*
5. Hibi, M., M. Murakami, M. Saito, T. Hirano, T. Taga, and T. Kishimoto. 1990. Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell 63: 1149.*
6. Geiger, T., T. Andus, J. Klapproth, T. Hirano, T. Kishimoto, and P. C. Heinrich. 1988. Induction of rat acute-phase proteins by interleukin 6 in vivo. *Eur. J. Immunol. 18:717.*
7. Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann. 1987. Interferon-$\beta$2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA 84:7251.*
8. van der Poll, T., M. Levi, C. E. Hack, H. ten Cate, S. J. van Deventer, A. J. Eerenberg, E. R. de Groot, J. Jansen, H. Gallati, H. R. Buller, et al. 1994. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J. Exp. Med. 179:1253.*
9. Shalaby, M. R., A. Waage, L. Aarden, and T. Espevik. 1989. Endotoxin, tumor necrosis factor-$\alpha$ and interleukin 1 induce interleukin 6 production in vivo. *Clin. Immunol. Immunopathol. 53:488.*
10. Sheron, N., J. N. Lau, J. Hofmann, R. Williams, and G. J. Alexander. 1990. Dose-dependent increase in plasma interleukin-6 after recombinant tumour necrosis factor infusion in humans. *Clin. Exp. Immunol. 82:427.*
11. Hack, C. E., E. R. De Groot, R. J. Felt-Bersma, J. H. Nuijens, R. J. Strack Van Schijndel, A. J. Eerenberg-Belmer, L. G. Thijs, and L. A. Aarden. 1989. Increased plasma levels of interleukin-6 in sepsis. *Blood 74:1704.*
12. Helfgott, D. C., S. B. Tatter, U. Santhanam, R. H. Clarick, N. Bhardwaj, L. T. May, and P. B. Sehgal. 1989. Multiple forms of IFN-$\beta$2/IL-6 in serum and body fluids during acute bacterial infection. *J. Immunol. 142:948.*
13. Waage, A., P. Brandtzaeg, A. Halstensen, P. Kierulf, and T. Espevik. 1989. The complex pattern of cytokines in serum from patients with meningococcal septic shock: association between interleukin 6, interleukin 1, and fatal outcome. *J. Exp. Med. 169:333.*
14. Calandra, T., J. Gerain, D. Heumann, J. D. Baumgartner, and M. P. Glauser. 1991. High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines: the Swiss-Dutch J5 Immunoglobulin Study Group. *Am. J. Med. 91:23.*
15. Casey, L. C., R. A. Balk, and R. C. Bone. 1993. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann. Intern. Med. 119:771.*
16. Damas, P., D. Ledoux, M. Nys, Y. Vrindts, D. De Groote, P. Franchimont, and M. Lamy. 1992. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. *Ann. Surg. 215:356.*
17. Gardlund, B., J. Sjolin, A. Nilsson, M. Roll, C. J. Wickerts, and B. Wretlind. 1995. Plasma levels of cytokines in primary septic shock in humans: correlation with disease severity. *J. Infect. Dis. 172:296.*
18. Preiser, J. C., D. Schmartz, P. Van der Linden, J. Content, P. Vanden Bussche, W. Buurman, W. Sebald, E. Dupont, M. R. Pinsky, and J. L. Vincent. 1991. Interleukin-6 administration has no acute hemodynamic or hematologic effect in the dog. *Cytokine 3:1.*
19. Starnes, H. F., Jr., M. K. Pearce, A. Tewari, J. H. Yim, J. C. Zou, and J. S. Abrams. 1990. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor-$\alpha$ challenge in mice. *J. Immunol. 145:4185.*
20. Smedegard, G., L. X. Cui, and T. E. Hugli. 1989. Endotoxin-induced shock in the rat: a role for C5a. *Am. J. Pathol. 135:489.*
21. Czermak, B. J., V. Sarma, C. L. Pierson, R. L. Warner, M. Huber-Lang, N. M. Bless, H. Schmal, H. P. Friedl, and P. A. Ward. 1999. Protective effects of C5a blockade in sepsis. *Nat. Med. 5:788.*
22. Huber-Lang, M., V. J. Sarma, K. T. Lu, S. R. McGuire, V. A. Padgaonkar, R. F. Guo, E. M. Younkin, R. G. Kunkel, J. Ding, R. Erickson, et al. 2001. Role of C5a in multiorgan failure during sepsis. *J. Immunol. 166:1193.*
23. Huber-Lang, M. S., J. V. Sarma, S. R. McGuire, K. T. Lu, R. F. Guo, V. A. Padgaonkar, E. M. Younkin, I. J. Laudes, N. C. Riedemann, J. G. Younger, and P. A. Ward. 2001. Protective effects of anti-C5a peptide antibodies in experimental sepsis. *FASEB J. 15:568.*
24. Guo, R. F., M. Huber-Lang, X. Wang, V. Sarma, V. A. Padgaonkar, R. A. Craig, N. C. Riedemann, S. D. McClintock, T. Hlaing, M. M. Shi, and P. A. Ward. 2000. Protective effects of anti-C5a in sepsis-induced thymocyte apoptosis. *J. Clin. Invest. 106:1271.*
25. Gerard, N. P., M. K. Hodges, J. M. Drazen, P. F. Weller, and C. Gerard. 1989. Characterization of a receptor for C5a anaphylatoxin on human eosinophils. *J. Biol. Chem. 264:1760.*
26. Gerard, N. P., and C. Gerard. 1991. The chemotactic receptor for human C5a anaphylatoxin. *Nature 349:614.*
27. Riedemann, N. C., R. F. Guo, T. A. Neff, I. J. Laudes, K. A. Keller, J. V. Sarma, M. M. Markiewski, D. Mastellos, C. W. Strey, C. L. Pierson, et al. 2002. Increased C5a receptor expression in sepsis. *J. Clin. Invest. 110:101.*
28. Schieferdecker, H. L., G. Schlaf, M. Koleva, O. Gotze, and K. Jungermann. 2000. Induction of functional anaphylatoxin C5a receptors on hepatocytes by in vivo treatment of rats with IL-6. *J. Immunol. 164:5453.*
29. Riedemann, N. C., R. F. Guo, I. J. Laudes, K. Keller, V. J. Sarma, V. Padgaonkar, F. S. Zetoune, and P. A. Ward. 2002. C5a receptor and thymocyte apoptosis in sepsis. *FASEB J. 16:887.*
30. Riedemann, N. C., R. F. Guo, V. J. Sarma, I. J. Laudes, M. Huber-Lang, R. L. Warner, E. A. Albrecht, C. L. Speyer, and P. A. Ward. 2002. Expression and function of the C5a receptor in rat alveolar epithelial cells. *J. Immunol. 168:1919.*
31. Becherer, J. D., and J. D. Lambris. 1988. Identification of the C3b receptor-binding domain in third component of complement. *J. Biol. Chem. 263:14586.*
32. Bennett, G. L., and R. Horuk. 1997. Iodination of chemokines for use in receptor binding analysis. *Methods Enzymol. 288:134.*
33. Eskandari, M. K., G. Bolgos, C. Miller, D. T. Nguyen, L. E. DeForge, and D. G. Remick. 1992. Anti-tumor necrosis factor antibody therapy fails to prevent lethality after cecal ligation and puncture or endotoxemia. *J. Immunol. 148:2724.*
34. Remick, D. G., D. E. Newcomb, G. L. Bolgos, and D. R. Call. 2000. Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. *Shock 13:110.*
35. Libert, C., A. Vink, P. Coulie, P. Brouckaert, B. Everaerdt, J. Van Snick, and W. Fiers. 1992. Limited involvement of interleukin-6 in the pathogenesis of lethal septic shock as revealed by the effect of monoclonal antibodies against interleukin-6 or its receptor in various murine models. *Eur. J. Immunol. 22:2625.*
36. Heremans, H., C. Dillen, W. Put, J. Van Damme, and A. Billiau. 1992. Protective effect of anti-interleukin (IL)-6 antibody against endotoxin, associated with paradoxically increased IL-6 levels. *Eur. J. Immunol. 22:2395.*
37. Gennari, R., and J. W. Alexander. 1995. Anti-interleukin-6 antibody treatment improves survival during gut-derived sepsis in a time-dependent manner by enhancing host defense. *Crit. Care Med. 23:1945.*
38. Remick, D., P. Manohar, G. Bolgos, J. Rodriguez, L. Moldawer, and G. Wollenberg. 1995. Blockade of tumor necrosis factor reduces lipopolysaccharide lethality, but not the lethality of cecal ligation and puncture. *Shock 4:89.*
39. Leon, L. R., A. A. White, and M. J. Kluger. 1998. Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am. J. Physiol. 275:R269.*
40. Kopf, M., H. Baumann, G. Freer, M. Freudenberg, M. Lamers, T. Kishimoto, R. Zinkernagel, H. Bluethmann, and G. Kohler. 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature 368:339.*