

C5a, a Therapeutic Target in Sepsis

Ren-Feng Guo* and Peter A. Ward

University of Michigan Medical School, Dept. of Pathology, Ann Arbor, Michigan 48109-0602, USA

Received: May 26, 2005; Accepted: July 14, 2005; Revised: July 27, 2005

Abstract: The complement activation product, C5a, is a potent inflammatory peptide with a broad spectrum of biological functions. Plasma levels of C5a are increased in sepsis, accompanied by increased content of C5a receptor (C5aR) in various organs. In the mouse and rat models of sepsis (cecal ligation and puncture, CLP), C5a blockade by anti-C5a antibody, anti-C5aR antibody or use of a C5aR antagonist (C5aRa) significantly improved survival in CLP animals. C5a blockade in sepsis attenuated the systemic inflammatory response syndrome (SIRS) by reducing plasma levels of IL-6 and decreasing bacteria counts in blood and organs. Anti-C5a treatment in CLP rodents markedly attenuated sepsis-induced defects in the coagulation/fibrinolytic system, while liver and kidney functions were remarkably preserved in contrast to CLP animals not receiving anti-C5a in which multi-organ failure occurs. In CLP rats treated with anti-C5a, thymus atrophy was diminished and thymocyte apoptosis was inhibited. Defective neutrophil functions (chemotaxis, phagocytosis, respiratory burst) caused by sepsis were significantly improved in CLP rats treated with anti-C5a. These data suggest during CLP-induced sepsis C5a has very harmful consequences and that its blockade might be a promising therapeutic strategy for the treatment of humans with sepsis. This review will summarize the beneficial effects of anti-C5a treatment in the rodent model of sepsis and will introduce the most recent patents on this line of research.

Key words: Sepsis, C5a, C5a receptor, neutrophils, innate immunity.

INTRODUCTION

Sepsis is a major clinical problem in humans. It affects more than 750,000 people annually, with a mortality rate ranging from 20-60%, despite intensive supportive therapy [1,2]. Sepsis is often accompanied by a systemic inflammatory response syndrome (SIRS), which describes a widespread inflammatory reaction that occurs following a variety of insults such as infection, pancreatitis, trauma, burns, etc. At the early onset of sepsis, the inflammatory system appears to be hyperactive, and inflammatory cells, especially macrophages, generate a large amount of inflammatory mediators, constituting the so-called "inflammatory cytokine storm" of sepsis. Well-described in animal models of sepsis are the many inflammatory mediators such as TNF- α and IL-1 occurring early in the development of sepsis [3-5]. However, most human clinical trials targeting blockade of these specific inflammatory mediators (e.g. TNF, IL-1) have failed [6]. Recently, activated protein C (APC) was approved by the Food and Drug Administration for treatment of sepsis in human adults [7]. APC has a variety of blocking effects, including inhibition of clotting factors Va and VIIa and plasminogen activator inhibitor-1. The success with APC has provided some hope for a newer and more effective approach that would be therapeutically useful in the treatment of human sepsis.

It is well known that sepsis triggers pathways of complement activation. Excessive activation of complement systems leads to high levels of complement activation products in the blood, including the potent pro-inflammatory

peptide C5a, which is associated with a severely compromised host defense system and widespread tissue injury. In this article, we provide an overview for the role of C5a in sepsis and potential therapeutic merits of blockade of C5a or C5aR in sepsis. Most data presented in this review are covered by US patent US6866845 and WO04043223A2.

BLOCKADE OF C5A OR C5AR IMPROVES SURVIVAL IN SEPSIS

During sepsis, high plasma levels of C3a, C4a, and C5a have been found during sepsis [8-11]. Alternative and classical pathways of complement activation are thought to be the major sources of complement activation products during sepsis. It would not be surprising that the lectin pathway also plays an important role, given the fact that the O-antigen region of LPS can cause activation of this pathway [12]. The assumption here is that LPS is a significant causative factor in sepsis. Therefore, all three complement activation pathways may be involved complement activation during sepsis, leading to the appearance in serum/plasma of complement activation products. Among the complement activation products, C5a is an exceptionally potent inflammatory peptide with a broad spectrum of biological functions. Regarding neutrophils, C5a induces their chemoattraction [13], an oxidative burst (consumption of O₂ and production of H₂O₂) [14], enhancement of phagocytosis and release of granule enzymes [15,16]. C5a is also a strong vasodilator [17]. More recently, the C5a receptor (C5aR) has been found to be up-regulated in major organs (lungs, liver, kidneys, heart) during sepsis [18], although the functional significance of this is not currently known.

Blockade of C5a in experimental sepsis or sepsis-like conditions has been proven to be beneficial. Infusion of

*Address correspondence to this author at the Department of Pathology, The University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, Michigan 48109-0602, USA; Tel: (734) 615-7766; Fax: (734) 764-4308; E-mail: grf@med.umich.edu; pward@umich.edu

monoclonal antibody against the C-terminal region of C5a has been shown to improve hemodynamic parameters in pigs infused with LPS or live *E. coli* [19]. In a primate (*Macaca fascicularis*) model of sepsis following a 30-minute infusion of liver *E. coli* (10^{10} /kg), administration of rabbit anti-human C5a polyclonal antibody improved animal survival rates [20]. Septic animals not treated with anti-C5a antibody had 75% mortality, decreased oxygenation, severe pulmonary edema, and profound hypotension. Septic primates treated with anti-C5a antibodies had improved physiological parameters; no increased extravascular lung water was found. Statistical analysis based on survival rate was not meaningful because of the very small number ($n=4$) of monkeys in each group. Convincing evidence for improved survival in CLP rats treated with anti-C5a was achieved by using a rat model of sepsis induced by cecal ligation and puncture (CLP) [21]. This model closely mimics the pathophysiology of sepsis in humans and has been widely used in sepsis studies. As shown in Fig. 1, the duration of observations in this model was 7-10 days. In the CLP group that received control rabbit IgG (400 μ g) at time 0, the survival rate was 66.7% (14 out of 21) 24 hours after CLP, and 2 animals out of 21 (9.5%) survived at day 8. In the CLP group receiving rabbit IgG antibody against C5a (400 μ g), the survival rate was substantially improved (Fig. 1A). By day 5, 50% of rats survived, and there were no further death in next 5 days. Comparison of the 10-day survival in the CLP group receiving control IgG to that of the group receiving IgG antibody against C5a produced p values of 0.012. As might be expected, depletion of complement (especially C3) prior to CLP greatly reduced survival (Fig. 1A), implying C3-dependent functions in innate immune defenses. Blockade of C5a receptor (C5aR) with anti-C5aR was also shown a promising therapeutic trend [18]. All mice subjected to CLP died within 7 days in the absence of a protective intervention. In the group treated with anti-C5a, the overall survival was 70% at day 7. In CLP mice treated with anti-C5aR at the initiation of CLP, the survival was similar (76%) within the first 3 days, and thereafter there were no additional deaths. In the CLP group receiving anti-C5aR 6 hours after CLP, no beneficial effect could be observed, and all animals died, as in the control IgG-injected group (Fig. 1B). Similar beneficial outcomes could be achieved by the treatment with the C5aR antagonist (C5aRa), a synthetic cyclic hexapeptide AcF[OpdChaWR] [22]. At a dose of 1-3 mg/kg, C5aRa treatment showed a significant improvement in survival rates in CLP mice. However, when administration of C5aRa was delayed until 6 hours after CLP, the protective effects were lost. Late blockade (6 hours after CLP) of C5aR by anti-C5aR and C5aRa resulted in no beneficial in survival, suggesting that the complement activation is a rapid process in this experimental model of sepsis.

ANTI-C5A TREATMENT ATTENUATES SIRS

It has been long assumed that endogenous mediators of inflammation play an important role in the development of sepsis, together with microbial products such as LPS. In sepsis, the loss of the balance between pro-inflammatory and anti-inflammatory mediators seem to occur, producing exaggerated inflammatory responses, followed by

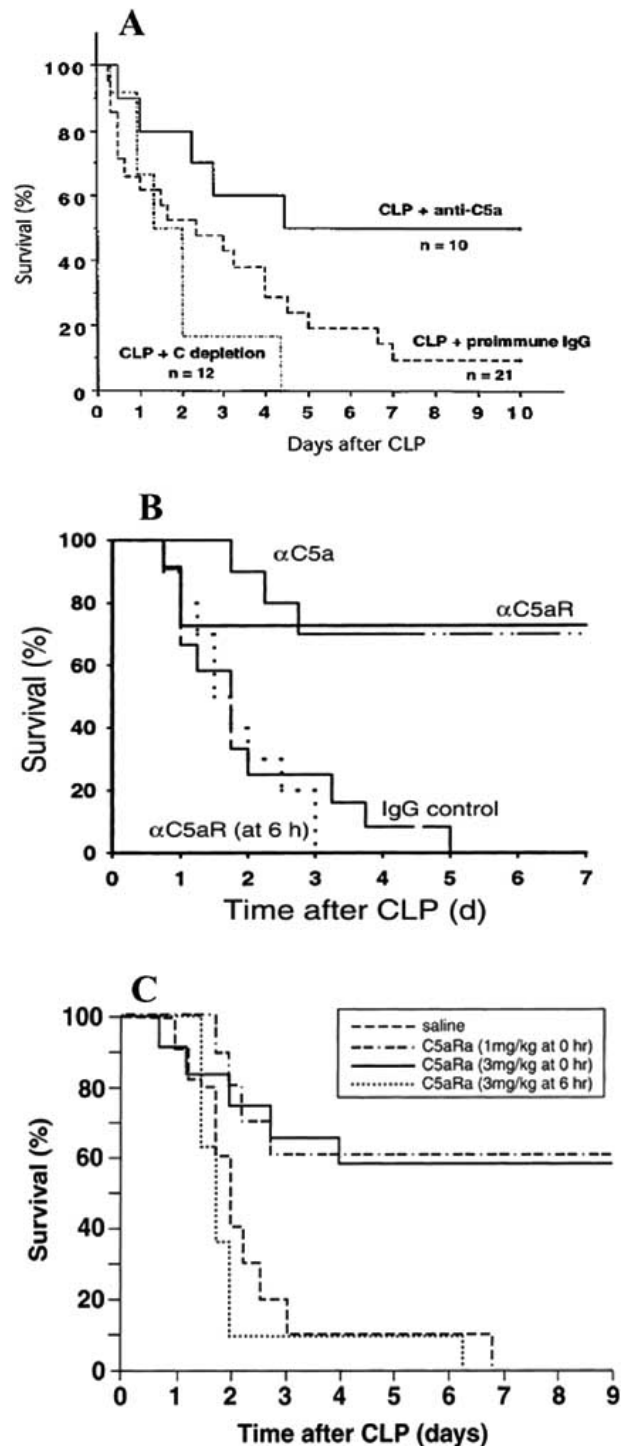


Fig. (1). Rates of survival in rats (A) or mice (B, C) after cecal ligation and puncture (CLP). **A.** Treatments included C3 depletion before CLP or an intravenous injection of normal rabbit IgG or anti-C5a IgG antibody (400 μ g) immediately after CLP. **B.** Mice were treated intravenously with anti-C5a antibody (20 μ g; $n = 10$), anti-C5aR (20 μ g; $n = 11$), or normal rabbit IgG (20 μ g; $n=12$) at the initiation of CLP. In another experiment, 20 μ g anti-C5aR ($n = 10$) was injected 6 hours after CLP. **C.** Mice were treated intravenously with either 200 μ l saline or C5aRa (1-3 mg/kg body weight). For each group, $n = 10$. The same protocols for C5a blockade were used in all subsequent figures.

immunosuppression, apoptosis, and organ dysfunction. Recent evidence suggests that C5a/C5aR signaling may influence the balance of the inflammatory network. It has been demonstrated that C5a stimulates the synthesis and release of pro-inflammatory cytokines such as TNF, IL-1, IL-6, and IL-8 from human leukocytes [23,24]. C5a produced a strong synergistic effect with LPS in production of TNF, macrophage inflammatory protein-2 (MIP-2), cytokine-induced neutrophil chemoattractant-1 (CINC), and IL-1 in rat alveolar epithelial cells (RAEC) [25]. Similarly, exposure of mouse dermal endothelial cells to C5a and LPS resulted in enhanced production of monocyte chemoattractant protein-1 (MCP-1) and MIP-2 when compared to the effects of C5a or LPS alone. Addition of physiological concentrations of C5a (low nM range) to human umbilical cord endothelial cells (HUVEC) caused strong up-regulation of IL-8, IL-1, and RANTES mRNA in a time- and dose-dependent manner [26]. These data suggest that the C5a/C5aR signaling pathway is involved in cytokine and chemokine production in a variety of cell types. Blockade of C5a with anti-C5a in sepsis resulted in a decrease of >75% in serum levels of IL-6 [24]. Blockade of C5a in CLP rats significantly reduced serum levels of IL-6 during sepsis [27]. The dependency of IL-6 production on C5a during sepsis was confirmed in C5aR-deficient mice. IL-6 levels in C5aR-deficient mice were significantly lower than that in control mice during sepsis. C5a stimulation of neutrophils elicited a rapid phosphorylation of p38-mitogen activated protein kinase (MAPK) and p44/p42 MAPK, which are critical in IL-6 production. Curiously, C5a can strongly suppress LPS-induced TNF production in neutrophils, while in alveolar macrophages C5a showed the opposite effect, with LPS and C5a synergistically inducing TNF production [28]. Given the fact that complement activation is an event occurring during the early phase of sepsis, C5a may come into play prior to appearance of most of the other inflammatory mediators. It is likely that C5a plays a key role in orchestrating the performance of the

cytokine network. Thus, anti-C5a treatment could be a reasonable strategy to attenuate SIRS.

ANTI-C5A TREATMENT REDUCES MULTI-ORGAN FAILURE (MOF) IN EXPERIMENTAL SEPSIS

Progressive MOF in humans with sepsis is associated with a high mortality rate [29,30]. Development of MOF in septic patients is often with defective blood/gas exchange in lung, these accompanied by activation of the clotting system, and defective function of the kidneys and liver [31]. The relationship between SIRS and multiple organ dysfunction syndrome (MODS), has been described as a patient going from sick to sicker to very sick [32]. MOF seems to be the final common pathway leading to death in modern intensive care units (ICU) [31]. MOF in either humans or animals often appears as a consequence of progressive development of hypotension, tissue hypoxia, complement activation, and an unregulated production of inflammatory mediators [10,33-35]. Accumulating evidence has established a link between C5a and MOF. Widespread upregulation of C5aR has been seen in organs from septic animals [18]. Anti-C5a treatment in the CLP model has been reported to attenuate the coagulopathy of sepsis and to improve the organ function (as described above).

Bacteremia is one of important features of sepsis. Blood cultures from septic (CLP) rats showed a significant number of aerobic and anaerobic bacterial colony forming units (CFU) [21]. In the CLP group receiving control IgG, the CFU value in blood was 740 ± 328 , whereas in the CLP group treated with anti-C5a the CFU value was profoundly reduced to 18 ± 10 (a 98% decrease) [21]. CLP rats treated with control IgG had very high CFU values (3×10^6 - 11×10^6 /g tissue) in spleen and liver (Fig. 2A). In contrast, CFU values from the anti-C5a group decreased almost to the basal levels [21]. Early respiratory alkalosis in septic animals 24 hour after CLP was documented by the rising arterial pH from 7.47 to 7.53. Septic rats 60 hour after CLP showed evidence of metabolic acidosis, with the blood pH values

Table 1. Effects of Anti-C5a on Parameters of MOF

Organ	Parameter	Groups		
		Sham+preimmune IgG	CLP+preimmune IgG	CLP+anti-C5a
Blood	Lactate (nmol/L)	2.87 ± 0.40	7.42 ± 1.31	3.54 ± 0.30
Liver	Bilirubin (mg/dl)	0.30 ± 0.01	0.53 ± 0.08	0.23 ± 0.03
	ALT (U/L)	46.6 ± 1.8	255.0 ± 65.2	63.6 ± 2.4
	AST (U/L)	74.0 ± 2.0	386.6 ± 71.6	128.0 ± 8.5
	LDH (U/L)	288.3 ± 85.8	926.0 ± 47.0	628.0 ± 11.5
Kidney	Creatinine (mg/dl)	0.43 ± 0.03	0.70 ± 0.05	0.43 ± 0.06
	BUN (mg/dl)	11.6 ± 1.4	44.5 ± 12.2	17.6 ± 0.7
	Urine protein (mg/dl)	1.30 ± 0.04	3.27 ± 0.29	1.57 ± 0.19
	Urine output (ml/36 h)	26.2 ± 0.8	6.6 ± 1.0	19.0 ± 1.0
	GFR (ml/min)	1.89 ± 0.03	0.49 ± 0.07	1.90 ± 0.24

falling from 7.48 to 7.34 [36]. In the CLP group treated with anti-C5a, all arterial values (pH, pCO₂, HCO₃⁻, pO₂) remained in the normal range, indicating that anti-C5a treatment was able to reverse the early respiratory alkalosis and late metabolic acidosis occurring in septic rats [36]. Sepsis is a leading cause of disseminated intravascular coagulation. During sepsis, tissue factor expression occurs on the surfaces of blood monocytes, endothelial cells and tissue macrophages, resulting in activation of the extrinsic coagulation pathway, with thrombin activation and fibrin formation [37]. Simultaneously, there is inhibition of natural anti-coagulant responses, all of which leads to excessive thrombin generation, fibrin formation, and consumption of clotting factors during sepsis. Anti-C5a significantly ameliorated the coagulation/fibrinolytic changes as well as evidence of disseminated intravascular coagulation in septic rats [38]. The changes in platelet counts, fibrinogen, factor VII:C, plasminogen, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor (PAI), as well as plasma thrombin-anti-thrombin (TAT) complexes and D-dimer, were all markedly attenuated in CLP rats treated with anti-C5a. C5a has been shown to induce tissue factor expression in endothelial cells and monocytes [39-41]. In addition, the effects of C5a on coagulation pathways may be also mediated by regulating the levels of chemokines and cytokines. For instance, C5a is a strong inducer of IL-8 production, and IL-8 can promote the formation of fibrin clots and induce thrombogenesis as well as proliferation and structural reorganization of endothelial cells [42]. Thus, the involvement of C5a in coagulation pathways could be direct or indirect. In septic rats, liver dysfunction was evidenced by elevations in serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), which suggests cell death. In animals treated with anti-C5a, most of these parameters (bilirubin, ALT, AST) remained in the normal range, and the rise in LDH levels was attenuated. In addition, kidney function was also protected by anti-C5a treatment, as reflected by unchanged levels of serum creatinine, blood urea nitrogen (BUN) and urine protein [36]. It is known that neutrophil accumulation in organs is associated with inflammatory tissue injury. C5a caused increased expression of both α_1 and α_2 integrins on blood neutrophils and enhanced adhesive interactions of neutrophils to HUVEC and to human bronchial epithelial cells [43,44]. The treatment of anti-C5a significantly reduced the upregulation of α_1 and α_2 integrins on blood neutrophils following sepsis [45]. Thus, anti-C5a may protect injury of various organs during sepsis by limiting neutrophil sequestration.

Atrophy of the thymus associated with thymocyte apoptosis has been observed during sepsis in rodents [46-48]. T-cell suppression and a decreased number in total T-lymphocytes are characteristic symptoms in MOF [49]. The widespread lymphocyte depletion induced by apoptosis maybe the cause of immunosuppression that occurs in sepsis. In the rat model of CLP-induced sepsis, thymus atrophy occurred as early as 12 hour after CLP and was maximally expressed at 24 hour. C5a blockade dramatically preserved thymic weight during CLP (Fig. 2B). Correspondingly, anti-C5a treatment resulted in a significant decrease in CLP-induced thymocyte apoptosis, by 80% (Fig. 2C). Anti-C5a

treatment in sepsis prevented thymocyte apoptosis by maintaining the integrity of mitochondria, preserving anti-apoptotic molecule Bcl-X_L, and inhibiting caspase-3, -6, and -9 activation [50].

BLOCKADE OF C5A IMPROVES NEUTROPHIL FUNCTION IN SEPSIS

Chemotactic responsiveness of neutrophils to C5a is defective in sepsis, perhaps in part because C5aR levels on neutrophils are significantly decreased in early sepsis [51,52]. During sepsis, blood neutrophils displayed defective phagocytosis and chemotaxis [53], and compromised H₂O₂ production [51] as well as defective assembly of NADPH oxidase [53]. All such defects were greatly ameliorated when CLP rats were treated with antibodies to either C5a or C5aR (Fig. 3), suggesting that during sepsis neutrophil innate immune functions are seriously compromised by excessive engagement of C5a/C5aR. C5a/C5aR interaction is a critical event in *E. coli*-induced up-regulation of CR3 (CD11b/CD18) and the subsequent oxidative burst and phagocytosis in neutrophils [16]. Interception of C5a/C5aR signaling in the whole blood by C5aRa significantly inhibited the *E. coli*-induced oxidative burst in neutrophils, markedly attenuated increased levels of CD11b on the neutrophil surface, and completely abolished both neutrophil and monocyte phagocytosis of *E. coli*. Under the same conditions, both granulocyte and monocyte phagocytosis was completely abolished by anti-CD11b antibody. These data suggest that optimal opsonization and subsequent phagocytosis of *E. coli* are dependent on C5a-induced upregulation of CR3 which then engages CD11b/CD18 on bacteria. Thus, C5a/C5aR signaling plays an essential role in host defense. However, excessive activation of C5a/C5aR signaling might be detrimental. Phorbol myristate acetate (PMA) stimulation of neutrophils results in phosphorylation of cytosolic p47^{phox} and its translocation to the cell membrane, which is essential for assembly of NADPH oxidase. Pre-exposure of neutrophils to C5a blocks phosphorylation of p47^{phox} and its translocation to the cell membrane in response to PMA, thereby leading to defective assembly of NADPH oxidase and a greatly depressed oxidative burst [53]. At the same time, phosphorylation of p42/p44 mitogen-activated protein kinase (MAPK) in neutrophils in response to PMA is also impaired by prior neutrophil contact with C5a. Since C5a is a strong inducer of MAP kinases and p42/p44 MAPK is an important kinase for p47^{phox} phosphorylation, the functional impairments in neutrophils exposed to C5a is likely due to paralysis of signaling cascades. As shown in Fig. 3A, neutrophils from CLP rats that had been treated with normal IgG showed compromised phagocytosis, while anti-C5a treatment of CLP rats resulted in a complete retention in neutrophil phagocytic activity. *In vitro* experiments demonstrated that pre-incubation of C5a with neutrophils impaired the phagocytic activity of neutrophils in a dose-dependent manner (Fig. 3B). Remarkably, neutrophil production of H₂O₂ and chemotactic responses were completely protected when anti-C5a was given intravenously at the start of CLP (Fig. 3C & D).

C5a/C5aR signaling in neutrophils appears to provide a survival signal for neutrophils. Apoptosis is considered to be an important mechanism for eliminating activated

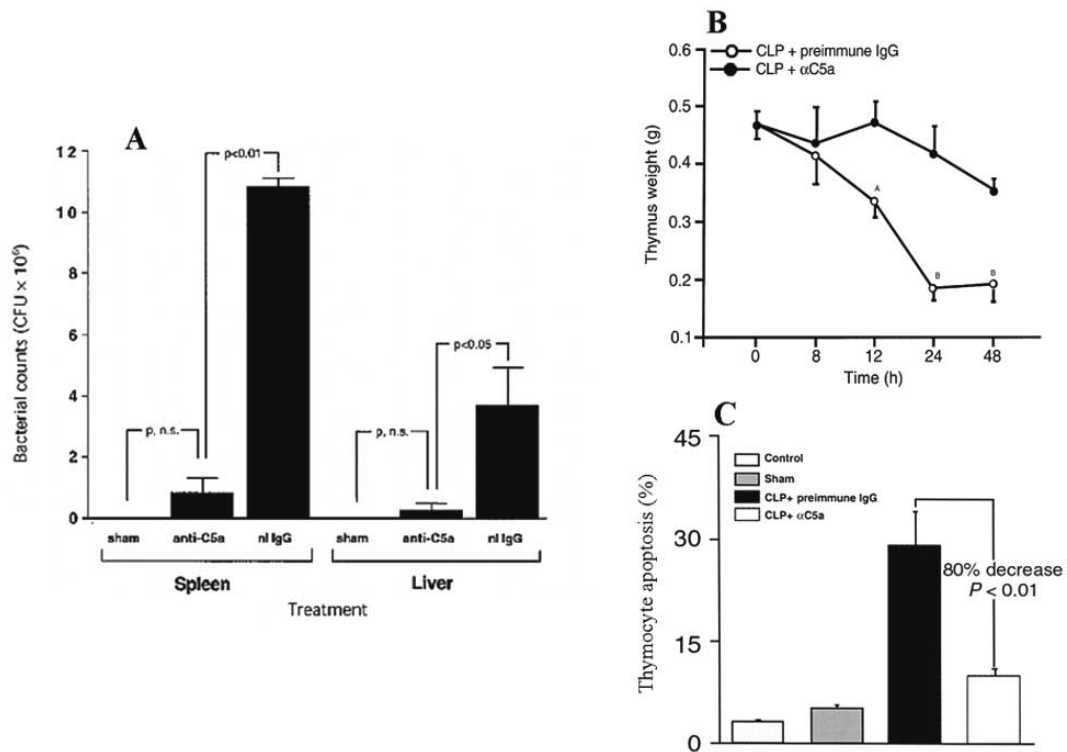


Fig. (2). Effects of anti-C5aR on MOF. **A.** Bacterial colony forming units (CFU) in spleen and liver in sepsis. CFUs in spleen and liver were determined using tissue homogenates 36 hour after CLP in rats pretreated at time 0 with either pre-immune IgG (nl IgG) or IgG antibody against C5a (anti-C5a). For each group, $n = 3$. **B.** The effects of C5a blockade on changes in thymus mass after CLP. At the indicated time points, the animals were sacrificed and the thymus glands weighed. For each vertical bar, $n = 4$ or 5 animals. ^A $P < 0.05$ and ^B $P < 0.01$ when compared to IgG control. **C.** Effects of C5a blockade on thymocyte apoptosis. Thymocytes were isolated 24 hour after CLP and analyzed for apoptosis by annexin V/propidium iodide staining. For each group, $n = 4$ animals.

neutrophils from inflamed tissues, preventing the release of the toxic cellular products from necrotic neutrophils. High levels of neutrophil sequestration may result in tissue damage by releasing a large amount of proteinases and free radicals, ultimately leading to MOF. Spontaneous apoptosis was significantly reduced in blood neutrophils obtained from patients with SIRS (8.6%) when compared to neutrophils from normal donors (34.9%) [54]. More than 50% of neutrophils isolated from normal donors undergo apoptosis during a 24-hour *in vitro* culture process (at 37°C). The presence of C5a in the cell culture media inhibits neutrophil apoptosis in a dose- and time-dependent manner [55]. The anti-apoptotic effects of C5a were markedly abrogated in the presence of wortmannin, a phosphatidylinositol-3 kinase (PI 3-K) inhibitor, suggesting that the PI3-K/Akt pathway is involved in induction of apoptosis. In addition, C5a caused phosphorylation of Akt and Bad proteins in neutrophils as well as decreased activity of caspase-9. These data suggest that neutrophils undergo spontaneous apoptosis via a mitochondria dependent pathway and that this pathway can be inhibited by C5a/C5aR signaling. As would be expected, blood neutrophils isolated from CLP rats had significantly less apoptotic cells after 24 hours of *in vitro* incubation in comparison to the blood neutrophils from normal rats. When CLP rats were treated with anti-C5a, the isolated cells regained the ability to undergo apoptosis, suggesting that long-life span neutrophils in sepsis are attributable, at least in

part, to C5a generated during sepsis (unpublished observations). Thus, anti-C5a treatment may prevent neutrophil accumulation in organs by maintaining a normal (short) life-span in neutrophils.

During the onset of experimental sepsis induced by CLP, C5aR content on neutrophils shows a dynamic expression pattern in that C5aR levels are decreased early in sepsis, reaching a nadir 24 hour after CLP, followed by a progressive and slow return thereafter [51]. This dynamic pattern of C5aR expression on neutrophils during sepsis is likely due to internalization of C5a/C5aR complexes followed by re-expression of C5aR on neutrophil surfaces. Intravenous blockade of C5a by anti-C5a antibody significantly preserved C5aR content on blood neutrophils [51]. Previously studies have suggested that, after binding of C5a to C5aR on neutrophils, the ligand/receptor complex is rapidly internalized, with C5aR being ultimately recycled to the cell surface [56-58]. C5aR expression on blood neutrophils reached the lowest point 24 hour after CLP. Simultaneously, innate immune functions (chemotaxis and oxidative burst) of neutrophils were seriously impaired [51]. Beyond 24 hour following CLP, C5aR content on blood neutrophils slowly elevated in surviving rats, being associated with an enhanced oxidative burst and improved chemotaxis. Interestingly, there was a positive correlation between the level of C5aR expression on neutrophils from CLP animals and animal survival. As shown in Fig. 4, all

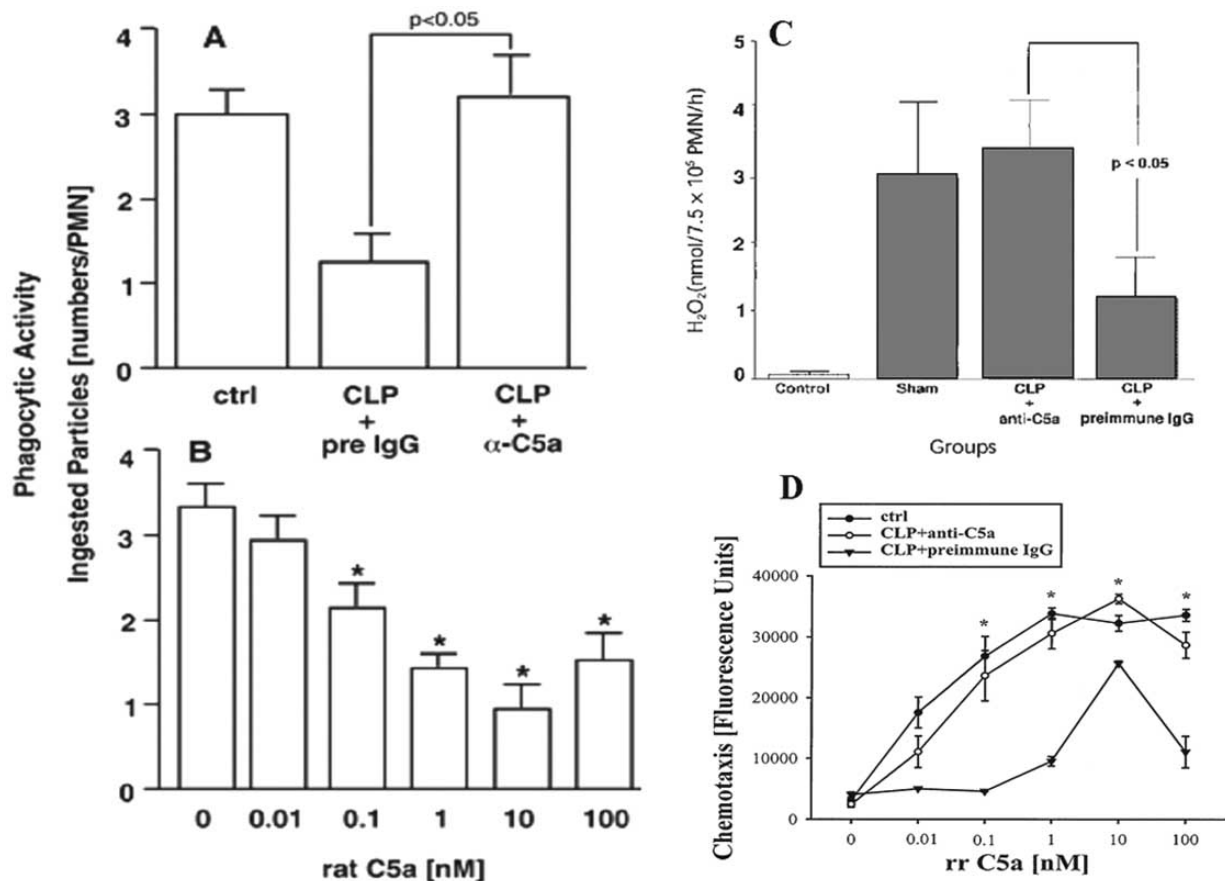


Fig. (3). Influence of neutrophil phagocytosis by exposure to C5a. **A.** Effects of C5a blockade on neutrophil phagocytosis in sepsis. Phagocytic activity of neutrophils was determined by the number of ingested IgG-opsonized zymosan particles per cell. Eight animals were used in each group. **B.** Phagocytic activity of normal rat neutrophils pre-incubated *in vitro* with different doses of C5a. * $P < 0.05$ vs control. **C.** H₂O₂ production by blood neutrophils. Neutrophils from the groups indicated were incubated with phorbol myristate acetate (PMA), and then H₂O₂ generation was measured. **D.** Chemotaxis of blood neutrophils in sepsis. Neutrophils were isolated from sham rats or CLP rats treated at time 0 with anti-C5a or control IgG. *In vitro* chemotactic responses to C5a were measured.

septic animals with C5aR levels higher than the overall median at 36 hour survived, whereas 67% of animals with C5aR levels lower than median failed to survive. These data establish a positive correlation between C5aR content on neutrophils and the functional capabilities of these cells in maintaining host defensive responsiveness, at least after CLP in rodents. Thus, *in vivo* blockade of C5a prevented the loss of C5aR on neutrophils and preserved neutrophil functions.

Since C5a stimulation of neutrophils elicits a strong inflammatory response, C5aR internalization could be a protective mechanism to attenuate the inflammatory reaction. However, this process may also compromise innate immune functions of neutrophils, given the fact that C5aR is important machinery in bacterial killing as described above. The levels of C5aR seem to be particularly important at the late stage of sepsis, because septic animals with high levels of C5aR survive, whereas those with low C5aR levels succumb. These observations suggest that C5a or C5aR blockade during sepsis may have desirable outcomes. A strategy that can effectively inhibit C5a-elicited inflammatory responses without affecting C5aR function might be preferential. From this perspective, the strategy of anti-C5a treatment might be more appealing than blockade

of C5aR. As shown in Fig. 1, blocking C5aR by anti-C5aR and C5aRa in the CLP model in rodents showed the same level of efficacy in improving the survival rate, suggesting that the management of inflammatory responses elicited by C5a at the early stages of sepsis may be critical for the outcome in septic animals.

SUMMARY OF RECENT PATENTS ON C5aR BLOCKING

Biological functions of C5a can be blocked directly by polyclonal or monoclonal antibodies. There are several patents filed related to this approach. Rollins *et al.* employ anti-C5 antibodies to prevent the formation of C5a and C5b [59]. These antibodies have been found to reduce complement activation, platelet activation, leukocyte activation, and platelet-leukocyte adhesion. Ward *et al.* utilize antibodies targeting the C-terminal truncated C5a peptides as a treatment for sepsis [60]. US4686100 applies an antibody specific for C5a or the des Arg derivative for the treatment of adult respiratory distress syndrome (ARDS) and sepsis [61]. Tanox Inc. applies anti-C5 monoclonal antibodies in treatment of delayed xenograft rejection or acute vascular rejection [62]. These antibodies are able to

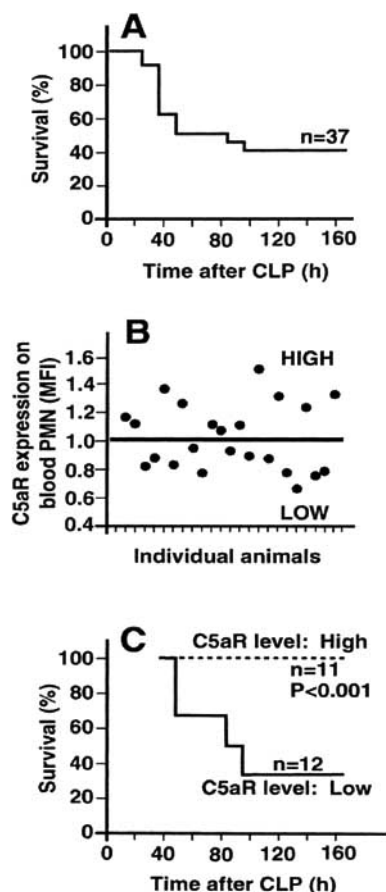


Fig. (4). Correlation of C5aR levels on neutrophils from CLP rats with survival. **A.** A survival curve was obtained over a 7 day interval. Blood samples were taken from tail veins 36 hour after CLP, and C5aR levels were evaluated by flow cytometry analysis. Based on the median (**B**), the animals were divided into two groups: animals with neutrophil C5aR levels higher than median (HIGH) and animals with neutrophil C5aR levels lower than median (LOW). **C.** Death rates in these two groups were monitored.

Table 2. Recent Patens on C5aR Blocking

Patent No.	Title	Pub.Date/Filed Date
WO 03062278A1	Monoclonal antibodies against extracellular loops of C5aR	2003-07-31/2003-01-24
US. 5,807,824	C5A receptor antagonists having substantially no agonist activity	1998-09-15/1995-06-05
US. 5,190,922	Terminally modified tri-, tetra- and pentapeptide anaphylatoxin receptor ligands	1993-03-03/1991-06-04
US. 6,777,422	Substituted tetrahydroisoquinolines as C5a receptor modulators	2004-08-17/2003-03-27
US. 6,858,637	Substituted biaryl amides as C5a receptor modulators	2005-02-22/2003-03-27
US. 6,884,815	High affinity small molecule C5a receptor modulators	2004-04-20/2000-09-28
US. 20040116424A1	Aryl imidazoles and related compounds as C5a receptor modulators	2004-06-17/2003-03-28
WO 05010030A2	C5a receptor antagonists	2005-02-03/2004-07-19
WO 04043223A2	Compositions and methods for the diagnosis and treatment of Sepsis	2004-05-27/2003-11-05

inhibit type II endothelial cell activation manifested by the suppression of E-selectin. However, no clinical data is available to assess the roles of these antibodies in human diseases. In addition, US4772584 describes an enzyme isolated from group A streptococci which inhibits the

binding of C5a to neutrophils by cleaving a six amino acid peptide from the C-terminus of C5a [63].

For clinical application, blocking C5aR appears to be an attractive strategy. Many patents have been filed or issued

for this line of invention. US5480974 and WO040432232A employ the antibody blocking approach to block C5aR [64, 65], while most of other patents describe the utilization of synthetic compounds or peptides to block C5aR [66-74]. NGD 2000-1 from Neurogen is the first oral C5aR antagonist tested in humans for the treatment of asthma [68-70]. In this exploratory trial, blockade of C5a receptors in patients with mild to moderate asthma did not demonstrate a therapeutic benefit. Australian biotech company Promics Pty. Ltd. has begun to investigate its C5a receptor antagonist [74], PMX53, in patients suffering chronic rheumatoid arthritis and inflammatory bowel disease.

CURRENT & FUTURE DEVELOPMENTS

Many clinical trials in targeting specific inflammatory mediators have failed in improving the survival of patients with sepsis. Recombinant activated protein C (APC) treatment reduced the mortality rate in patients with sepsis from 31% to 25%. Although such efficacy is debatable, the FDA approved APC for clinical usage in adult humans with sepsis in the absence of other effective treatment. Obviously, the understanding of the pathogenesis of sepsis and the treatment of sepsis are still in the formative stage. The accumulating data suggest that anti-complement strategy should be considered for treatment of humans with sepsis. As noted above, C5a blockade in experimental sepsis attenuates SIRS development, reduces MOF improves organ function, inhibits thymocyte apoptosis, and improves neutrophil functions by preserving oxidative pathways. Since the pathogenesis of sepsis is multi-factorial, involving an "inflammatory cytokine storm", complement activation, coagulation, and cell apoptosis, etc., an intervention that can positively influence these important aspects of sepsis development should be considered. Since C5a has been involved such a broad range of biological activities in the development of sepsis, the strategy of blockade of C5a-C5aR signaling is attractive for further investigation in treatment of sepsis in human. To date, no clinical trial has been initiated in this line of research, mainly due to the lack of reliable blocking reagents for humans.

ACKNOWLEDGEMENTS

We thank Beverly Schumann for secretarial assistance. This work is supported by the National Institutes of Health (grant GM-61656)

REFERENCES

- [1] Stone R. Search for sepsis drugs goes on despite past failures. *Science* 1994; 264: 365-367.
- [2] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; 348: 138-150.
- [3] Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* 1985; 229: 869-871.
- [4] Tracey KJ, Fong Y, Hesse DG, *et al.* Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987; 330: 662-664.
- [5] Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* 1990; 348: 550-552.
- [6] Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat Med* 2003; 9: 517-524.
- [7] Bernard GR, Vincent JL, Laterre PF, *et al.* Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001; 344: 699-709.

- [8] del Balzo U, Polley MJ, Levi R: C3a-induced contraction of guinea pig ileum consists of two components: fast histamine-mediated and slow prostanoid-mediated. *J Pharmacol Exp Ther* 1989; 248: 1003-1009.
- [9] Smedegard G, Cui LX, Hugli TE. Endotoxin-induced shock in the rat. A role for C5a. *Am J Pathol* 1989; 135: 489-497.
- [10] Bengtson A, Heideman M. Anaphylatoxin formation in sepsis. *Arch Surg* 1988; 123: 645-649.
- [11] Nakae H, Endo S, Inada K, Takakuwa T, Kasai T, Yoshida M. Serum complement levels and severity of sepsis. *Res Commun Chem Pathol Pharmacol* 1994, 84: 189-195.
- [12] Zhao L, Ohtaki Y, Yamaguchi K, *et al.* LPS-induced platelet response and rapid shock in mice: contribution of O-antigen region of LPS and involvement of the lectin pathway of the complement system. *Blood* 2002; 100: 3233-3239.
- [13] Hugli TE. The structural basis for anaphylatoxin and chemotactic functions of C3a, C4a, and C5a. *Crit Rev Immunol* 1981; 1: 321-366.
- [14] Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An *in vitro* model of immune vascular damage. *J Clin Invest* 1978; 61: 1161-1167.
- [15] Goldstein IM, Weissmann G. Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes. *J Immunol* 1974; 113: 1583-1588.
- [16] Mollnes TE, Brekke OL, Fung M, *et al.* Essential role of the C5a receptor in *E coli*-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002; 100: 1869-1877.
- [17] Schumacher WA, Fantone JC, Kunkel SE, Webb RC, Lucchesi BR. The anaphylatoxins C3a and C5a are vasodilators in the canine coronary vasculature *in vitro* and *in vivo*. *Agents Actions* 1991; 34: 345-349.
- [18] Riedemann NC, Guo RF, Neff TA, *et al.* Increased C5a receptor expression in sepsis. *J Clin Invest* 2002; 110: 101-108.
- [19] Mohr M, Hopken U, Oppermann M, *et al.* Effects of anti-C5a monoclonal antibodies on oxygen use in a porcine model of severe sepsis. *Eur J Clin Invest* 1998; 28: 227-234.
- [20] Stevens JH, O'Hanley P, Shapiro JM, *et al.* Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. *J Clin Invest* 1986; 77: 1812-1816.
- [21] Czermak BJ, Sarma V, Pierson CL, *et al.* Protective effects of C5a blockade in sepsis. *Nat Med* 1999; 5: 788-792.
- [22] Huber-Lang MS, Riedemann NC, Sarma JV, *et al.* Protection of innate immunity by C5aR antagonist in septic mice. *FASEB J* 2002; 16: 1567-1574.
- [23] Strieter RM, Kasahara K, Allen RM, *et al.* Cytokine-induced neutrophil-derived interleukin-8. *Am J Pathol* 1992; 141: 397-407.
- [24] Hopken U, Mohr M, Struber A, *et al.* Inhibition of interleukin-6 synthesis in an animal model of septic shock by anti-C5a monoclonal antibodies. *Eur J Immunol* 1996; 26: 1103-1109.
- [25] Riedemann NC, Guo RF, Sarma VJ, *et al.* Expression and function of the C5a receptor in rat alveolar epithelial cells. *J Immunol* 2002; 168: 1919-1925.
- [26] Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *FASEB J* 2003; 17: 1003-1014.
- [27] Riedemann NC, Guo RF, Laudes IJ, *et al.* C5a receptor and thymocyte apoptosis in sepsis. *FASEB J* 2002; 16: 887-888.
- [28] Riedemann NC, Guo RF, Bernacki KD, *et al.* Regulation by C5a of neutrophil activation during sepsis. *Immunity* 2003; 19: 193-202.
- [29] Deitch EA. Multiple organ failure. Pathophysiology and potential future therapy. *Ann Surg* 1992; 216: 117-134.
- [30] Baue AE, Durham R, Faist E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? *Shock* 1998; 10: 79-89.
- [31] Baue AE. Multiple organ failure--the discrepancy between our scientific knowledge and understanding and the management of our patients. *Langenbecks Arch Surg* 2000; 385: 441-453.
- [32] Baue AE. Sepsis, systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and multiple organ failure: are trauma surgeons lumpers or splitters? *J Trauma* 2003; 55: 997-998.

- [33] Ebong SJ, Call DR, Bolgos G, *et al.* Immunopathologic responses to non-lethal sepsis. *Shock* 1999; 12: 118-126.
- [34] Faist E, Wichmann MW: [Immunology in the severely injured]. *Chirurg* 1997; 68: 1066-1070.
- [35] Nakae H, Endo S, Inada K, Yoshida M. Chronological changes in the complement system in sepsis. *Surg Today* 1996; 26: 225-229.
- [36] Huber-Lang M, Sarma VJ, Lu KT, *et al.* Role of C5a in multiorgan failure during sepsis. *J Immunol* 2001; 166: 1193-1199.
- [37] Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood* 2003; 101: 3765-3777.
- [38] Laudes IJ, Chu JC, Sikranth S, *et al.* Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. *Am J Pathol* 2002; 160: 1867-1875.
- [39] Ikeda K, Nagasawa K, Horiuchi T, Tsuru T, Nishizaka H, Niho Y. C5a induces tissue factor activity on endothelial cells. *Thromb Haemost* 1997; 77: 394-398.
- [40] Muhlfelder TW, Niemetz J, Kreutzer D, Beebe D, Ward PA, Rosenfeld SI. C5 chemotactic fragment induces leukocyte production of tissue factor activity: a link between complement and coagulation. *J Clin Invest* 1979; 63: 147-150.
- [41] Carson SD, Johnson DR. Consecutive enzyme cascades: complement activation at the cell surface triggers increased tissue factor activity. *Blood* 1990; 76: 361-367.
- [42] Horuk R. The interleukin-8-receptor family: from chemokines to malaria. *Immunol Today* 1994; 15: 169-174.
- [43] Molad Y, Haines KA, Anderson DC, Buyon JP, Cronstein BN. Immunocomplexes stimulate different signalling events to chemoattractants in the neutrophil and regulate L-selectin and beta 2-integrin expression differently. *Biochem J* 1994; 299 (Pt 3): 881-887.
- [44] Jagels MA, Daffern PJ, Hugli TE. C3a and C5a enhance granulocyte adhesion to endothelial and epithelial cell monolayers: epithelial and endothelial priming is required for C3a-induced eosinophil adhesion. *Immunopharmacology* 2000; 46: 209-222.
- [45] Guo RF, Riedemann NC, Laudes IJ, *et al.* Altered neutrophil trafficking during sepsis. *J Immunol* 2002; 169: 307-314.
- [46] Wang SD, Huang KJ, Lin YS, Lei HY. Sepsis-induced apoptosis of the thymocytes in mice. *J Immunol* 1994; 152: 5014-5021.
- [47] Ayala A, Herdon CD, Lehman DL, Ayala CA, Chaudry IH. Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators. *Blood* 1996; 87: 4261-4275.
- [48] Barke RA, Roy S, Chapin RB, Charboneau R. The role of programmed cell death (apoptosis) in thymic involution following sepsis. *Arch Surg* 1994; 129: 1256-1261; discussion 1261-1252.
- [49] Papatheanassoglou ED, Moynihan JA, Ackerman MH. Does programmed cell death (apoptosis) play a role in the development of multiple organ dysfunction in critically ill patients? a review and a theoretical framework. *Crit Care Med* 2000; 28: 537-549.
- [50] Guo RF, Huber-Lang M, Wang X, *et al.* Protective effects of anti-C5a in sepsis-induced thymocyte apoptosis. *J Clin Invest* 2000; 106: 1271-1280.
- [51] Guo RF, Riedemann NC, Bernacki KD, *et al.* Neutrophil C5a receptor and the outcome in a rat model of sepsis. *FASEB J* 2003; 17: 1.
- [52] Seely AJ, Naud JF, Campisi G, *et al.* Alteration of chemoattractant receptor expression regulates human neutrophil chemotaxis *in vivo*. *Ann Surg* 2002; 235: 550-559.
- [53] Huber-Lang MS, Younkin EM, Sarma JV, *et al.* Complement-induced impairment of innate immunity during sepsis. *J Immunol* 2002; 169: 3223-3231.
- [54] Jimenez MF, Watson RW, Parodo J, *et al.* Dysregulated expression of neutrophil apoptosis in the systemic inflammatory response syndrome. *Arch Surg* 1997; 132: 1263-1269; discussion 1269-1270.
- [55] Perianayagam MC, Balakrishnan VS, King AJ, Pereira BJ, Jaber BL. C5a delays apoptosis of human neutrophils by a phosphatidylinositol 3-kinase-signaling pathway. *Kidney Int* 2002; 61: 456-463.
- [56] Van Epps DE, Simpson S, Bender JG, Chenoweth DE. Regulation of C5a and formyl peptide receptor expression on human polymorphonuclear leukocytes. *J Immunol* 1990; 144: 1062-1068.
- [57] Naik N, Giannini E, Brouchon L, Boulay F. Internalization and recycling of the C5a anaphylatoxin receptor: evidence that the agonist-mediated internalization is modulated by phosphorylation of the C-terminal domain. *J Cell Sci* 1997; 110: 2381-2390.
- [58] Gilbert TL, Bennett TA, Maestas DC, Cimino DF, Prossnitz ER. Internalization of the human N-formyl peptide and C5a chemoattractant receptors occurs *via* clathrin-independent mechanisms. *Biochemistry* 2001; 40: 3467-3475.
- [59] Rollins, S., Smith, B.R., Squinto, S. P.: US5853722 (1998).
- *[60] Ward, P.A., Huber-Lang, M., Sarma, V.: US6866845 (2005).
- [61] Raffin, T.A., Stevens, J.H.: US4686100 (1987).
- [62] Fung, M.S., Sun, B.N., Sun, C.R.: US6534058 (2003).
- [63] Cleary, P.P., Wexler, D.E.: US4772584 (1988).
- [64] Morgan, E. L., Ember, J.A., Hugli, T.E.: US5480974 (1996).
- *[65] Guo, R.-F., Reidemann, N.C., Ward, P.A.: WO04043223A2 and WO04043223A3 (2004).
- [66] Hahn, G.S.: US4692511 (1987).
- [67] van Oostrum, Jan., Boyar, W.C., Galakatos, N.G., Schmitz, A., Van Heeke, G.: US5807824 (1998).
- [68] Luly, J.R., Kawai, M., Wiedeman, P.E.: US5190922 (1993).
- [69] Lee, K., Mitchell, S., Ohliger, R., Zhang, L.Y., Zhao, H., Currie, K.: US6777422 (2004).
- [70] Gao, Y., Hutchison, A.J., Pringle, W.C., Thurkauf, A., Yoon, Zhao, H.: US6858637 (2005).
- [71] Thurkauf, A., He, X.-S., Zhao, H., *et al.*: US6884815 (2005).
- [72] Luke, G.P., Maynard, G.D., Mitchell, S., *et al.*: US20040116424A1 (2004).
- [73] Hummel, G., Locrdi, E., Polakowski, T., Scharn, D., Schnatbaum, K.: WO05010030A2 (2005).
- *[74] Fairlie, D., Taylor, S.M., Finch, A.M., Wong, A.: US6821950 (2004).