Beneficial and Detrimental Effects of Plasmin(ogen) during Infection and Sepsis in Mice

Yongzhi Guo*, Jinan Li*, Elin Hagström, Tor Ny*

Introduction

Sepsis is the culmination of complex interactions between the infecting microorganism and the host immune, inflammatory, coagulation and anti-coagulation responses [1]. Sepsis caused by infections with bacteria such as Staphylococcus aureus is a life-threatening condition that may lead to septic shock, resulting in multiple organ failure and death [2]. It is well known that disorders of coagulation and fibrinolysis play a major role in the development of organ dysfunction during sepsis [3]. Plasmin, a potent serine protease in fibrinolysis and the key component of the plasminogen activator (PA) system, is generated by conversion from its precursor, plasminogen, by either of two physiological PAs, tissue-type PA (tPA) and urokinase-type PA (uPA) [4]. Besides fibrinolysis, plasmin also degrades a large range of extracellular matrix substrates and activates pre-matrix metalloproteinases [5]. Plasmin has therefore been suggested to be an important upstream regulator of extracellular matrix remodeling in many tissue degradation-related innate immune processes such as cell migration, tissue remodeling, inflammation, and complement activation [6–8].

In addition to its roles in extracellular proteolysis, the PA system is also involved in generation of pro-inflammatory responses in the extracellular environment. Studies using plasminogen-deficient (plg−/−) mice have provided evidence supporting a role of the PA system in mediating the migration of inflammatory cells towards inflammatory sites [9]. In vitro studies have also indicated that plasmin cleaves components of the complement system, thereby releasing chemotactic complement fragments [10,11]. Moreover, recent in vitro studies suggest that the PA system appears to be involved in the intracellular signaling events during inflammation. For instance, plasmin can activate the p38 mitogen-activated protein kinase (MAPK), Janus kinase (JAK), signal transducers and activators of transcription (STAT) signaling pathways in monocytes, which have been shown to be important for the inflammatory response [12]. Plasmin is also known to stimulate the release of cytokines and other inflammatory mediators by different cell types [13]. During severe infection, uncontrolled release of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) may cause a so-called cytokine storm [14]. An uncontrolled cytokine storm leads to sepsis, and is therefore fatal. However, although various mechanisms underlying the inflammatory response during infection have been proposed, the possible functional roles of the PA system during infection, and during sepsis especially, remain largely unknown.

In the current study, we have used single gene-deficient mice lacking plasminogen (plg−/−), uPA (uPA−/−), tPA (tPA−/−), and doubly deficient mice lacking both tPA and uPA (tPA−/−/uPA−/−) to study the functional roles of plasmin during S. aureus-induced infection and sepsis. Our data show that the plasmin shows contrasting roles in infection and sepsis.

Results

Reduced survival rate upon induction of infection and increased survival rate upon induction of sepsis in plg−/− mice in comparison to WT mice

To reveal the functional roles of plasmin in infection and sepsis, we used WT, plg−/−, and plg−/− mice, which exhibit 100%, 50%,
and 0% of the normal serum plasminogen level, respectively. Infection and sepsis were achieved by intravenous injection of
1$x10^7$ or 1.6$x10^8$ CFU of $S. aureus$, respectively, and the survival of mice was thereafter followed for 25 days. As shown in Figure 1A,
in the first 4 days after bacterial injection no mice died in the infection group. From day 5 to day 25, the survival rates of WT,
plg$^{+/+}$, and plg$^{-/-}$ mice were 86.7%, 80%, and 50%, respectively. The survival rate in plg$^{-/-}$ mice upon induction of infection was

significantly lower ($P=0.04$) than that in the WT mice. A contrasting result was obtained when sepsis was induced
(Figure 1B and Table 1). In this model, the median survival time in plg$^{-/-}$ mice was 156 h, which was significantly longer ($P=0.009$)
than that in WT mice (40 h). Interestingly, the median survival time and the survival curve of plg$^{-/-}$ mice were intermediate as
compared to WT and plg$^{+/+}$ mice. These data show that plasminogen deficiency has contrasting effects on the survival
during infection and sepsis induced by *S. aureus*, and the serum levels of plasminogen affect the survival phenotype.

PA-deficient mice have delayed onset of death and an improved survival

Plasminogen is converted to the active enzyme plasmin by either uPA or tPA. We therefore studied *S. aureus*-induced sepsis in tPA−/− or uPA−/− mice, and also in doubly deficient (tPA−/−/uPA−/−) mice. As shown in Figure 1B and Table 1, the tPA−/− and uPA−/− mice showed delayed onset of death and significantly increased survival rates as compared to WT mice (log-rank test *P* > 0.04). Moreover, when tPA−/−/uPA−/− mice were used to induce sepsis, they showed the longest median survival time compared to all other genotypes. The delayed onset of death in these tPA−/−/uPA−/− mice was even more evident than in plg−/− mice (Figure 1B and Table 1). These data suggest that lack of tPA and/or uPA in mice significantly enhances the survival rate, and prolongs the onset of death in *S. aureus*-induced sepsis, thus confirming the notion that active plasmin plays a key role in the mortality of sepsis.

Comparison of viable bacterial counts in mouse organs after induction of infection and sepsis

In order to study whether there was a difference in the presence of bacteria in different organs in WT and plg−/− mice after induction of infection, both genotypes of mice were inoculated with 1 × 10^7 CFU of *S. aureus*, and 24 h later numbers of viable bacteria were counted from various organs. As shown in Figure 2A, the bacterial counts in liver, kidney, spleen, and blood were similar in WT and plg−/− mice. The bacterial counts in brain, however, were significantly higher in WT mice than in plg−/− mice. These results suggest that in the blood and in most organs, the ability to kill bacteria was similar in WT and plg−/− mice at this early stage of infection. The difference in bacterial counts in the brain suggests that plasminogen may be required for bacterial invasion through the blood-brain barrier (BBB) [15,16].

We also investigated the presence of viable bacteria in various organs after induction of sepsis in WT, tPA−/−, uPA−/−, tPA−/−/uPA−/−, and plg−/− mice. As shown in Figure 2B, the numbers of viable bacteria in the blood were not significantly different in all genotypes studied at 24 h after bacterial injection. However, as shown in Figure 2C–2F, the viable bacterial counts in the spleen, brain, liver, and kidney of WT, tPA−/−, and uPA−/− mice were significantly higher than those in tPA−/−/uPA−/− mice and plg−/− mice. The viable bacterial counts in all organs studied were not significantly different in WT, tPA−/−, and uPA−/− mice. The bacterial counts in the brains of tPA−/−/uPA−/− mice were significantly higher than those in plg−/− mice, but this was not the case for other organs (Figure 2D). These data suggest that in sepsis, the bacterial killing ability is improved in mice with reduced levels of functional plasmin. Taken together, our data show that plasmin exhibits contrasting functions in bacterial killing in infection and sepsis.

| Genotypes   | Number of mice used | Median survival time (h) | Mean onset time of death (h) |
|-------------|---------------------|--------------------------|-----------------------------|
| WT          | 28                  | 40*                      | 64.0±8.8                    |
| tPA−/−/uPA−/−| 10                 | 40                       | 54.7±10.7                  |
| tPA−/−      | 13                  | 132                      | 88.5±15.9                  |
| uPA−/−      | 15                  | 132                      | 86.8±14.0                  |
| plg−/−      | 20                  | 126                      | 70.3±13.7                  |
| plg−/−      | 10                  | 156                      | 122±10.0                   |
| tPA−/−/uPA−/−| 7                   | 168                      | 141.0±9.0                  |

*Data presented as Mean ± SD.

*P* < 0.05. WT group was compared with plg−/− group.

doi:10.1371/journal.pone.0024774.t001

Higher serum levels of TNF-α, IL-6, and IL-10 in WT mice than in plg−/− mice during sepsis

*In vitro* studies have indicated that plasmin stimulates the expressions of several cytokines [13]. A dramatic increase in inflammatory cytokine levels is one of the major clinical features of sepsis [17,18]. To study how plasminogen affects the cytokine production, we studied the profiles of inflammatory cytokines in the sera of WT and plg−/− mice during infection and sepsis. As shown in Figure 3A (sepsis) and 3B (infection), serum levels of TNF-α reached a maximum level at 2 h after the injection of *S. aureus*, and gradually fell thereafter in both genotypes of mice and in both infection and sepsis. After induction of sepsis (Figure 3A), significantly higher TNF-α levels were seen in WT mice than in plg−/− mice at all 4 time points studied. In contrast, in the infection model (Figure 3B), the TNF-α levels were either comparable (at 6 h and 24 h) or significantly lower (at 2 h and 36 h) in WT mice than in plg−/− mice.

As shown in Figure 3C and 3D, IL-6 levels were comparable in WT mice and plg−/− mice at 6 h, for both the infection model and the sepsis model. However, 24 h after induction of sepsis, WT mice had 8-fold higher levels of IL-6 than plg−/− mice (Figure 3C), while no difference was observed at the same time point in the infection model (Figure 3D). At 24 h after induction of sepsis, the IL-6 levels in the sera of plg−/− mice were also significantly lower than those in WT mice, but significantly higher than those in plg−/− and tPA−/−/uPA−/− mice (Figure 3E).

As shown in Figure 3F, at 24 h after induction of sepsis, the IL-10 levels were significantly higher in WT mice than in plg−/− mice, while the levels of IL-10 were similar between the two genotypes in the infection model. Furthermore, in both genotypes of mice, IL-10 levels were significantly higher in sepsis than in infection. Taken together, these data demonstrate that during sepsis, plg−/− mice have significantly lower cytokine levels than WT mice. In addition, during sepsis, since the IL-6 levels are highest in WT mice, intermediate in plg−/− mice, and lowest in both tPA−/−/uPA−/− and plg−/− mice, the increase in cytokine levels appears to be related not only to levels of plasminogen but also to levels of active plasmin.
Improved survival in septic mice treated with IL-6 antibodies

IL-6 is an important pro-inflammatory cytokine that is upregulated during sepsis [19]. As we found that the serum levels of IL-6 were significantly higher in WT mice than in plg<sup>−/−</sup> mice 24 h after induction of sepsis, we tested the hypothesis that neutralization of IL-6 may delay the onset of death, or improve the survival rate in our sepsis model. As shown in Figure 4 and Table 2, when WT mice were treated with an anti-IL-6 neutralizing antibody (1.33 mg per kg body weight) at 1 h before and 24 h after the induction of sepsis, the onset of death was delayed for a period of 45 h, and the survival rate was significantly elevated as compared to mice treated with control IgG (P<0.05). WT mice treated with anti-IL-6 antibody showed a similar survival curve to that of plg<sup>−/−</sup> mice, which suggests that lack of plasminogen improves the chances of survival from sepsis by reducing the production of IL-6.

Figure 2. Bacterial counts in different organs and in different genotypes of mice. A. Bacterial counts in brain, liver, kidney, spleen, and blood of WT and plg<sup>−/−</sup> mice 24 h after inoculation with 1×10<sup>7</sup> CFU of <i>S. aureus</i>. B.–F. Bacterial counts in blood, spleen, brain, liver, and kidney 24 h after inoculation of 1.6×10<sup>8</sup> CFU of <i>S. aureus</i> to different genotypes of mice. The means ± SD of 8 mice are shown. NS = not significant, * = P<0.05 was considered significant, by One-way ANOVA, with Bonferroni’s Multiple Comparison test.
doi:10.1371/journal.pone.0024774.g002
Activation of STAT3 during infection and sepsis

STAT3 is a key molecule in mediating signaling of several inflammatory cytokines during an acute inflammatory response [20]. To study if plasminogen deficiency affects STAT3 activation, we measured the levels of phosphorylated and total STAT3 in spleen neutrophils obtained 24 h after infection and sepsis induction (Figure 5A and 5B). In the infection model, levels of STAT3 and phosphorylated STAT3 are either moderately increased (in WT mice) or remained unchanged (in plg-/- mice) as compared to uninfected controls. In the sepsis model, levels of total and phosphorylated STAT3 in WT mice increased significantly upon induction of sepsis. However, the levels remained largely unchanged in plg-/- mice under sepsis challenge, indicating that plasmin is involved in the activation of the STAT3 signaling pathway in sepsis, which may contribute to the differences in cytokine levels, bacterial killing ability, and mortality between WT and plg-/- mice during infection and sepsis.

Comparison of intensity of C5a on blood neutrophils in WT and plg-/- mice during sepsis

Studies using a murine cecal ligation and puncture sepsis model have shown that exposure of neutrophils to C5a at concentrations

Figure 3. Serum levels of TNF-α, IL-6, and IL-10 during infection and sepsis. Blood samples were taken at the indicated time points. A. TNF-α levels in sera from WT and plg-/- mice after induction of sepsis. B. TNF-α levels in sera from WT and plg-/- mice after induction of infection. C. IL-6 levels in sera from WT and plg-/- mice after induction of sepsis. D. IL-6 levels in sera from WT and plg-/- mice after induction of infection. E. IL-6 levels in sera from WT, plg-/-, tPA-/-/uPA-/-, and plg-/- mice after induction of sepsis. F. IL-10 levels in sera from WT and plg-/- mice after induction of infection or sepsis. The means ± SD of 5 mice are shown. NS = not significant, * = P<0.05 was considered significant, and ** = P<0.01 was considered extremely significant, by 2-tailed t-test.

doi:10.1371/journal.pone.0024774.g003
occuring in human plasma during sepsis leads to neutrophil dysfunction and paralysis of signaling pathways [21]. We therefore investigated whether plasminogen deficiency may influence the surface deposition of C5a on blood neutrophils after induction of sepsis. Twenty-four and 36 h after induction of sepsis, the intensity of C5a on neutrophils was determined. As shown in Figure 6, the intensity of C5a on neutrophils was lower in plg\(^{-/-}\) mice than in WT mice at 24 h after sepsis induction and was further reduced at 36 h. The diminished deposition of C5a on neutrophils in plg\(^{-/-}\) mice during sepsis suggests that C5a may contribute to the underlying molecular mechanisms in our sepsis model.

### Discussion

In the present study, we report that plasmin plays a beneficial role in \(S.\) \textit{aureus}-induced infection, but a detrimental role in \(S.\) \textit{aureus}-induced sepsis. Several pro- and anti-inflammatory cytokines showed different profiles in WT and plg\(^{-/-}\) mice during infection and sepsis. In sepsis, blockade of IL-6 improved the survival of WT mice to a level that is close to plg\(^{-/-}\) mice. Furthermore, depression of the active complement mediator C5a on the surface of neutrophils as well as activation of STAT3 is important in regulating cytokine expression, inflammatory signal transduction, bacterial killing ability, and mice survival rate during infection and sepsis.

We have previously shown that plasminogen levels are important for the incidence and severity of collagen II-induced arthritis [22]. Similarly, in the current study, our data with the sepsis model showed three types of survival phenotypes: (1) tPA\(^{-/-}\)/uPA\(^{-/-}\) and plg\(^{-/-}\) mice, which cannot form active plasmin, have the highest survival rate, the longest median survival time, and the latest time of onset of death; (2) WT and tPA\(^{-/-}\)/uPA\(^{-/-}\) mice have the lowest survival rate, the shortest median survival time, and the earliest time of onset of death; and (3) plg\(^{-/-}\), uPA\(^{-/-}\), and tPA\(^{-/-}\) mice have intermediate survival rate and time of onset of death. Because both tPA and uPA can activate plasminogen to functional plasmin, tPA or uPA singly deficient mice still have the capacity to convert plasminogen to active plasmin. Furthermore, tPA\(^{-/-}\)/uPA\(^{-/-}\) mice have normal levels of plasminogen, but they cannot activate plasminogen to active plasmin. Thus, the survival data from the sepsis study suggest that it is the relative plasmin levels that determine the survival outcomes during sepsis.

During infection, however, WT and plg\(^{-/-}\) mice showed similar survival rates. These data indicate that unlike in sepsis, the average time of onset of death in the infection model was less dependent on the level of plasminogen. Furthermore, in sepsis, mice with normal or apparently normal ability to form plasmin (WT, tPA\(^{-/-}\), and uPA\(^{-/-}\) mice) all had significantly impaired bacterial killing ability as compared to mice that are unable to form plasmin (plg\(^{-/-}\) or plg\(^{-/-}\)/uPA\(^{-/-}\) or plg\(^{-/-}\)/tPA\(^{-/-}\) mice). Moreover, the IL-6 levels in plg\(^{-/-}\) mice were approximately half of those in WT mice at 24 h after induction of sepsis, indicating that the relative levels of plasminogen also affect cytokine production. Based on these results, we propose that the available level of plasmin is critical for the onset of death, bacterial killing ability, and cytokine production in the development of sepsis.

STAT3 is a key molecule in mediating the signaling of many inflammatory cytokines such as TNF-\(\alpha\), IL-6, and IL-10 during inflammatory responses. TNF-\(\alpha\) and IL-6 have been considered to be the primary mediators of sepsis [23], and a positive correlation has been found between serum IL-6 and TNF-\(\alpha\) levels and multiple organ failure [24,25]. IL-10 has a pronounced anti-inflammatory effect by reducing the level of superoxide production in neutrophils, which interferes with neutrophil-mediated cellular cytotoxicity [26]. Previous in vitro studies have shown that plasmin stimulates the expression of cytokines in human monocytes [13].

### Table 2. Median survival time and mean onset time of death with or without treatment of anti-IL-6 antibody (Ab) during sepsis.

| Mouse group          | Number of mice used | Median survival time (h) | Mean onset time of death (h)¹ |
|----------------------|---------------------|--------------------------|-------------------------------|
| WT with control IgG  | 14                  | 52²                      | 64±12.4                       |
| WT with anti-IL-6 Ab | 9                   | 160                      | 100.8±19.5                    |
| plg\(^{-/-}\) with PBS | 10                  | 156                      | 120.0±13.5                    |

¹Data presented as Mean ± SD.
²* P<0.05. Anti-IL-6 Ab-treated group was compared to control IgG-treated group.
In our studies, we found that levels of total and phosphorylated STAT3 remained largely unchanged in plg−/− mice undergoing sepsis, which is in contrast to the dramatic increased STAT3 activation in WT mice undergoing sepsis. In addition, we also found that TNF-α, IL-6, and IL-10 levels were significantly lower in plg−/− mice than in WT mice during sepsis. In tPA−/−/uPA−/− mice, the IL-6 was at a low level similar to that in plg−/− mice.

It is well known that the cytokine storm during sepsis induces an overwhelming inflammatory response, which leads to multiple organ failure and subsequent death. Blocking cytokines such as TNF-α, IL-6 and IL-1 did show benefit in patients with bacteremia [27]. Diminished cytokines levels in plg−/− and tPA−/−/uPA−/− mice indicate that there is a strong correlation between levels of active plasmin, cytokine expression and STAT3 activation. The data presented here suggest that reducing or removing functional plasmin in mice, leads to a higher survival rate during sepsis due to an impaired cytokine production. Studies of how plasmin regulate cytokine expression and downstream signal pathways during sepsis are being carried out in our laboratory.

Several studies have shown that mice with low complement activity have impaired host defense during infection [28–30], whereas during sepsis excessive complement activation leads to compromised innate immune functions [31]. Blockade of C5a or the C5a receptor with antibodies has been shown to greatly improve survival of rodents during sepsis [32,33]. In the current study, the contrasting phenotypes in terms of survival rate and cytokine expression between plg−/− and WT mice during infection and sepsis are in accordance with the results of previous studies in mice with dysfunction of the complement system [34]. We have also shown that both the phosphorylated and total protein expression levels of STAT3 in spleen neutrophils of plg−/− mice were markedly lower than those in WT mice after induction of sepsis. Thus, one possible explanation is that plasmin is important in mediating complement activation, which subsequently activates the STAT3 signaling pathway and contributes to the different phenotypes observed in plg−/− and WT mice during infection and sepsis.

In summary, the current study reveals a contrasting role of plasminogen deficiency in infection and sepsis, and suggests that pro-inflammatory plasmin plays deleterious roles during systemic inflammation (sepsis). This pro-inflammatory function of plasmin is distinctly different from its classical roles as a protease that degrades fibrin and extracellular matrix. Our findings may be potentially useful for the development of novel therapeutic strategies against infection and sepsis in humans.

**Materials and Methods**

**Experimental animals**

Plasminogen-heterozygous (plg+/−) mice [35] of mixed genetic background (129×C57BL/6) were intercrossed to generate wild-type (WT), plg−/− and plasminogen-deficient (plg−/−) mice. tPA-deficient (tPA−/−) and uPA-deficient (uPA−/−) mice [36] were backcrossed 10 times to mice of C57BL/6 genetic background. The tPA/uPA doubly deficient (tPA−/−/uPA−/−) mice were generated by crossing tPA−/− mice with uPA−/− mice. The mice were genotyped by PCR and by measuring plasma levels of...
plasminogen, as described previously [37]. Male mice 8–12 weeks of age, with a body weight 22 grams or more were used for the experiments. In control experiments, the survival rates during infection and sepsis were also determined in female mice. However, no gender-related differences were found (Data not shown). The regional ethical committee of Umeå University approved all experimental protocols (Approval ID: A116-05).

**Bacterial strains**

The Phillips strain of *S. aureus* isolated from a patient with osteomyelitis was kindly supplied by Dr. Hook, Gothenburg, Sweden. *S. aureus* was grown either in LB broth or on LB agar plates.

**Models for induction of infection and sepsis**

**Infection model.** Mice were challenged by intravenous injection with 1 × 10⁶ CFU of *S. aureus* Phillips. The survival rate was examined over the whole experiment (up to 25 days).

**Sepsis model.** Mice were challenged by intravenous injection with 1.6 × 10⁸ CFU of *S. aureus* Phillips. The survival rate was examined over the whole experiment (up to 8 days).

**Bacterial counts in blood and different organs**

Twenty-four hours after injection of the bacteria in amounts compatible with either infection or sepsis, the mice were killed and the blood, brain, spleen, liver, and kidney were collected and homogenized in 2 ml sterile PBS. After serial dilution, the homogenates and the blood were spread on LB agar plates and incubated at 37°C for 24 h. Colonies were counted and expressed as CFU/organ.

**ELISA**

At the indicated time points after induction of infection or sepsis, approximately 600 μl blood were collected, placed on ice and allowed to clot before centrifugation at 3000 g for 10 min. Serum samples were then used for ELISA. The serum levels of TNF-a, IL-6, and IL-10 were measured using ELISA kits according to the instructions of the manufacturer (Nordic Biosite, Taby, Sweden).

**Western blot analysis**

Twenty-four hours after injection of infection or sepsis, spleens were collected and homogenized on ice, and the neutrophils were isolated by Percoll gradients (P4937; Sigma-Aldrich, Stockholm, Sweden). Neutrophils were lysed with 1% NP-40 and protease inhibitor cocktail. Thereafter, protein concentrations were measured by the BCA protein assay method (Pierce Biotechnology, Rockford, IL). Thirty micrograms of protein was electrophoresed on SDS-PAGE (7,5%), and transferred to polyvinylidene difluoride membranes (Amersham, UK) for western blot. Anti-STAT3 and anti-phosphorylated-STAT3 (Tyr705) antibodies were obtained from Cell Signaling Technology (Boston, MA). The mouse monoclonal antibodies to β-actin were purchased from Sigma-Aldrich, Sweden. Western blot analyses were carried out as described by Persaud et al [38].

**Detection of C5a on blood neutrophils during sepsis by flow cytometry**

Flow cytometric staining was performed on whole blood from 5 WT mice and 5 pIgR−/− mice at 24 and 36 h, respectively, after sepsis induction. Leukocytes isolation was essentially followed by Zhang et al [39]. Cells were incubated with rat antibody to mouse neutrophils (MCA71G; Serotec, UK) and goat antibody to mouse C5a (AP2150; Minneapolis, MN) for 30 min on ice. After washing the secondary antibodies with FACS buffer, FITC-labeled sheep anti-rat IgG diluted 1:100 (AAR10F; Serotec, UK) or PE-labeled donkey anti-goat IgG diluted 1:200 (Rockland Immunonochemicals, PA) was added. The cells were washed once with FACS buffer and resuspended in 500 μl PBS. The samples were analyzed by double-colour fluorescence flow cytometry with cytomics FC500 (Beckman Coulter) for the detection of C5a intensity on blood neutrophils.

**IL-6 blockade in WT mice**

WT mice 1 h prior to and 24 h after bacterial challenge

A rat monoclonal antibody (anti-IL-6; BD PharMingen, San Diego, CA) that specifically neutralize recombinant mouse IL-6 were used. The antibody were given to WT mice at a dose of 1.33 mg per kg body weight at 1 h prior to and 24 h after the induction of sepsis. As controls, 14 WT mice were given control IgG (R3-34; BD PharMingen, San Diego, CA) at the same dosage. The mice were monitored every 8 h, and were killed 192 h after induction of sepsis.

**Statistical analysis**

Results of bacterial counts are expressed as the mean ± SD. Data sets were analyzed using One-way ANOVA, with Bonferroni’s Multiple Comparison test. Results of cytokine levels are expressed as the mean ± SD. Data sets were analyzed using 2-tailed t-test. To compare the survival curves among different genotypes of mice, log-rank test was used. P values less than 0.05 were considered significant.

**Author Contributions**

Conceived and designed the experiments: YG TN JL. Performed the experiments: YG EH JL. Analyzed the data: YG JL TN EH. Contributed reagents/materials/analysis tools: YG JL TN EH. Wrote the paper: YG JL TN.

**References**

1. Russell JA (2006) Management of sepsis. N Engl J Med 355: 1699–1713.
2. Hinshaw LB (1996) Sepsis/septic shock: participation of the microcirculation: an abbreviated review. Crit Care Med 24: 1072-1078.
3. Amaral A, Opal SM, Vincent JL (2004) Coagulation in sepsis. Intensive Care Med 30: 1032–1040.
4. Collien D (1999) The plasminogen (fibrinolytic) system. Thromb Haemost 82: 259-270.
5. Behrendt N, Alexander CM, Werb Z (1992) Metalloproteinases mediate extracellular matrix degradation by cells from mouse blastocyst outgrowths. Development 114: 447–456.
6. Beck JM, Preston AM, Gyetko MR (1999) Urokinase-type plasminogen activator in inflammatory cell recruitment and host defense against Pneumocystis carinii in mice. Infect Immun 67: 879-884.
7. Paul R, Winkler F, Bayerlein I, Popp B, Pfister HW, et al. (2005) Urokinase-type plasminogen activator receptor regulates leukocyte recruitment during experimental pneumococcal meningitis. J Infect Dis 191: 776–782.
8. Winkler F, Kastenbauer S, Koedel U, Pfister HW (2002) Role of the urokinase plasminogen activator system in patients with bacterial meningitis. Neurology 59: 1350–1355.
9. Buusnill SJ, Plopis VA, Castellino FJ, Tang L, Eaton JW, et al. (2004) A central role for plasminogen in the inflammatory response to biomaterials. J Thromb Haemost 2: 1788-1805.
10. Schafft WT, Eisenberg PR (1997) Direct induction of complement activation by pharmacologic activation of plasminogen. Coron Artery Dis 8: 9–18.
11. Lachmann PJ, Paugham MK, Oldroyd RG (1982) Breakdown of C5 after pharmacologic activation of plasminogen. J Exp Med 156: 205–216.
12. Burysek L, Syvorots T, Simmet T (2002) The serine protease plasmin triggers expression of MCP-1 and CD40 in human primary monocytes via activation of p38 MAPK and janus kinase JAK/STAT signalling pathways. J Biol Chem 277: 35309–35317.
13. Syvorots T, Jedruch M, Roehweder A, Schule A, Simmet T (2001) Plasmin-induced expression of cytokines and tissue factor in human monocytes involves AP-1 and IKKbeta-mediated NF-kappaB activation. Blood 97: 3941–3950.
14. Remick DG, Bolges G, Copeland S, Siddiqui J (2005) Role of interleukin-6 in mortality from and physiologic response to sepsis. Infect Immun 73: 2751–2757.
15. Lahteenmaki K, Edelman S, Korhonen TK (2005) Bacterial metastasis: the host plasminogen system in bacterial invasion. Trends Microbiol 13: 79–85.
16. Huang SH, Simo MF, Kim KS (2000) Bacterial penetration across the blood-brain barrier during the development of neonatal meningitis. Microbes Infect 2: 1237–1244.

17. Tyburski JG, Dente C, Wilson RF, Steffes G, Declin J, et al. (2001) Differences in arterial and mixed venous IL-6 levels: the lungs as a source of cytokine storm in sepsis. Surgery 130: 748–751; discussion 751-742.

18. Muhl H, Pfischelter J (2006) Controlling the cytokine storm by insulin: glycogen synthase kinase-3 as a target in systemic inflammation. Crit Care Med 34: 1567–1569.

19. Magushiana MO, Ballet DE, Cooper PA, Trualer J, Cory BJ, et al. (2000) Serial interleukin 6 measurements in the early diagnosis of neonatal sepsis. J Trop Pediatr 46: 267–271.

20. Levy DE, Lee CK (2002) What does Stat3 do? J Clin Invest 109: 1143–1148.

21. Huber-Lang MS, Younkin EM, Sarma JV, McGuire SR, Lu KT, et al. (2002) Complement-induced impairment of innate immunity during sepsis. J Immunol 169: 3223–3231.

22. Li J, Ny A, Leonardsson G, Nandakumar KS, Holmdahl R, et al. (2005) The plasminogen activator/plasmin system is essential for development of the joint inflammatory phase of collagen type II-induced arthritis. Am J Pathol 166: 783–792.

23. Leon LR, White AA, Kluger MJ (1998) Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. Am J Physiol 275: R269–277.

24. Geppert A, Steiner A, Zorn G, Delle-Karth G, Koreny M, et al. (2002) Multiple organ failure in patients with cardiogenic shock is associated with high plasma levels of interleukin-6. Crit Care Med 30: 1987–1994.

25. Giamarellos-Bourboulis EJ, Bolanos N, Laoudes IJ, Keller KA, et al. (2002) Increased C5a receptor expression in sepsis. J Clin Invest 110: 101–108.

26. Riedemann NC, Guo RF, Bernacki KD, Reuben JS, Laudes IJ, et al. (2003) Regulation by C5a of neutrophil activation during sepsis. Immunity 19: 183–202.

27. Persaud DR, Yousefi V, Haunerland N (2003) Efficient isolation, purification, and characterization of the Helicoverpa zea VHDL receptor. Protein Expr Purif 32: 260–264.

28. Zhang J, Dong Z, Zhou R, Luo D, Wei H, et al. (2005) Isolation of lymphocytes and their innate immune characterizations from liver, intestine, lung and uterus. Cell Mol Immunol 2: 271–280.