Functions of the complement components C3 and C5 during sepsis

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ABSTRACT **Activation of the complement system is a key event in the pathogenesis of sepsis. Nevertheless, the exact mechanisms remain inadequately understood. In the current study, we examined the role of complement C3 and C5 in sepsis in wild-type and C3- or C5-deficient mice induced by cecal ligation and puncture. When compared to wild-type mice, $C5^{-/-}$ showed identical survival, and $C3^{-/-}$ presented significantly reduced survival. Interestingly, this was associated with significant decreases in plasma levels of proinflammatory mediators. Moreover, although septic $C3^{-/-}$ animals displayed a 10-fold increase of blood-borne bacteria, $C5^{-/-}$ animals exhibited a 400-fold increase in bacteremia when compared to wild-type mice. These effects were linked to the inability of $C5^{-/-}$ mice to assemble the terminal membrane attack complex (MAC), as determined by complement hemolytic activity (CH-50). Surprisingly, although negative control $C3^{-/-}$ mice failed to generate the MAC, significant increases of MAC formation was found in septic $C3^{-/-}$ mice. In conclusion, our data corroborate that hemolytic complement activity is essential for control of bacteremia in septic mice. Thus, during sepsis, blockade of C5a or its receptors (rather than C5) seems a more promising strategy, because C5a-blockade still allows for MAC formation while the adverse effects of C5a are prevented.—Flierl, M. A., Rittirsch, D., Nadeau, B. A., Day, D. E., Zetoune, F. S., Sarma, J. V., Huber-Lang, M. S., Ward, P. A. Functions of the complement components C3 and C5 during sepsis. *FASEB J.* 22, 3483–3490 (2008)**

Key Words: survival · cytokines · chemokines · colony-forming units · CH-50

SEPSIS REMAINS AN ENIGMATIC, poorly understood disease (1). It is now becoming increasingly clear that sepsis might not be a disease *per se* but represents a complex accumulation of symptoms forming a multifaceted entity that can be described only by basic clinical parameters (2). However, these crude definitions fail to be consistent, because patients might present with either hyperthermia or hypothermia, leukocytosis or leukopenia, bacteremia or lack of bacteremia (2, 3). Thus, some clinicians preferably refer to this complex of symptoms as "sepsis syndrome." It is of concern that doctors have seen a rapid increase in hospitalization and mortality rates for severe sepsis in the United States between 1993 and 2003 while mortality rates only slightly decreased (4). During this 11-year period, the hospitalization rate has almost doubled and is considerably higher than it has been previously predicted, making septicemia now the 10th leading cause of death in the United States. (5).

Encroachment of pathogens prompts the complement cascade, which plays a decisive role in the host's immune response (1, 6). Its activation can be triggered *via* 3 different pathways, converging to form the C3-convertase, which cleaves C3 into C3a and the opsonizing C3b (7, 8). The C5-convertase subsequently cleaves C5 into the anaphylatoxin C5a and C5b and thereby initiates the formation of the terminal "membrane attack complex" (MAC), consisting of C5b, C6, C7, C8, and C9. However, during sepsis, when complement is excessively activated, the initially beneficial effects can rapidly turn into a severe threat to the host. In particular, disproportionately elevated levels of the anaphylatoxin C5a have been described as "too much of a good thing" (9) and to reveal a "dark side in sepsis" (10), contributing to immunoparalysis (11), multiorgan dysfunction (12), thymocyte apoptosis (13, 14), and deterioration of the coagulatory/fibrinolytic system (15). Clinical studies have confirmed elevated levels of complement activation products during sepsis, which have been linked to poor outcome (16–19). Accordingly, C5a blockade has been shown to be protective in cecal ligation and puncture (CLP) -induced sepsis (20) and to prevent multiorgan failure in septic rats (12, 21, 22). On the other hand, mice deficient for C3 have been described to show higher susceptibility to gram-negative sepsis and endotoxin shock (23, 24). Emerging evidence also suggests that

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doi: 10.1096/fj.08-110595

C3a might have anti-inflammatory properties in addition to its proinflammatory functions (24).

In the current study, we sought to evaluate the impact of the complement components C3 and C5 on inflammation and bacterial clearance, including the underlying mechanisms during experimental sepsis using C3- or C5-knockout mice.

MATERIALS AND METHODS

Experimental sepsis

All procedures were performed in accordance with the National Institutes of Health guidelines and University Committee on Use and Care of Animals, University of Michigan. Specific pathogen-free, 9- to 10-wk-old male wild-type mice (WT; Jackson Laboratories, Bar Harbor, ME, USA), $C3^{-/-}$ mice (as described previously; ref. 25) or $C5^{-/-}$ mice (congenic strains *B10.D2/oSn* and *B10.D2/nSn;* Jackson Laboratories) were used. All mice were on a *C57BL/6* background. Intraperitoneal ketamine (100 mg/kg body weight) (Fort Dodge Animal Health, Fort Dodge, IA, USA) was used for anesthesia and intraperitoneal xylazine (13 mg/kg body weight) (Bayer Corp., Shawnee Mission, KS, USA) for sedation. Experimental sepsis was induced by CLP as described previously (26). Briefly, after abdominal midline incision, the cecum was exposed, ligated, and punctured through and through with a 19-gauge needle, and a small portion of feces was pressed out to ensure persistence of the punctures. After repositioning of the bowel, the abdomen was closed in layers using 6-0 surgical sutures (Ethicon Inc., Somerville, NJ, USA) and metallic clips. Before and after the surgery, animals had unrestricted access to food and water. To induce intermediate-grade CLP, ~50% of the cecum was ligated, whereas, during high-grade CLP, 75% of the cecum was ligated. Kaplan-Meier survival curves were analyzed for WT, $C3^{-/-}$, and $C5^{-/-}$ mice (n=15/group).

Plasma collection

At given time points after CLP, whole blood was collected by cardiac puncture into syringes containing anticoagulant citrate dextrose (ACD; Baxter, Deerfield, IL, USA) in a 9:1 ratio. Samples were centrifuged (2500 rpm, 10 min, 4°C); plasma was obtained and immediately stored at −80°C until further analysis.

Cytokine/chemokine measurements

Plasma was harvested as described above. The following mouse ELISA kits were used: IL-1β, IL-6, MCP-1, MIP-2, TNF-α, IL-10, and sTNF RII (all R&D Systems, Minneapolis, MN, USA). Measurements were performed according to the manufacturer's protocols. Antibodies for IL-1Ra were obtained from R&D Systems, and an ELISA was established using the same protocol as for the commercially available kits. For quantification of HMGB1 in mouse plasma, a commercially available ELISA assay was used (Shino-Test Corporation, Kanagawa, Japan).

Determination of colony-forming units (CFUs) in blood

Blood was obtained by cardiac puncture and was drawn into syringes containing 10% citrate. Following appropriate dilution in 0.9% saline, 100 μl samples were plated in duplicates on 5% sheep blood agar (Remel, Lenexa, KS, USA) and incubated for 24 h at 37°C under aerobic conditions. CFUs were then determined and multiplied by the dilution factor.

Assessment of neutrophil counts

Plasma was obtained by cardiac puncture and diluted 1:10 in PBS containing 5% BSA. Samples were then analyzed for neutrophil concentrations using a Coulter counter (ADVIA 120 Hematology System; Bayer, Norwood, MA, USA).

Depletion of the complement component 6 (C6)

C6 was depleted as described previously (27). Briefly, WT mice were injected with 40 μg of polyclonal anti-mouse C6 (Cell Sciences, Canton, MA, USA) intraperitoneally daily for 5 consecutive days.

ELISA measurements of mouse C5a

Plasma levels of mouse C5a were determined as described previously (28–30). As capture antibody, purified monoclonal anti-mouse C5a IgG (5 μg/ml, BD Pharmingen, San Diego, CA, USA) was used. After blocking, diluted plasma samples and recombinant mouse C5a (as standards) were applied, and biotinylated monoclonal anti-mouse C5a antibody (500 ng/ml; BD Pharmingen) was used for detection.

Serum collection

Whole blood was collected by cardiac puncture and pipetted into microtubes specially prepared for serum collection (Sarstedt, Nümbrecht, Germany). Serum was obtained according to the manufacturer's protocol and stored at −80°C until further analysis.

Hemolytic complement assay (CH-50)

Hemolytic activity of mouse serum was assessed as described previously (31). Briefly, rabbit red blood cells (Colorado Serum Company, Denver, CO, USA) were sensitized with goat antiserum to rabbit red blood cells (MP Biomedicals, Solon, OH, USA) and exposed at 30°C for 60 min to serial dilutions of serum samples in gelatin veronal buffer containing Ca^{2+}/Mg^{2+} (Sigma, St. Louis, MO, USA). Following a centrifugation step (2500 *g,* 5 min), absorbance of the supernatant fluid was measured at 405 nm, and concentration inducing 50% of hemolysis (CH-50) was determined. For some experiments, serum was heat inactivated by 30 min incubation at 56°C.

Measurement of thrombin activity

We performed the thrombin assay as described previously (30). Briefly, plasma was obtained at indicated time points by cardiac puncture. S-2238 (H-D-phenylalanyl-L-pipecolyl-L-arginine-*p*-nitroaniline dihydrochloride; DiaPharma, West Chester, OH, USA) was used as a substrate. After thrombin cleavage of the substrate, the amount of *p*-nitroaniline dihydrochloride (pNA) formed was measured by absorbance at 405 nm in a kinetic profile. A standard curve was generated using known amounts of thrombin (Sigma-Aldrich), and corresponding pNA formed and the activity present in the sample were determined from the standard curve. We performed the assay in the presence of aprotinin (Roche, Indianapolis, IN, USA) to inhibit proteinase activities other than that of thrombin.

Statistical analysis

All values are expressed as means ± SE. Data were analyzed with a 1-way ANOVA, and individual group means were then compared with a Student-Newman-Keuls test. Differences were considered significant when $P \leq 0.05$. For analysis of survival curves, log-rank and −2 log-rank tests were used. Outcomes in different animals were compared using χ^2 and Fisher's exact tests.

RESULTS

Absence of C3 decreases survival whereas lack of C5 does not alter survival during experimental sepsis

Intermediate-grade CLP was employed, in which 50% of the cecum was ligated, resulting in a 40–50% survival over a 9 day period. Results derived from intermediate-grade sepsis are described in **Fig. 1*A***. Survival by day 9 was 46% in WT mice, 14% in $C3^{-/-}$ littermates, and 43% in $C5^{-/-}$ animals.

A recent report illustrated that there are striking differences between a moderate and severe version of CLP (32). Therefore, we also employed the more severe form of CLP. In this high-grade version of CLP, 75% of the cecum was ligated, resulting in 100% lethality by day 3. As illustrated in Fig. 1*B*, all animals succumbed to CLP within 54 h. Despite this lack of difference in the overall survival between $C5^{-/-}$, $C3^{-/-}$, and WT animals, $C3^{-/-}$ mice seemed to be the most susceptible to the lethal consequences of sepsis. In summary, in intermediate-grade as well as high-grade sepsis, no significant difference was seen in survival between WT mice and $C5^{-/-}$ mice, whereas $C3^{-/-}$ were more susceptible to CLP challenge.

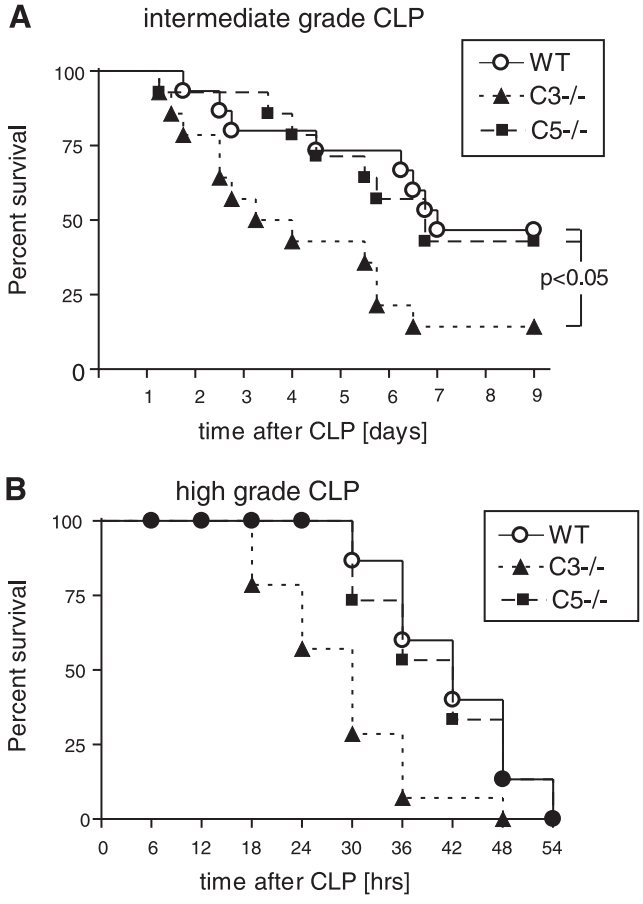

Figure 1. Survival of WT, $C3^{-/-}$, and $C5^{-/-}$ mice following CLP. Mice were subjected to CLP. Kaplan-Meier survival curves were analyzed for WT, $C3^{-/-}$, and $C5^{-/-}$ mice (n=15 per group and condition). Intermediate-grade CLP (*A*) was initiated by ligation of 50% of the cecum, whereas during high-grade CLP (*B*), 75% of the cecum was ligated.

TABLE 1. *Plasmatic inflammatory mediators following CLP*

Mediator	Change (%)	
	$C3^{-/-}$	$C5^{-/-}$
IL-1β	−90 ± 6*	−89 ± 5*
IL-6	−53 ± 13*	−60 ± 11*
MCP-1	−65 ± 13*	−36 ± 9*
MIP-2	−37 ± 8*	−53 ± 16*
TNF-α	+11 ± 26	+64 ± 30
HMGB-1	−6 ± 23	−15 ± 29
IL-1Ra	+811 ± 141*	−24 ± 37
IL-10	+64 ± 85	−72 ± 11*
sTNF RII	+119 ± 12*	+2 ± 5

Data presented as mean ± SE; change vs. 24 h CLP in WT mice. Negative and positive control values (pg/ml): IL-1β: 35 ± 1, 882 ± 200; TNFα: 19 ± 2, 147 ± 37; IL-10: 10 ± 1, 217 ± 56; IL-6: 240 ± 10, 603 ± 73; MCP-1: 40 ± 10, 2300 ± 300; MIP-2: 860 ± 30, 66,500 ± 2700; HMGB-1: 4700 ± 1700, 25,300 ± 6900; IL-1Ra: 480 ± 10, 2100 ± 600; sTNF RII: 3000 ± 200, 9400 ± 400. *$P < 0.05$ *vs.* 24 h CLP in WT mice.

C3 and C5 deficiency diminishes plasma levels of proinflammatory cytokines and chemokines during CLP-induced sepsis

WT, $C3^{-/-}$, and $C5^{-/-}$ animals were subjected to intermediate-grade CLP, and blood samples were collected by cardiac puncture 24 h after CLP because cytokines and chemokines have been shown to peak 24 h after initiation of CLP sepsis (unpublished data). Plasma was analyzed by ELISA. **Table 1** displays changes (as percentage) in mediator levels obtained from septic $C3^{-/-}$ and $C5^{-/-}$ mice in comparison to septic WT littermates. With the exception of TNF-α and HMGB1, the levels of proinflammatory mediators (IL-1β, IL-6, MCP-1, and MIP-2) were significantly decreased in C3- and C5-deficient animals during CLP-induced sepsis. The plasma levels of the anti-inflammatory mediators IL-1 receptor antagonist (IL-1Ra), soluble TNF-α receptor II (sTNFα RII), and IL-10 were significantly elevated in C3-deficient mice, whereas IL-1Ra and IL-10 levels were significantly decreased in animals lacking C5. In summary, the inflammatory response as indicated by the systemic release of cytokines/chemokines is widely dampened in the absence of C3 or C5. However, the reduction of plasma mediators during sepsis is not reflected in survival rates.

Lack of C5 and C3 greatly impairs bacterial clearance during sepsis

To evaluate whether C3- and C5-deficient animals displayed altered systemic bacterial clearance due to an inability to form the terminal MAC, we first sought to identify the peak of systemic CFUs in blood following CLP. WT mice were subjected to intermediate-grade CLP, and blood samples were obtained 0, 6, 12, 24, and 48 h after induction of CLP, plated on sheep-blood agar, and incubated for 24 h under aerobic conditions. As shown in **Fig. 2*A***, systemic CFUs peaked 24 and 48 h after CLP. Therefore, we chose 24 h post-CLP for all subsequent CFU experiments. WT, $C3^{-/-}$, and $C5^{-/-}$ animals were subjected to CLP, and blood samples obtained after 24 h were evaluated for CFUs. WT mice displayed a significant increase of CFUs following sepsis challenge (Fig. 2*B*). Septic $C3^{-/-}$ animals showed a ~4-fold increase in levels when compared to WT littermates ($P<0.01$), whereas septic $C5^{-/-}$ mice had significantly increased systemic CFUs, displaying a 400-fold increase when compared to WT littermates and a 100-fold increase over $C3^{-/-}$ mice (Fig. 2*B; P*<0.01 *vs.* WT and $C3^{-/-}$). To determine whether these significant increases in CFUs were caused by the absence of hemolytic complement activity, WT mice were C6 depleted with a neutralizing antibody (resulting in a 65% reduction of hemolytic activity; ref. 27) and then subjected to CLP. CFUs were evaluated 24 h thereafter. As demonstrated in Fig. 2*C,* C6-depleted animals exhibited significant increases in CFUs (10-fold) when compared to WT mice, suggesting that the absence of the MAC significantly increases CFUs during CLP. Blood neutrophil counts were determined in WT, $C3^{-/-}$, and $C5^{-/-}$ mice. Although there were no significant changes of neutrophil numbers between WT, $C3^{-/-}$, and $C5^{-/-}$ negative control animals, septic WT and $C3^{-/-}$ mice displayed statistically significant increases of blood neutrophils. In sharp contrast, $C5^{-/-}$ mice revealed severe neutropenia following CLP (Fig. 2*D*).

Finally, in plasma from WT mice obtained 24 h after CLP, significantly elevated levels of C5a (Fig. 2*E*) were seen. Interestingly, $C3^{-/-}$ mice had similarly increased levels of C5a (Fig. 2*E*), possibly triggered *via* a newly discovered complement activation pathway involving thrombin (30). As shown in **Fig. 3**, there may be a "shortcut" in activation of complement, because septic $C3^{-/-}$ mice displayed significantly increased thrombin activity when compared to WT littermates. As expected, $C5^{-/-}$ mice failed to generate C5a during sepsis with plasma levels ranging at baseline 24 h after CLP (Fig. 2*E*).

Absence of C5 but not C3 deficiency results in decreased hemolytic complement activity (CH-50)

To evaluate whether the reduced capability to form the MAC might be related to the extent of the bacterial

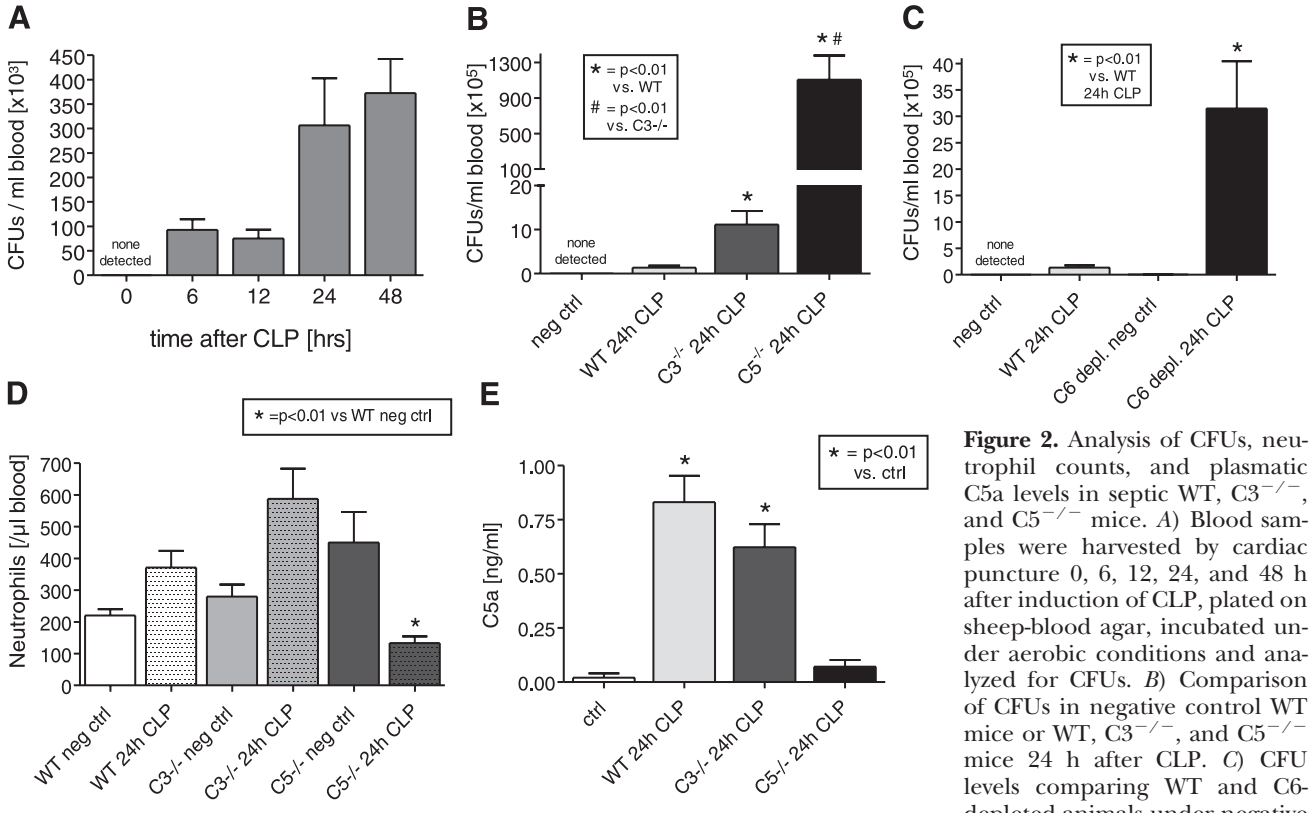

Figure 2. Analysis of CFUs, neutrophil counts, and plasmatic C5a levels in septic WT, $C3^{-/-}$, and $C5^{-/-}$ mice. *A*) Blood samples were harvested by cardiac puncture 0, 6, 12, 24, and 48 h after induction of CLP, plated on sheep-blood agar, incubated under aerobic conditions and analyzed for CFUs. *B*) Comparison of CFUs in negative control WT mice or WT, $C3^{-/-}$, and $C5^{-/-}$ mice 24 h after CLP. *C*) CFU levels comparing WT and C6-depleted animals under negative control conditions or 24 h after CLP. *D*) Neutrophil counts were obtained from WT, $C3^{-/-}$, and $C5^{-/-}$ mice under negative control conditions or 24 h after initiation of CLP. *E*) Plasma levels of C5a in WT, $C3^{-/-}$, and $C5^{-/-}$ mice following CLP as determined by ELISA.

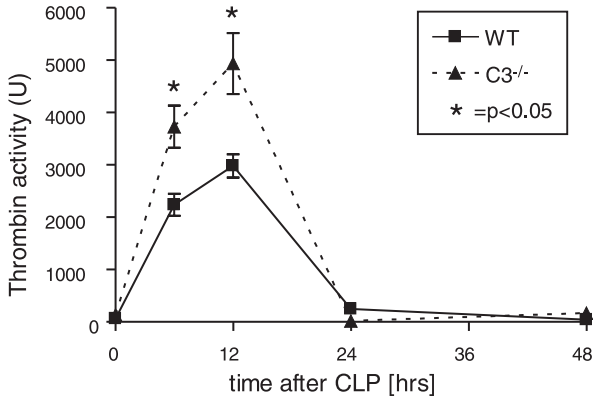

Figure 3. Analysis of thrombin activity in WT and $C3^{-/-}$ mice following CLP. Plasma was obtained from $C3^{-/-}$ or WT mice by cardiac puncture and subjected to analysis for thrombin activity as a function of time after CLP.

load in the blood compartment after CLP, sera from WT, $C3^{-/-}$, $C5^{-/-}$, and C6-depleted mice were analyzed for their hemolytic activity by CH-50. This assay determines the exact serum dilution, which lyses 50% of sensitized rabbit red blood cells. As expected, WT mice displayed a robust left shift of the CH-50 curve 24 h after CLP, indicating severe complement consumption (**Fig. 4*A***). As expected, hemolytic activity was severely impaired in $C3^{-/-}$ negative control mice (Fig. 4*B*). However, following CLP, serum from $C3^{-/-}$ mice exhibited a right shift (Fig. 4*B*), indicating that septic $C3^{-/-}$ mice are able to produce hemolytic activity, which is in line with the observation that $C3^{-/-}$ mice generated C5a after CLP (Fig. 2*E*). To exclude assay-dependent variation, we heat-inactivated sera obtained from septic $C3^{-/-}$ mice and subjected them to CH-50 analysis (Fig. 4*B*). As expected, no hemolytic activity was found. In contrast, $C5^{-/-}$ animals failed to display hemolytic complement activity under either negative control or septic conditions (Fig. 4*C*). In additional experiments, CH-50 was assessed using sera from C6-depleted mice. Although preIgG-treated mice demonstrated a typical WT CH-50 curve, C6-depleted animals showed a significant left shift of the CH-50 curve (Fig. 4*D*), indicating that the hemolytic complement ability in C6-depleted mice was significantly reduced. Heat-inactivated serum obtained from C6-depleted animals failed to show any hemolytic activity (Fig. 4*D*). In a second set of experiments, C6-depleted WT mice subjected to CLP showed a further left shift in the CH-50 when compared to non-CLP C6-depleted mice (Fig. 4*E*). Figure 4*F* summarizes the actual CH-50 values as means ± SE and displays significant differences between CH-50 curves. Most strikingly, $C3^{-/-}$ mice failed to generate hemolytic complement activity under negative control conditions but were able to create significant hemolysis following CLP-induced sepsis, perhaps because of increased plasma activity of thrombin (Fig. 3).

DISCUSSION

Recent research clearly indicates that the sepsis syndrome involves a complex and intricate interplay of

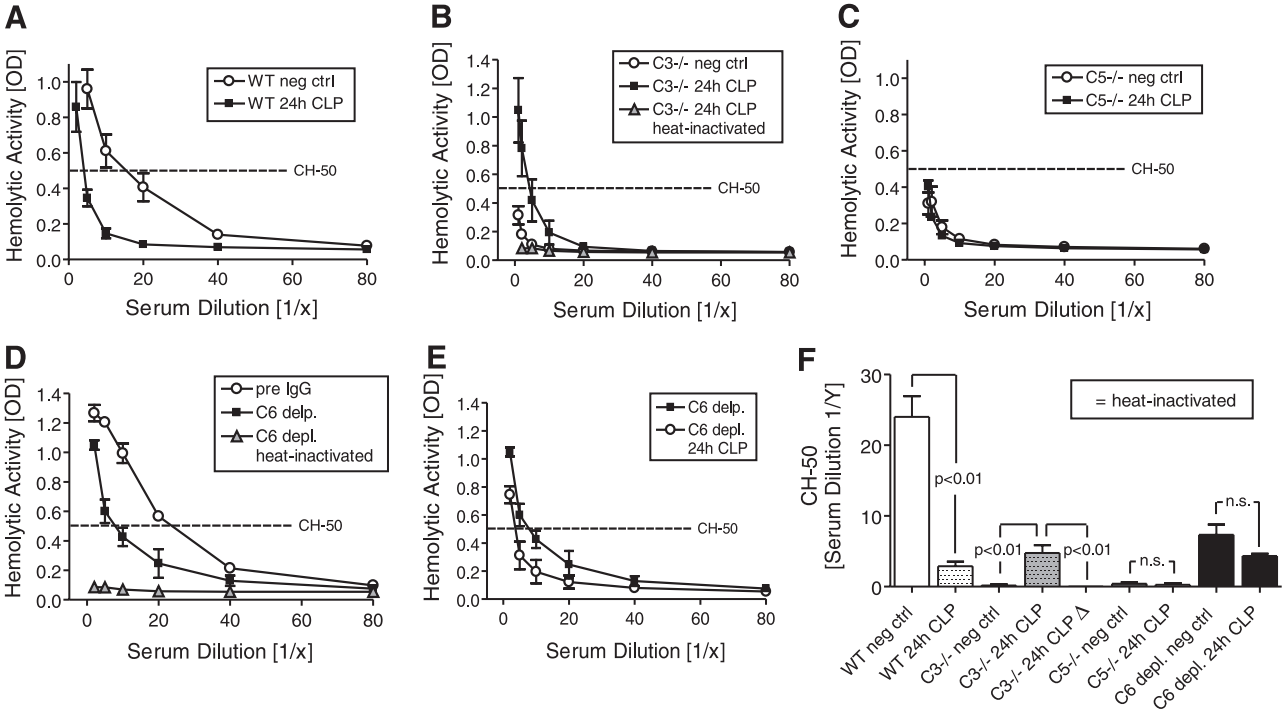

Figure 4. Analysis of serum complement hemolytic activity (CH-50) in healthy and septic WT, C6-depleted, $C3^{-/-}$, and $C5^{-/-}$ mice. Sera were obtained by cardiac puncture and subjected to CH-50 analysis as described in the Methods. *A–C*) Analysis of wild-type (*A*), $C3^{-/-}$ (*B*), or $C5^{-/-}$ (*C*) animals under negative control and septic conditions. *D*) Effect of C6-depleting antibody or isotype IgG on CH-50 values. *E*) Comparison of CH-50 values of C6-depleted mice under negative control conditions or following CLP. *F*) Actual CH-50 values of the curves *A–E*, including display of statistical differences.

various endogenous cascade systems. In the present study, we subjected WT mice and mice deficient in complement C3 or C5 to experimental sepsis in order to further define the role of these complement components during CLP.

Surprisingly, $C5^{-/-}$ mice displayed similar survival when compared to WT animals, although excessive levels of the cleavage product of C5, C5a, play a detrimental role during sepsis (12, 20–22). We found in the present study that, during experimental sepsis, absence of C5 or C3 greatly enhanced bacteremia (~400- and 4-fold, respectively) in comparison to WT animals (Fig. 2*B*). Once microbes enter the bloodstream, various components of the activated complement system, such as C1q, C3b/iC3b, C4b, and mannose-binding lectin, rapidly opsonize these pathogens (33). This opsonization of pathogens helps bind bacteria to the complement receptors 1 (CR1) and 3 (CR3, CD11b/CD18) expressed on leukocytes (34), inducing phagocytosis and oxidative burst. C3, C5, C5a, and C5aR have been shown to be critically involved in the up-regulation of the phagocytosis receptor CD11b together with induction of the oxidative burst, because blockade of any of these components leads to pronounced inhibition of phagocytosis and oxidative burst (35, 36). Thus, the massive bacteremia observed in the genetic absence of C5 or C3 during sepsis (Fig. 2*B*) might be related to the inability of these knockout animals to either induce phagocytosis or initiate the oxidative burst.

Although negative control $C3^{-/-}$ animals failed to show hemolytic complement activity (CH-50), sera from septic $C3^{-/-}$ mice showed a significant right shift of the CH-50 curve, indicating a significantly increased capability of complement-mediated hemolysis under septic conditions. When these septic sera obtained from $C3^{-/-}$ mice were heat inactivated, this increase in hemolytic activity was abolished (Fig. 4*B*). The significant differences of blood-borne bacteria (Fig. 2*B*) between $C3^{-/-}$ mice and $C5^{-/-}$ littermates (~100-fold) might be explained by the observation that septic $C3^{-/-}$ mice showed full production of C5a in plasma (similar to WT mice, Fig. 2*E*). This is consistent with a recent report describing a novel pathway of complement activation, where, in the absence of C3, thrombin can function as a C5-convertase, directly cleaving C5 into C5b and biologically active C5a (30). As shown in Fig. 3, C3-deficient mice after CLP displayed significantly increased thrombin activity when compared to WT animals, indicating that this "shortcut" complement activation pathway is fully functional during CLP sepsis. Thus, $C3^{-/-}$ mice not only can generate C5a *via* this novel pathway, but they can also produce C5b for MAC assembly. This is underscored by our data in Fig. 4.

In addition, the hemolytic complement activity (CH-50) in $C5^{-/-}$ mice was virtually absent (Fig. 4*C*), suggesting that the tremendous bacterial load in septic $C5^{-/-}$ mice may be further accentuated by the inability to generate C5b, a crucial component of the terminal MAC. To confirm the role of hemolytic complement activity in sepsis, we depleted WT mice of another protein necessary for MAC assembly, C6, and subjected these animals to CH-50 and CFU analysis. As expected, C6-depleted mice displayed significantly reduced CH-50 values (Fig. 4*D*) and exhibited significant increases in blood-borne bacteria (Fig. 2*C*). In contrast to our findings, a previous study described that antibody-induced blockade of C5 or absence of C6 protected rats from sepsis mortality and attenuated the bacterial load in liver and spleen after CLP (37). The authors concluded that the MAC does not contribute to bacterial clearance during sepsis, although supportive evidence was not provided. Furthermore, as suggested by the authors, it is possible that the $C6^{-/-}$ rats have developed unknown compensatory adaptive responses.

However, many findings obtained from murine sepsis models have failed to be translatable into clinical efficacy, raising the question as to how valid and useful widely used septic animal models are (38). It is generally agreed that, of all sepsis models (such as live bacteria, endotoxin, LPS challenge, and CLP), the CLP animal model seems to most closely resemble the immunopathologic responses of human sepsis, including hypothermia, decreased activity, weight loss, reduced hemoglobin, decreased platelet, lymphocyte, monocyte and neutrophil counts, as well as the cytokine/chemokine profile (39–41). Interestingly, we found similarities in human case reports about patients with hereditary deficiency of C3 or C5, who suffer from recurrent infectious episodes. A C3-deficient patient showed reduced CH-50 values (42), but normal complement-mediated passive hemolysis (43), whereas in C5-deficient patients, MAC-mediated hemolysis failed to occur in either test (43, 44).

The greatly attenuated surge in proinflammatory mediators in septic C3- or C5-deficient mice is a surprising observation, challenging the pathophysiological role of the previously described "cytokine-chemokine storm" as a centerpiece of the systemic inflammatory response syndrome and sepsis (45, 46). Based on the cytokine-chemokine storm concept, several clinical trials have tried to block proinflammatory cytokines (such as TNF or IL-1) during sepsis, but none of these trials have been successful (1). Thus, there is now increasing evidence that the cytokine storm is less mechanistic for sepsis and sepsis-related deaths, but might rather be an endogenous secondary response to overwhelming inflammatory insult (47). Although $C3^{-/-}$ and $C5^{-/-}$ mice in our studies showed an ameliorated mediator response, they ultimately encountered identical lethality in CLP-induced sepsis. Thus, as a matter of debate, the cytokine-chemokine storm might not be linked to septic lethality, but rather represents a resulting inflammatory reaction. In contrast to the broad reduction of proinflammatory mediators in $C3^{-/-}$ and $C5^{-/-}$ mice, 2 anti-inflammatory mediators (IL-10 and IL-1Ra) were found to be differently regulated in these knockout mice. $C5^{-/-}$ mice displayed significant reductions of

the anti-inflammatory mediator IL-10 in contrast to $C3^{-/-}$ or WT mice (Table 1). It has been shown that exogenous IL-10 reduces mortality in experimental sepsis and that IL-$10^{-/-}$ mice display much greater susceptibility to endotoxemia (48–50). Because $C5^{-/-}$ mice displayed significantly reduced levels of IL-10 as well as severe bacteremia, it is tempting to speculate whether there is a link between these two observations.

Unexpectedly, septic $C3^{-/-}$ mice exhibited a robust increase of plasmatic IL-1Ra when compared to WT or $C5^{-/-}$ littermates (Table 1). In line with our findings in $C3^{-/-}$ mice (high plasma levels of IL-1Ra, increased bacteremia, reduced CLP survival), treatment of WT mice with IL-1Ra before CLP resulted in decreased proinflammatory cytokine response, augmented bacterial load, and increased mortality (51). Moreover, following endotoxemia challenge in mice, administration of recombinant IL-1Ra was reported to partially block increased mucosal production of C3 in the small intestine (52). Furthermore, when mice were injected with IL-1Ra, reduced serum levels of C3 was found in an animal model of myasthenia gravis (53). However, the association between C3 and IL-1Ra has yet to be fully understood.

Collectively, animals seem to succumb to sepsis because of very different pathophysiological causes. Septic WT mice encounter not only mild bacteremia (Fig. 2*A*, B) and an exacerbated inflammatory mediator response (54), but also excessive levels of C5a, resulting in immunoparalysis of neutrophils (11). C3-deficient mice seem to struggle with a lethal combination of impaired bacterial clearance because of reduced phagocytotic activity, impaired oxidative burst (35, 36), and diminished hemolytic complement activity, as well as C5a-induced immunoparalysis of neutrophils. Although $C5^{-/-}$ mice cannot produce C5a during sepsis, this putative "advantage" seems to be traded off for an overshooting, uncontrollable bacteremia because of impaired bacterial clearance (35, 36) and deficient bactericidal complement activity.

In conclusion, our data corroborate the importance of the complement system in the pathogenesis of sepsis. Bactericidal, lytic complement activity seems indispensable for controlling murine systemic bacterial infections. In line with a previous report in a meningococcal model of sepsis (55), our data imply that blockade of C5a or its receptors rather than C5 should be pursued for complement inhibition in sepsis, because only this strategy allows formation of the MAC, while at the same time C5a is prevented from revealing its harmful consequences. FJ

The authors thank Beverly Schumann and Sue Scott for the preparation of this manuscript. This work was supported by U.S. National Institutes of Health grants GM29507, GM61656, and HL-31963 (to P.A.W.) and Deutsche Forschungsgemeinschaft grants DFG HU 823/2–1, HU 823/2–2, and DFG HU 823/2–3 (to M.H.-L.).

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Received for publication March 20, 2008.
Accepted for publication May 22, 2008.