Interleukin-32γ Transgenic Mice Resist LPS-Mediated Septic Shock

Sun Jong Kim¹, Siyoung Lee², Areum Kwak², Eunsom Kim², Seunghyun Jo³, Suyoung Bae³, Youngmin Lee⁴, Soyoon Ryoo³⁵, Jida Choi²⁶*, and Soohyun Kim²*

¹Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, Kunkuk University Hospital, and ²Laboratory of Cytokine Immunology, Department of Biomedical Science and Technology, Konkuk University, Seoul 143-701, Republic of Korea
²Department of Bioequivalence Division for Drug Evaluation, Ministry of Food and Drug Safety, Chungcheongbuk-do 363-700, Republic of Korea
³Department of Medicine, Pusan Paik Hospital, College of Medicine, Inje University, Busan 633-165, Republic of Korea
⁴Department of Veterinary Bacterial Disease, and ⁶Division of Veterinary Epidemiology, Animal and Plant Quarantine Agency, Gyeonggi-do 480-757, Republic of Korea

Introduction

Interleukin-32 (IL-32) is a cytokine and inducer of various proinflammatory cytokines such as TNFa, IL-1β, and IL-6 as well as chemokines. There are five splicing variants (α, β, γ, δ, and ε) and IL-32γ is the most active isoform. We generated human IL-32γ transgenic (IL-32γ TG) mice to express high level of IL-32γ in various tissues, including immune cells. The pathology of sepsis is based on the systemic inflammatory response that is characterized by upregulating inflammatory cytokines in whole body, particularly in response to gram-negative bacteria. We investigated the role of IL-32γ in a mouse model of experimental sepsis by using lipopolysaccharides (LPS). We found that IL-32γ TG mice resisted LPS-induced lethal endotoxemia. IL-32γ reduced systemic cytokines release after LPS administration but not the local immune response. IL-32γ TG increased neutrophil influx into the initial foci of the primary injected site, and prolonged local cytokines and chemokines production. These results suggest that neutrophil recruitment in IL-32γ TG occurred as a result of the local induction of chemokines but not the systemic inflammatory cytokine circulation. Together, our results suggest that IL-32γ enhances an innate immune response against local infection but inhibits the spread of immune responses, leading to systemic immune disorder.

Keywords: Interleukin-32, inflammatory cytokine, IL-32γ transgenic mice, lipopolysaccharides, mouse model of sepsis

Interleukin-32 (IL-32) is a cytokine and inducer of various proinflammatory cytokines, TNFα, IL-1β, and IL-6, as well as chemokines leading to inflammatory disorders [6, 23, 25, 41]. IL-32 has been discovered originally in activated natural killer cells and T cells, however, IL-32 expression is also detected in other immune cells such as macrophages and monocytes [5, 10, 16]. Additionally, non-immune cells, including keratinocytes, human umbilical vein endothelial cells, and pancreatic cells, are also sources of IL-32 [27, 32, 35]. Although a specific receptor for IL-32 has not been demonstrated, IL-32 has been involved in many inflammatory disorders and infections and its expression has correlated with disease severity [3, 5, 19].

Septic shock is a systemic inflammatory response caused by an overwhelming infection that leads to a serious disorder with high rates of morbidity and mortality, despite the advance of antibiotics and progress in the management of intensive care patients [4]. The most frequent microbial pathogens responsible for septic shock are gram-negative bacteria, which can directly evoke a strong innate immune response in hosts [37]. Lipopolysaccharide (LPS), a main component of gram-negative bacteria outer membranes,
IL-32 binds specifically to Toll-like receptor (TLR)-4 expressed in various immune and non-immune cells and induces a robust immune reaction via induction of proinflammatory cytokines such as TNFα, IL-1β, IL-6, and chemokines, including other inflammatory factors [14, 24, 39]. IL-32 consists of several isoforms (α, β, γ, δ, and ε), which show no significant difference in functions and act as a secretary or intracellular protein [2, 5]. Intracellular IL-32 is upregulated in activated NK and T cells by IFNγ-producing cells and implicated in cell apoptosis [16, 32]. Endogenous IL-32 also exhibits anti-inflammatory properties by promoting the production of IL-10 and by decreasing the induction of IL-12 in dendritic cells [20]. In contrast, secreted forms of IL-32 act as a proinflammatory cytokine and induce TNFα or IL-8 in monocytes and macrophages derived from human peripheral blood mononuclear cells (PBMCs) and mouse bone marrow cells [2, 25].

Sepsis or endotoxemia, the events that follow in the presence of excessive levels of endotoxin due to bacterial overgrowth, consequently lead to failure to maintain a proper balance between excessive and inadequate inflammation and immune responses. [7]. LPS-induced endotoxemia often provokes a life-threatening inflammatory disorder characterized by fever, hypotension, disseminated intravascular coagulation, multiple organ failure, shock, and, in 25–40% of cases, death [30]. This phenomenon can be experimentally reproduced in animals by administration of a high dose of exogenous LPS purified from gram-negative bacteria [15]. High concentrations of inflammatory cytokines in the blood stream sometimes indicate an increased risk of mortality but targeting to antagonize their activities has only limited success [34, 38]. Furthermore, mortality is observed in later period of infection and has been ascribed to the second phase of immune suppression that follows the excessive immune responses in the early phase [21]. Increasing evidence suggests that the complex syndrome of sepsis develops when the initial host response is limited to contain the primary infection, resulting in widespread inflammation and organ failure [22].

As no analog of IL-32 has yet been reported in mice, investigators have assessed the in vivo effect of endogenous IL-32 by generating different isoforms of IL-32 transgenic mice (IL-32TG). Different types of IL-32TG have shown hyperactivation in inflammatory circumstances and, as a result, developed severe clinical signs in the mouse model of sepsis [26, 33, 42]. However, our previous study indicated that IL-32/TG mice presented fast and severe development of experimental colitis, but had benefits for recovery and survival [2]. This updated report suggested that IL-32 would have dual effects in a certain immune response in vivo, although IL-32 is originally reported to escalate an inflammation as an inflammatory cytokine. We therefore investigated the role of IL-32γ in experimental sepsis, a complicated inflammatory condition. The present study described here is designed to determine the effect of IL-32γ in experimental sepsis using IL-32γTG and to elucidate the function of IL-32γ to induce immune responses against infection.

Materials and Methods

Mice

We generated IL-32γTG mice, which overexpressed human IL-32γ, on a C57BL/6 background under a control of a ubiquitous chicken β-actin promoter in the previous study as described [6]. Wild-type (WT) C57BL/6 mice were obtained from Orient (Seoul, Korea). Eight week-old WT and IL-32γTG mice were used for this study. Experimental procedures followed the recommendations for animal use and welfare, as dictated by institutional and national guidelines.

Induction of Endotoxemia

Endotoxemia was induced in mice by intraperitoneal (i.p.) injection of low dose (3 mg/kg) or high dose (30 mg/kg) of LPS (E. coli O127:B9; Sigma-Aldrich, St. Louis, MO, USA). All injected mice were closely monitored every 2 to 3 h for the first 16 h and every 12 h thereafter for at least 80 h. In an additional experiment, we collected blood from mice at 1, 4, and 17 h after intraperitoneal injection, and the serum was obtained after clotting for 2 h at 4°C. Samples were centrifuged for 10 min at 12,000 rpm and supernatants were stored at –20°C until assay.

Cytokine ELISA

Murine TNFα, IL-6, IL-10, MIP-2, and human IL-32 quantities in samples were determined by standard sandwich ELISA. Development kits of mouse cytokines were used according to manufacturer instructions (R & D systems, Minneapolis, MN, USA). Human IL-32γ concentrations in transgenic mice were determined by a human IL-32 specific sandwich ELISA kit (YbdY, Seoul, Korea). Briefly, 96-well plates were coated with capture antibody overnight at 4°C. After blocking for 1 h with 3% BSA/PBS, samples and standards were incubated for 2 h at room temperature. The ELISA plates were then incubated with detection antibody for 2 h, and streptavidin/peroxidase for mouse cytokine ELISA or peroxidase-conjugated anti-rabbit IgG was incubated for 45 min. Washing the plate four times with 0.05% Tween/PBS was performed before the addition of all reagents in each procedure. Finally, the color reaction was developed with 3,3',5,5'-tetramethyl-benzidine liquid substrate (Sigma-Aldrich), and then stopped with 0.1 M H2SO4. The absorbance at 450 nm was measured using an ELISA plate reader (Molecular Devices, Orleans, CA, USA).
Bone Marrow-Derived Macrophages Culture

For in vitro studies, macrophages were differentiated from bone marrow cells. Whole bone marrow cells were flushed from femurs and incubated in a differential medium (RPMI 1640, 10% FBS, 30% L929 cell cultured supernatant, 100 U/ml penicillin, and 100 µg/ml streptomycin) for 7 days to allow for proliferation and differentiation. Differentiated bone marrow-derived macrophages (BMDMs) were seeded at a density of 4 x 10^4 cells in 96-well plates for cytokine assays, and in 6-well plates for western blot assay. BMDMs were allowed to adhere for 24 h before use in the assays. Cells were incubated with or without LPS (100 ng/ml) at 37°C under humidified 5% CO₂. Supernatants were collected at different time points, centrifuged at 2,000 rpm, and stored at −20°C until assay of cytokines by ELISA. For detection of phosphorylated NF-κB and p38MAPK, the cells were stimulated as indicated in the Figure legends and lysed directly with RIPA buffer (Cell Signaling, Danvers, MA, USA) containing protease inhibitor cocktail (Sigma-Aldrich). Cell lysates were centrifuged at 14,000 rpm for 5 min, and the supernatant was transferred and kept until use for western blot assay.

Western Blot

For assessing LPS-induced signaling, BMDMs from IL-32/TG and WT were collected after LPS stimulation at different time points. The concentration of total protein was determined using a BCA kit (GE Healthcare, Piscataway, NJ, USA) and 30 µg of protein was resolved on 10% SDS-PAGE gel and transferred to nitrocellulose membranes (GE healthcare). Membranes were blocked in 3% BSA/TBST (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were then incubated with primary antibody for phosphorylated NF-κB and p38MAPK (Cell Signaling), appropriate secondary antibody conjugated to horseradish peroxidase (Cell Signaling), and visualized using ECL plus (GE Healthcare). Signals were detected by a LAS-3000 mini CCD camera (Fuji Film, Tokyo, Japan). The membranes were reprobed with goat anti-IL-32 antibody (YbdY) and then normalized finally with goat anti-actin (Santa Cruz Biotechnology).

Kupffer Cell Staining

Kupffer cell numbers in the liver were determined by intravenously administering India ink (50 µl) via the tail vein to mice. After 20 min, the livers were removed and fixed in chilled 4% (w/v) paraformaldehyde (Sigma) in PBS, pH 7.4, for tissue section. The fixed tissues were embedded in paraffin blocks, sectioned at 5 µm using a microtome, and placed on glass slides. Tissue sections were deparaffinized with xylene and covered with mounting solution and a cover slide. Kupffer cells in liver sections were quantitated by counting the number of India ink-positive cells in microscopic low-power fields (× 100).

FACS Staining

Thioglycollate-elicited peritoneal macrophages were isolated from peritoneal cavities after intraperitoneal injections of 3 ml of 3% thioglycollate broth, followed by peritoneal lavage with PBS 4 days later. Peritoneal cells after LPS (30 mg/kg) injection were harvested by peritoneal lavage with 3 ml of PBS at the times indicated in the Figures and legends. Pulmonary macrophages were obtained from broncho-alveolar lavage with PBS. Spleen cells were mechanically disaggregated by mincing the tissues and incubated with hypotonic solution for lysing red blood cells. The cells were washed with PBS, counted, and subjected to FACS analysis. Examination of the cells by flow cytometry was done on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA) and the data were analyzed on CellQuest software (Becton Dickinson). Isolated cells were washed once in ice-cold PBS containing 2% FBS and 0.1% NaN₃. Nonspecific binding was blocked by incubation of cells in 10% normal mouse serum/PBS for 15 min, followed by incubation with Gr-1 and F4/80 antibodies conjugated by fluorescence (eBioscience, San Diego, CA, USA) for 30 min. Cells were subsequently washed with PBS containing 2% FBS and 0.1% NaN₃, resuspended, and analyzed by FACS.

RT-PCR

Total RNA was isolated with Tri-Reagent (MRC, Canada) from the peritoneal cells. MMLV-RTase (Beams Bio, Korea) converted 2 µg of total RNA to first strand cDNA, and then PCR was performed at 94°C for 45 sec, 59°C for 40 sec, and 72°C for 30 sec for 35 cycles, with chemokine-specifric primers and GAPDH primers. Pairs of mouse MCP-1 sense primer, 5’-ACGCG GCCATCAC-3’ and reverse primer, 5’-CGGGTCAACTTC ACATTCAAAG-3’; mouse RANTES sense primer, 5’-CTTGCA GAGGGCTCGAGAC-3’ and reverse primer, 5’-ATTITCCTC GAAGGAGGTG-3’; mouse MIP-2 sense primer, 5’-ACACCTCAG CTTGCCCAT-3’ and reverse primer, 5’-CAGGTCTAGTTAG CCTTGCC-3’; and GAPDH sense primer, 5’-ACACATTCC CATGGATGC-3’ and reverse primer, 5’-TCCACACCCCTGTG CTGTA-3’, were used for the RT-PCR. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide.

Statistical Analysis

Graphs were created with the GraphPad 4.0 Prism software. All results were expressed as means ± SEM. Survival curves were analyzed by Log-rank (Mantel-Cox) test, and ELISA data were analyzed by the Student t-test, with p < 0.05 as the minimum level of significance.

Results

IL-32/TG Mice Are Less Sensitive to Endotoxic Shock Induced by LPS Challenge

A high-dose LPS-induced mortality of WT and IL-32/TG was analyzed in Fig. 1. IL-32/TG mice survived longer than WT mice after LPS administration. In the first 24 h after LPS injection, WT mice succumbed to LPS with a survival...
rate of 40%, whereas the IL-32γTG group showed high survival rate (86.6%) at the same time point. The difference between IL-32γTG and WT mice was sustained to 42 h of LPS administration as indicated by the asterisk (Fig. 1). There was statistically significant difference between Kaplan-Meier survival curves between IL-32γTG and WT mice, although a final lethality of IL-32γTG was equal to WT mice at the end of the study. This suggests that IL-32γ protects mice from LPS-induced sepsis leading to death.

LPS-Mediated Inflammatory Cytokine Productions Decreased in Sera of IL-32γTG Mice

We examined the induction of cytokines by a high-dose LPS (30 mg/kg) injection in sera of WT and IL-32γTG mice. We measured the cytokine level, including TNFα, IL-6 and IL-10, in the sera of LPS-injected mice by a specific ELISA kit. As shown in Fig. 2, the levels of these cytokines increased in the sera of the WT mice and IL-32γTG mice in response to LPS at different time points. For TNFα, there was an early induction at 1 h after the injection of LPS in both groups, but IL-32γTG produced less TNFα compared with WT, was appeared at 4 and 17 h after LPS administration (Fig. 2A). Unlike TNFα, the level of IL-6 increased progressively reaching the maximum at 4 h after LPS administration. The level of IL-6 in IL-32γTG mice was lower than in WT at 17 h (Fig. 2B). In addition, we measured the level of the anti-inflammatory cytokine IL-10. Interestingly, the level of IL-10 was also lower in IL-32γTG than in WT mice at 4 and 17 h after LPS administration (Fig. 2C).

We also tested the induction of cytokines by a low-dose LPS (3 mg/kg) injection in sera of WT and IL-32γTG mice. As shown in Figs. 3A and 3B, the patterns of TNFα and IL-6 production were similar to the high-dose LPS-injected mice, but a difference between IL-32γTG and WT mice was observed with IL-6 production at 1 h after LPS administration (Fig. 3B). Interestingly, the low-dose LPS-induced IL-10 was observed at 1 h after LPS and the statistical significance was obtained only at 1 h after LPS injection (Fig. 3C). We also determined the IL-32 level in sera during LPS endotoxemia in IL-32γTG mice by a specific sandwich ELISA kit. As shown in Figs. 2D and 3D, LPS injection influenced the serum IL-32 level in IL-32γTG mice. In the high-dose LPS (30 mg/kg), IL-32γ was slightly decreased until 4 h, and then recovered at 17 h after LPS injection, whereas in the low-dose LPS (3 mg/kg), the IL-32γ level was reduced at 1 h, rebounded at 4 h, and significantly decreased at 17 h after LPS injection (Fig. 3D).
In order to test whether the attenuated response to LPS in IL-32γTG mice is due to an intrinsic defect of target cells, we isolated and cultured BMDMs from WT and IL-32γTG mice in the presence or absence of LPS. The differentiation time and the cell numbers were equal in WT and IL-32γTG bone marrow cells (data not shown). After LPS treatment, supernatants were collected at different time points to measure the levels of MIP-2, TNFα, IL-6, and IL-10. The production of TNFα and IL-10 (data not shown) was not significantly different (Fig. 4B). However, MIP-2 was slightly enhanced in IL-32γTG macrophages compared with that of the control WT mice at every time point (Fig. 4A) but significance was obtained at 4 and 24 h. We found that at the early time after LPS stimulation, BMDMs of IL-32γTG released higher IL-6 than that of WT at 4 and 9 h, but final production after 24 h was not different between WT and IL-32γTG mice (Fig. 4C).

To assess the possibility that LPS signaling may be altered in BMDMs from IL-32γTG, we collected the BMDMs after LPS stimulation and studied the amount of phosphorylated NF-κB p65 (p-NF-κB) and p38MAPK (p-p38MAPK) with protein of BMDMs (Fig. 4D). The activity of these signaling molecules induced by LPS reached a peak level at 30 min in BMDMs of IL-32γTG, but at 60 min in BMDMs of WT.

**Fig. 3.** IL-32γTG mice reduced levels of proinflammatory cytokines in the sera after a low-dose LPS administration. WT (C57BL/6) (n = 4) and IL-32γTG mice (n = 4) were injected i.p. with LPS (3 mg/kg), and sera were collected 0, 1, 4, and 17 h post-injection. The levels of (A) TNFα, (B) IL-6, (C) IL-10, and (D) IL-32γ were assessed by sandwich ELISA as described in Materials and Methods. Student’s t-test, ***p < 0.001. One of three representative experiments is shown.

**Fig. 4.** BMDMs from IL-32γTG mice exhibit equivalent responses to LPS compared with WT. Bone marrow cells collected from WT (n = 3) and IL-32γTG mice (n = 3) were differentiated to macrophages, and cultured in the presence or absence of LPS (100 ng/ml). Supernatants were collected at the indicated time and used for analysis of (A) MIP-2, (B) TNFα, and (C) IL-6 levels by ELISA. Student’s t-test *p < 0.05; **p < 0.01. Representative data are shown of three independent experiments. (D) Activation of the LPS-induced signaling pathway was determined at different time points by detecting the phosphorylated protein levels in the BMDMs isolated from WT and IL-32γTG mice using western blot analysis. One of two representative experiments is shown.
addition, the phosphorylation level of NF-κB in IL-32γTG was weaker than in WT at 60 min (Fig. 4D, upper panel). In contrast, the phosphorylation level of p38MAPK in IL-32γTG was higher than that of WT at 30 min (Fig. 4D, the second panel).

**Tissue Macrophage Distribution Is Not Influenced by IL-32γTG**

Next, we compared the macrophage distribution in the liver, lung, peritoneal cavity, and spleen from WT and IL-32γTG mice. To determine Kupffer cell distribution in the liver, animals were injected intravenously via the tail vein with India ink. Liver sections of WT and IL-32γTG mice revealed that Kupffer cell distribution and its number were similar (Fig. 5A). In addition, IL-32γTG mice had normal compositions of alveolar, peritoneal, and splenic macrophages as determined by FACS staining with Gr-1, a neutrophil marker, and F4/80, a macrophage marker, compared with WT (Figs. 5B–5D). In the steady state of peritoneal lavage, macrophage cells recovered (~2 × 10⁶ cells/ per mouse) ~50% equally in both IL-32γTG and WT mice (Figs. 5E and 5F).

**Neutrophil Migration into the Primary Injected Site Was Elevated in IL-32γTG**

To confirm whether IL-32γ affects immune cell migration following administration of LPS (3 mg/kg), we examined the population of total peritoneal cells in the peritoneal wash fraction labeled with antibodies to Gr-1 and F4/80.

---

**Fig. 5.** Comparison of macrophage distribution and migration in vivo in WT and IL-32γTG mice.
The macrophage proportion in tissue was examined in the liver, lung, peritoneal cavity, and spleen from WT and IL-32γTG mice. (A) The numbers of Kupffer cells were determined by India ink staining as described in Materials and Methods. Representative pictures are shown of three independent experiments. Bars indicate the mean ± SEM. The macrophage percentage in (B) lung, (C) peritoneal cavity, and (D) spleen was analyzed by FACS. Isolated cells from each tissue were stained with Gr-1 and F4/80 Abs conjugated by fluorescence. Macrophage was identified by F4/80 staining alone. (E) The numbers of peritoneal cells and (F) percentage of peritoneal macrophages are presented by graphs. Bars indicate the mean ± SEM.
from IL-32γTG and WT mice at 2 and 6 h post-injection. As shown in Fig. 6A, we observed a dramatic increase in the percentage of Gr-1+ cells in IL-32γTG mice compared with WT mice at 2 and 6 h after LPS administration. On the contrary, the populations of F4/80-expressing macrophages were decreased in IL-32γTG mice relatively. To determine whether recruitment of neutrophils to the peritoneal cavity is the result of responses to a chemokine, the major chemoattractant for neutrophil, MIP-2, was checked in the peritoneal lavage and serum by ELISA. The serum level of LPS-induced MIP-2 in IL-32γTG was decreased, as with other proinflammatory cytokines (Fig. 6B). In contrast, there was no difference in the amounts of MIP-2 in the peritoneal exudates between IL-32γTG and WT mice after LPS injection. Furthermore, the expressions of several chemokines by RT-PCR were examined in the peritoneal cells from mice after LPS administration. Interestingly, the expressions of MCP-1, RANTES, and MIP-2 were found in IL-32γTG mice, but these chemokines expressions were absent in WT (Fig. 6C). The amounts of MIP-2 and TNFα production in the peritoneal lavage from LPS-injected mice were diminished significantly. Unlike the serum level of

![Graphs and figures]

**Fig. 6.** Analysis of early alteration of neutrophil and macrophage populations in the peritoneal cavity of mice injected with LPS. Peritoneal lavage was performed with WT and IL-32γTG mice at 0, 2, and 6 h after LPS administration, and peritoneal cells were separated by centrifugation. (A) Flow cytometric analysis of Gr-1 and F4/80 staining. Gr-1+ neutrophils, upper left; F4/80+ macrophages, lower right. (B) MIP-2 expression induced in WT and IL-32γTG mice in response to LPS injection. The levels of the neutrophil attracting chemokine MIP-2 in the blood and peritoneal fluid (PF) were determined by ELISA. Data are means ± SEM. *, p < 0.05. (C) Induction of several chemokines in peritoneal cells from WT and IL-32γTG mice by LPS. Expression of chemokines was analyzed using RT-PCR with total RNA isolated in the peritoneal lavage cells at 2 h post-injection. (D) MIP-2, (E) TNFα, and (F) IL-6 production into the peritoneal lavages from WT and IL-32γTG mice by LPS injection. At 2 and 6 h time points, the peritoneal lavages were collected and analyzed by ELISA. Data are shown as means with SEM.
cytokines, the cytokines in the peritoneal lavage of IL-32γTG was similar to WT at 2 and 6 h post-injection (Figs. 6D and 6E). Interestingly, IL-6 production in the peritoneal cavity of IL-32γTG mice by LPS was sustained longer than in WT mice (Fig. 6F). The cytokine profiling of the peritoneal exudates suggests that IL-32γTG elicits active immune responses against LPS locally.

Discussion

In this study, we found that IL-32γTG mice were more resistant to a lethal dose of LPS injected via the intraperitoneal route than WT and achieved long-term survival. Moreover, the levels of serum cytokines, including TNFα, IL-6, and IL-10, in IL-32γTG were lower than in WT mice. Proinflammatory cytokines mediate many of the immune pathological features of septic shock [18]. This is remarkable in severe sepsis, in which the excessive production of proinflammatory cytokines causes capillary leakage, tissue injury, and lethal organ failure [29]. Therefore, the suppression of proinflammatory cytokines has a beneficial effect to reduce the collateral damage and mortality from cytokine overwhelming.

Sepsis arises if a local infection progresses into a systemic inflammatory condition. The failure of an initial and appropriate host response in the inflamed site causes the amplification and dysregulation of inflammatory responses. Neutrophils have a crucial arm in the primary defense mechanism against bacteria invading, as shown by neutropenia such as leukemia and drug-induced neutropenia, which augments susceptibility to infection and to sepsis [8]. Moreover, in some diseases that have a high risk to infection, such as diabetes [28] and acquired immunodeficiency syndrome [31], an impairment of neutrophil migration is reported. Indeed, IL-32γ dramatically enhanced neutrophil recruitment into the LPS-injected site, which correlated with high and continuous chemokines production by LPS in the peritoneal cavity but not in the blood stream. We detected more chemokine mRNAs in the peritoneal cells of IL-32γTG (Fig. 6C). These results indicated that the resident peritoneal cells might be the source of the chemokines in the peritoneal cavity, and IL-32γTG improved the chemokine production in the primary LPS-injected site, which is similar to local infection, but not in the blood stream like systemic circulation. The induction of high levels of chemokines in the peritoneal cavity relative to the blood circulation would establish a chemokine gradient that results in recruiting neutrophils sufficiently into the peritoneal site.

LPS resistance of IL-32γTG mice is mainly due to their impaired cytokine production. Mononuclear cells play a key role, releasing the classic proinflammatory cytokines IL-1, IL-6, and TNFα, and macrophages are the first targets of LPS [17]. However, macrophages from IL-32γTG mice responded to LPS normally and settled in tissues properly compared with WT mice. Therefore, the resistance of IL-32γTG mice to LPS could be related to their ability to block the process of LPS-induced systemic immune response rather than a direct result of their low induction of inflammatory factors. Cytokine production in the peritoneal cavity of IL-32γTG mice was also not reduced compared with controls and, even in case of IL-6, sustained higher than WT mice (Fig. 6F). The results indicate that the immune responses in the initial site would be strongly elicited in IL-32γTG mice contrary to the entire body. Consequently, our data suggest that IL-32γ could attenuate widespread inflammation by enhancing chemokine-mediated neutrophil influx and production of proinflammatory cytokines in the primary infectious foci.

Experimental sepsis models have provided the knowledge of specific inflammatory mediators and adhesion molecules implicated in sepsis, but conclusions so far have yet to have clinical benefits. Antibody therapies against TNFα and IFNγ protect baboons [40] and mice [11] from bacterial infection, but antagonizing TNFα is ineffective in humans with sepsis [1, 9] and is even harmful [13]. Similarly, the supplementation of IFNγ enhances neutrophil function in several infectious models of mice [12] but does not improve patients’ outcomes [36]. A fact commonly ignored is that, in humans, neutrophils are the major population of leukocytes in the blood, whereas in the mouse, usually used as an animal model of sepsis, neutrophils are a minor population. Current treatment trials based on the previously established pathology are inadequate to reduce the high mortality rate.

In conclusion, these present data suggested that LPS-induced immune responses were suppressed in IL-32γTG mice by restraining systemic inflammation, but local immune responses were not affected in the primary injected area. Our findings on the mechanism of IL-32γ in the LPS-induced mouse model of septic shock will provide new insights on the role of IL-32γ in sepsis and other inflammatory diseases.

Acknowledgments

This paper was supported by Konkuk University in 2013.
References


