Protective effects of anti-C5a peptide antibodies in experimental sepsis[1]

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SPECIFIC AIMS

Our recent studies of experimental sepsis, induced by cecal ligation and puncture (CLP) in rats, showed that anti-C5a antibody protects against the lethal effects of sepsis. The main aim of the current study was to obtain maximally protective antibodies to rat C5a that would protect in the CLP model and to determine to what extent delayed treatment with anti-C5a would still afford protection against sepsis-induced lethality.

PRINCIPAL FINDINGS

1. Antibodies to different peptide regions of rat C5a have differential inhibitory activities on the functional (chemotactic) activity of rat C5a

Rabbit polyclonal antibodies were developed to the following peptide regions of rat C5a: amino-terminal region (A), residues 1–16; middle region (M), residues 17–36; and the carboxyl-terminal region (C), residues 58–77 (**Fig. 1**). With the use of rat neutrophils, the chemotactic activity of rat C5a was significantly inhibited by antibodies with the following descending rank order: anti-C > anti-M >> anti-A. The antibodies by themselves did not evoke a significant response in rat neutrophils.

2. The anti-C5a antibodies do not inhibit hemolytic activity of rat serum

To assess to what extent anti-C5a preparations (anti-C5a M or C) might interfere with the hemolytic activity of rat serum (implying a blocking effect on the parent molecule, C5) and assembly of C5b-9 (membrane attack complex), whole hemolytic activity was measured in presence or absence of these antibodies. There were no significant differences in hemolysis of sensitized sheep erythrocytes in the presence of fresh rat serum containing preimmune IgG or anti-C5a M or C antibodies, which suggests that these protective antibodies do not block activation of the complement system.

3. Differential protective effects of C5a antibodies during experimental sepsis

Our original studies of experimental sepsis (cecal ligation and puncture in rats) showed that anti-C5a antibodies could protect against the lethal effects of sepsis (Czermak et al. *Nature Medicine* **5,** 788–92, 1999). To determine the targets on rat C5a that are most effective for blockade, antibodies against A, M, and C peptides of rat C5a (Fig. 1) or preimmune IgG were administered immediately after the CLP procedure. The relative protective efficacies (10-day survival rates of animals) of anti-C5a preparations (in descending order of efficacy) were anti-C > anti-M >> anti-A. Dose-dependent protective effects against the lethal complications of sepsis in CLP animals was found when antibodies to the M or C regions was used.

4. Anti-C5a M or C antibodies confer protective effects during sepsis even after onset of symptoms of sepsis

To determine if delayed infusion of antibodies could still confer protective effects *in vivo* after CLP, 600 μg of the most protective antibodies (anti-C5a M or C antibodies) were intravenously infused 6, 12, or 24 h after onset of CLP. When infused immediately after CLP (0 h, **Fig. 2*A***), anti-C5a M or C-treated groups exhibited highly protective effects (leading to 80% survival). When infusion was delayed until 6 h after CLP (Fig. 2*B*), a time when clinical symptoms of sepsis were evident, both antibodies still conferred significant protection. When infused 12 h after CLP, the majority of animals exhibited typical clinical signs of sepsis: decreased physical activity, piloerection, cessation of grooming behavior, glazed eyes with crusting exudates, tachypnea, and reduced urinary output. Under these conditions, infusion of anti-C5a M and anti-C5a C still

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.00–0653fje; to cite this article, use *FASEB J.* (January 19, 2001) 10.1096/fj.00–0653fje

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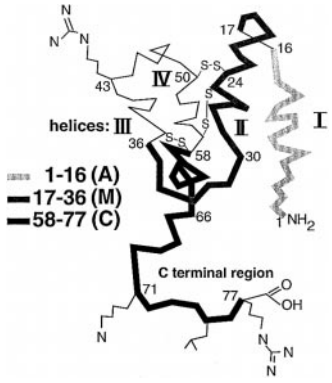

Figure 1. Location of selected peptide regions for antibody targeting (A = amino-terminal, M = middle peptide region and C = carboxy-terminal region), based on the antigenic index and predicted molecular structure of C5a.

significantly improved survival at 10 days (37% and 46%, respectively) as compared with the preimmune IgG-treated group (12%) (Fig. 2*C*). The protective effects of the anti-C5a M and C peptide antibodies were lost, if infusion was delayed until 24 h after CLP (data not shown). Although the anti-C5a protective effects were diminished when delayed until late symptoms of sepsis, these data suggest that delayed infusion of anti-C5a, even in the presence of overt clinical signs of sepsis, confers protective effects.

CONCLUSIONS

Abundant evidence suggests that homeostatic control of the inflammatory system has been lost during sepsis, as is reflected by cytokine and chemokine appearance and unremitting data of complement activation. In experimental sepsis induced by CLP, we have recently demonstrated in rats that extensive complement activation occurs, which leads to C5a deposition on blood neutrophils and a loss of the respiratory burst in these cells and results in defective oxygen-dependent bacterial killing and a high mortality rate. A rabbit polyclonal antibody to the middle peptide region (M) of rat C5a caused protective effects during sepsis. To establish the most protective anti-C5a antibody preparation as well as the dose of anti-C5a that provides optimal protection against the lethal effects of sepsis, we raised polyclonal antibodies against different peptide regions (A,M,C) of the rat C5a molecule (Fig. 1). IgG antibodies targeting

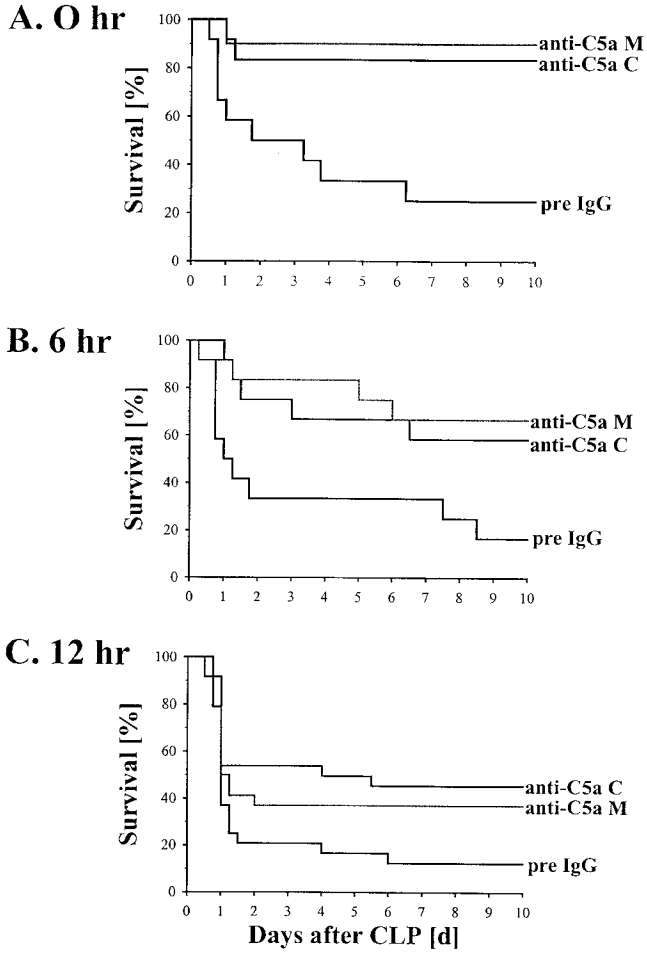

Figure 2. Ten-day survival rates of rats with sepsis; dependency on the time of intravenous infusion of anti-C5a antibodies, which were raised against the middle peptide region, residues 17–36 (anti-C5a M) or carboxyl-terminal region residues 58–77 (anti-C5a C). Animals received either 600 μg of preimmune IgG or 600 μg anti-C5a M or C antibody immediately after onset of CLP (*A*), 6 h after CLP (*B*), or 12 h after CLP (*C*).

the amino-terminal residues 1–16 (A region of C5a) did not prevent the lethal consequences of experimental sepsis, whereas antibodies against the middle core residues 17–36 of the M region or against the carboxyl-terminal residues 58–77 (C region) greatly improved

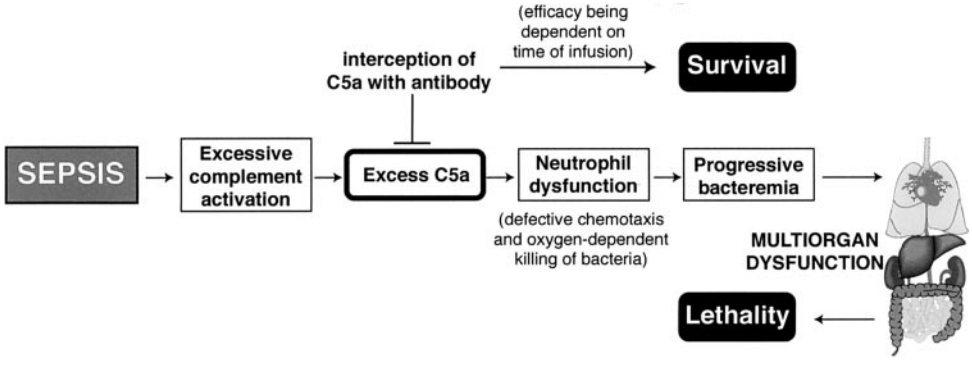

Figure 3. Schematic diagram of protective effects of anti-C5a administration from lethal consequences of sepsis.

survival of CLP-rats in a dose dependent manner. Although the amino-terminal region of C5a contributes to the full potency of the intact C5a molecule and serves to stabilize a biologically favorable C5a conformation, blocking antibody to the amino terminal region (residues 1–16) was not an effective target, as defined by lack of protective effects in CLP-induced sepsis.

In the present study, antibodies against different peptide regions of C5a suppressed the chemotactic activity of rat neutrophils to rat C5a in a descending order of potency: CMA, which mirrors the protective *in vivo* effects of these antibodies. Because both recruitment and activation of neutrophils during sepsis are essential for local (e.g., peritoneal) remote organ (e.g., lung, liver, kidney) injury, effective blockade of anti-C5a antibodies preserves vital protective functional responses of neutrophils, especially the ability of these cells to activate NADPH oxidase, result in less organ injury and improved survival.

We have shown previously that injection of anti-C5a immediately after sepsis-induction provides protection against its detrimental effects. However, the present data suggest that even after a considerable delay (6–12 h) in administering antibodies, which were targeted against the M or C terminal regions of C5a, there was still a significant improvement of survival (Fig. 2 and **Fig. 3**). In the CLP model, relatively rapid development of polymicrobial sepsis occurs within the first 12 h resulting in lethal complications. The early events are characterized by a hyperdynamic phase (2–10 h after CLP), followed by a hypodynamic phase (after 16 h). The hyperdynamic phase of sepsis is compressed in the CLP model in rats into a rather brief period (16 h) relative to that observed in humans, which often appears to extend over a three-day period. The data in the rat model suggest that anti-C5a intervention is effective during the hyperdynamic phase, although the protective effects diminish with time (Fig. 2). These data suggest that, in humans with sepsis, there may be a 'window' of time of several days during which such an intervention may be useful. FJ