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IL-17 Receptor Signaling Is Required to Control Polymicrobial Sepsis

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Sepsis is a systemic inflammatory response resulting from the inability of the host to contain the infection locally. Previously, we demonstrated that during severe sepsis there is a marked failure of neutrophil migration to the infection site, which contributes to dissemination of infection, resulting in high mortality. IL-17 plays an important role in neutrophil recruitment. Herein, we investigated the role of IL-17R signaling in polymicrobial sepsis induced by cecal ligation and puncture (CLP). It was observed that IL-17R-deficient mice, subjected to CLP-induced non-severe sepsis, show reduced neutrophil recruitment into the peritoneal cavity, spread of infection, and increased systemic inflammatory response as compared with C57BL/6 littermates. As a consequence, the mice showed an increased mortality rate. The ability of IL-17 to induce neutrophil migration was demonstrated in vivo and in vitro. Beside its role in neutrophil recruitment to the infection focus, IL-17 enhanced the microbicidal activity of the migrating neutrophils by a mechanism dependent on NO. Therefore, IL-17 plays a critical role in host protection during polymicrobial sepsis.

Neutrophils are the major leukocytes promptly recruited to the inflamed site in response to infection or tissue injury. These cells are ideally suited to the elimination of pathogenic bacteria due to their capability of phagocytosis, releasing the stores of granular lytic enzymes and antimicrobial polypeptides into the phagolysosome (1). In this way, migrating neutrophils may control bacterial growth and, consequently, prevent bacterial dissemination and death (2–8). However, once the host fails to restrict the pathogens to a localized area, they and/or their products may spread systemically, resulting in an overproduction of cytokines/chemokines, systemic inflammatory response syndrome, septic shock, and death (9–11).

In fact, the importance of neutrophil recruitment to the infection focus in the development of sepsis has been clearly demonstrated in experimental and human sepsis by our laboratory. We showed that the failure of neutrophil migration is observed in severe polymicrobial sepsis (5, 7, 12), Gram negative or positive (6, 8). The impairment of neutrophil migration was associated with a decrease in the rolling and adhesion of neutrophils on endothelial cells, leading to a failure of bacterial clearance in the infection focus, increased number of bacteria in blood, high levels of circulating cytokines/chemokines, and mortality. Conversely, in mice subjected to non-severe sepsis, in which neutrophil migration failure was not observed, bacterial infection was restricted to the peritoneal cavity and, consequently, no mortality was observed (5–8, 12). Furthermore, neutrophils obtained from septic patients showed reduced chemotaxis in response to IL-8, leukotriene B4, and fMLP, and the intensity of the reduction of neutrophil chemotaxis correlated with the severity of the sepsis (13, 14). The mechanisms involved in neutrophil migration failure are not completely understood. However, it is known that systemic activation of TLRs by bacteria and/or their products results in excessive production of circulating cytokines/chemokines which stimulate the expression of inducible NO synthase, leading to high amounts of NO, which in turn result in the reduction of neutrophil/endothelium interaction and chemotaxis (5, 6, 8, 14–17).

IL-17 is the founding member of a new multimember cytokine family consisting of IL-17 (IL-17A) and IL-17B to IL-17F (18–25). IL-17 (IL-17A) is a proinflammatory cytokine produced mainly by the memory T cells known as Th17 (18, 26–29), a Th cell lineage distinct from Th1 and Th2 cells, which is negatively regulated by IFN-γ and IL-4 (30, 31). IL-17 plays a particularly significant role in regulating neutrophil recruitment and granulopoiesis. IL-17 up-regulates G-CSF and overexpression of IL-17 causes neutrophilia in mice (32). In addition, it has been demonstrated that IL-17 is involved in neutrophil recruitment via the production of several cytokines and CXC chemokines such as Gro-α (keratinocyte-derived chemokine (KC),3 CXCL1), LIX (CXCL5), and MIP-2 (CXCL2) (33–37). Recent reports indicate that IL-17 is critical in the clearance of several pathogens, including Klebsiella pneumoniae, Escherichia coli, Candida albicans, and Toxoplasma gondii (35, 38, 39). It was demonstrated that IL-17R-deficient mice showed an impaired host defense against K. pneumoniae, which was associated with a marked reduction in neutrophil recruitment to the lung (35). Moreover, it was also shown that the production of IL-17 after i.p. inoculation of E. coli is

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3 Abbreviations used in this paper: KC, keratinocyte-derived chemokine; BHI, brain-heart infusion; CLP, cecal ligation and puncture; NS-CLP, non-severe septic injury; S-CLP, severe septic injury; WT, wild type; KO, knockout; AG, aminoguanidine; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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critical for the local infiltration of neutrophils and control of bacteria growth (40). On the other hand, other authors demonstrated that the blockade of IL-17A was protective in cecal ligation and puncture (CLP)-induced sepsis. These authors associated the protective effects of IL-17A blockade with a reduction in the levels of bacteremia and of systemic proinflammatory cytokines and chemokines (41).

Therefore, in the present study using IL-17R-deficient mice, we investigated the role of IL-17R signaling in neutrophil migration to the infection focus and in the outcome of polymicrobial sepsis induced by CLP. We found that the absence of IL-17R signaling results in an impairment of neutrophil migration toward the infection focus followed by bacteremia and an increase in systemic inflammatory response, resulting in high mortality. Furthermore, IL-17 increased the microbicidal activity of neutrophils, by a NO-independent mechanism. Thus, our data provide clear evidence for a critical role of IL-17R signaling in the protective response against polymicrobial sepsis.

Materials and Methods

Mice

Adult male C57BL/6 wild-type (WT) mice were bred in a specific pathogen-free animal facility at the Transgenose Institut (Centre National de la Recherche Scientifique, Orleans, France). Adult male IL-17R knockout (KO) mice were on a C57BL/6 genetic background, as described previously (35), and provided by Amgen. Mice were maintained in a temperature-controlled (23°C) facility with a strict 12 h light-dark cycle and were given free access to food and water. The experiments were performed with gender-matched mice ages 10–12 wk. All animal experiments complied with the French government’s ethical and animal experiment regulations.

Drugs and reagents

Percoll, RPMI 1640, HBSS medium, and aminoguanidine were purchased from Sigma-Aldrich. Aminoguanidine was dissolved in saline. IL-17 was purchased from R&D Systems.

Induction of sepsis using CLP model

Sepsis was induced by the CLP model (42). Briefly, mice were anesthetized with ketamine and xylazine diluted in PBS (1.25 mg/ml ketamine:0.5 mg/ml xylazine) by the i.p. administration of 200 µl, and a 1-cm midline incision was made on the anterior abdomen. The cecum was exposed and ligated below the ileocecal junction without causing bowel obstruction. A triple puncture was made using a 30-gauge needle to induce non-severe septic injury (NS-CLP) and a double puncture with an 18-gauge needle to induce severe septic injury (S-CLP). Pressure was applied (the cecum was squeezed) to allow cecum contents to be expressed through the punctures. The cecum was placed back in the abdominal cavity and the peritoneal wall and skin incision were closed. All animals received 1 ml of saline s.c. immediately after the surgery. Sham-operated animals (controls) underwent identical laparotomy but without cecum ligation or puncture.

Materials and Methods

Effect of IL-17 in the microbicidal activity of neutrophils

Cecal bacteria isolation. For the killing assay, bacteria were isolated from the cecum of WT mice. Briefly, the cecum contents of three mice were removed, diluted in PBS, and filtered through sterile gauze. An aliquot of cecal content suspension was diluted in BHI medium (Difco Laboratories) and incubated for 20 h at 37°C and 5% CO2. The suspension was centrifuged (10 min, 2000 x g) and washed twice with PBS. The bacterial suspension was lyophilized (model CT 110; Hotovac) and stored at ~70°C. All steps were performed in sterile conditions. For bacterial counts, the lyophilized contents were diluted in BHI medium, homogenized, and incubated for 20 h at 37°C. The bacterial suspension was then centrifuged (10 min, 2000 x g) and the pellet was washed twice with PBS and resuspended in 10 ml of PBS. The number of CFU was determined through serial log dilutions and plating on Mueller-Hinton agar dishes (Difco Laboratories). CFU were counted after 18 h and the results are expressed as the log CFU/ml.

Isolation of neutrophils. WT mice were injected i.p. with thioglycolate (3%) to obtain peritoneal neutrophils. The peritoneal cells were harvested 6 h later by washing the peritoneal cavities with RPMI 1640 medium. Cell viability (trypan blue exclusion) was >98% and the cell population consisted of macrophages and neutrophils, with the latter representing >85% of total leukocytes.

Bacterial killing by neutrophils. The isolated WT and IL-17R KO neutrophils (1 x 10⁶/ml) were incubated in Eppendorf tubes for 1 h at 37°C and 5% CO2 in antibiotic-free RPMI 1640 with RPMI 1640 (control), 100 ng/ml IL-17, or 300 ng/ml IL-17. In addition, neutrophils from WT mice were incubated with aminoguanidine (AG; 300 µM), an inhibitor of NO synthase, or with IL-17 (100 ng/ml) plus AG (300 µM). Afterward, the bacterial suspension (2 x 10⁷/ml) was added to the neutrophil culture followed by incubation for 3 h at 37°C with mild shaking. As control, the bacterial suspension was incubated in the same experimental condition in the absence of neutrophils. At the end of these incubation times, the tubes were centrifuged (400 x g) and the pellet was lysed in 0.2% Triton X-100. Bacterial viability was assessed by serial log dilutions and plating on Mueller-Hinton agar dishes (Difco Laboratories). CFU were counted after 18 h and the results expressed as number of viable bacteria (log CFU/ml).

Determination of cytokine and chemokine levels

The cytokine and chemokine levels were detected in peritoneal exudate and in serum of mice, 6 h after CLP surgery, by ELISA according to the recommendations of the manufacturer (with detection limits in pg/ml). TNF-α, MIP-2, KC, and IL-17 were all purchased from R&D Systems. The results are expressed as pg/ml of peritoneal exudate or blood.

Leukogram

Animals were anesthetized with ketamine and xylazine and a sample of blood (diluted in EDTA) was collected 6 h after sepsis induction. Total counts were performed using a Neubauer chamber. Differential cell counts were conducted on cytospin slides (cytospin at 700 rpm for 10 min at room temperature) stained with Diff-Quik (Merz & Dade). Differential counts were performed under oil immersion microscopy, where 100 cells were counted for the determination of the percentage of neutrophils present in blood. The results are expressed as % SEM of the neutrophil number per ml.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity

The levels of AST and ALT (U/ml) were measured in the serum of mice 6 h after surgery as biochemical indicators of liver injury. The determinations were made using commercial colorimetric kits (Labtest). The results are expressed as means ± SEM.

Neutrophil isolation and chemotaxis

C57BL/6 mice were sacrificed by cervical dislocation. Bone marrow neutrophils were isolated as previously described (44). A modified Boyden chamber assay to examine the neutrophil chemotacticant response to IL-17 was performed using a 48-well microchamber (NeuroProbe). The stimulus IL-17 was diluted in RPMI 1640 and RPMI 1640 alone was used as a negative control. The stimuli and negative control were added to the lower chambers of the apparatus. A 5-µm pore polycarbonate membrane (NeuroProbe) was placed between the upper and lower chambers and 5 x 10⁵ cells
in a volume of 50 μl were added to the top chambers of the apparatus. Cells were allowed to migrate into the membrane for 1 h per treatment at 37°C with 5% CO₂. Following incubation, the chamber was disassembled and the membrane was scraped and washed in PBS to remove nonadherent cells before being fixed in methanol and stained using the Dif-Quik system (Dade Behring). Each well-associated membrane area was scored using light microscopy to count the intact cells present in five random fields. The results were expressed as the number of neutrophils per field.

**Statistical analysis**

The data (except for the survival curves and bacterial counts) were reported as the means ± SEM of values obtained from two different experiments. The means of different treatments were compared by ANOVA followed by Bonferroni’s t test for unpaired values (GraphPad Prism Software version 3). Bacterial counts were reported as median log CFU and were analyzed by the Mann-Whitney U test (GraphPad Prism Software version 3). The neutrophil chemotaxis data were analyzed by ANOVA with Tukey’s correction (GraphPad Prism Software version 3). The survival rate was expressed as the percentage of live animals, and the Mantel-Cox log rank test was used to determine differences between survival curves (GraphPad Prism Software version 3). A p value of 0.05 or less was considered significant.

**Results**

**IL-17R signaling is required for resistance against polymicrobial sepsis**

Initial experiments were designed to determine the involvement of IL-17R signaling in the outcome of polymicrobial sepsis. For this purpose, IL-17R gene-deficient (IL-17R KO) mice were subjected to NS-CLP or S-CLP polymicrobial sepsis using the CLP model. As shown in Fig. 1A, IL-17R-deficient mice displayed a significant reduction in survival rate following NS-CLP. At 60 h after NS-CLP, 100% of WT mice were alive compared with 80% in IL-17R KO mice. The detrimental effect of the absence of IL-17R signaling increased thereafter. On the third and fourth days after NS-CLP, the survival rates in WT mice stayed at 100%, whereas 60 and 40% survival rates were observed in IL-17R KO mice, respectively. With regard to S-CLP, both WT and IL-17R KO mice showed almost the same survival rate curves during the observation period. At 7 days after CLP, both groups had survival rates of only 20%. The sham-operated mice (WT and IL-17R KO) survived for the whole period of observation.

To identify the mechanisms underlying the susceptibility of IL-17R KO mice to NS-CLP, we investigated the neutrophil recruitment to the peritoneal cavity after sepsis induction. Fig. 1B shows that the WT animals subjected to NS-CLP showed a marked neutrophil migration into the peritoneal cavity 6 h after surgery, whereas IL-17R KO mice subjected to the same procedure displayed a significant reduction in neutrophil migration. Confirming previous results (4, 5, 7, 8, 12), Fig. 1B also shows that WT animals subjected to S-CLP failed to recruit neutrophils to the infection focus. Although it was not statistically significant, the IL-17R-deficient mice subjected to S-CLP showed a greater failure of neutrophil recruitment compared with WT mice. It was also observed that compared with sham-operated mice, WT animals subjected to NS-CLP produced significant amounts of IL-17A at the infection focus (Fig. 1C). Taken together, these results demonstrate that IL-17A is produced in CLP sepsis and IL-17R signaling plays a critical role in neutrophil recruitment to the infection focus and consequent host survival.

**IL-17R KO mice subjected to NS-CLP had increased bacteria levels in peritoneal exudate and in blood**

To associate the migration of neutrophils with the control of infection focus, we examined the capacity of IL-17R-defective mice to control bacterial dissemination. For this, we determined the bacterial load in the peritoneal exudate and in blood 6 h after...
non-severe sepsis induction. As shown in Fig. 2, IL-17 R KO mice subjected to NS-CLP had a marked increase in the amount of bacteria in peritoneal exudate and in blood. IL-17 R KO and their respective WT controls were subjected to sham surgery, NS-CLP, or S-CLP sepsis induced by the CLP model. Bacterial counts in the peritoneal exudate (A) and in blood (B) were performed 6 h after sepsis induction. The results are expressed as the median log of CFU/ml peritoneal exudate or blood (n = 10 animals/experimental group). *, p < 0.05 compared with sham group; #, p < 0.05 compared with WT mice subjected to NS-CLP; **, p < 0.05 compared with IL-17 R KO mice subjected to NS-CLP; and §, p < 0.05 compared with WT mice subjected to S-CLP (Mann-Whitney U test).

IL-17 affects neutrophil microbicidal activity

In addition, we explored whether IL-17, besides having the ability to recruit neutrophils to the infection focus, also increases the microbicidal activity of this leukocyte subtype. As shown in Fig. 3, neutrophils harvested from IL-17 R-defective mice demonstrated reduced killing activity compared with WT neutrophils, suggesting a physiological role of IL-17 R signaling in the microbicidal activity of neutrophils. Moreover, WT neutrophils incubated with IL-17 (100 or 300 ng/ml) for 3 h showed a significant increase in bacterial killing compared with WT neutrophils incubated with medium plus bacteria; **, p < 0.05 compared with WT neutrophils incubated with IL-17 (100 ng/ml) plus bacteria; and ##, p < 0.05 compared with WT neutrophils incubated with IL-17 (300 ng/ml) plus bacteria (Mann-Whitney U test).

IL-17R signaling is required for the control of systemic inflammatory response

To further extend the importance of IL-17 R signaling in the outcome of sepsis, we evaluated the systemic inflammatory response in mice subjected to sepsis. We therefore determined the systemic concentrations of TNF-α (index of systemic cytokine production) and the hepatic enzymes AST and ALT (index of organ dysfunction). As shown in Fig. 4A, the serum levels of TNF-α 6 h after surgery were significantly increased in IL-17 R gene-deficient mice subjected to NS-CLP compared with the WT NS-CLP group. Furthermore, the IL-17 R KO mice subjected to non-severe infection showed a significant increase in serum levels of AST and ALT.

FIGURE 2. IL-17R-deficient mice subjected to NS-CLP showed increased amounts of bacteria in peritoneal exudate and in blood. IL-17 R KO and their respective WT controls were subjected to sham surgery, NS-CLP, or S-CLP sepsis induced by the CLP model. Bacterial counts in the peritoneal exudate (A) and in blood (B) were performed 6 h after sepsis induction. The results are expressed as the median log of CFU/ml peritoneal exudate or blood (n = 10 animals/experimental group). *, p < 0.05 compared with sham group; #, p < 0.05 compared with WT mice subjected to NS-CLP; **, p < 0.05 compared with IL-17 R KO mice subjected to NS-CLP; and §, p < 0.05 compared with WT mice subjected to S-CLP (Mann-Whitney U test).

FIGURE 3. IL-17 increases neutrophil microbicidal activity. Neutrophils harvested from WT and IL-17 R KO mice were incubated in vitro for 1 h at 37°C and 5% CO2 with antibiotic-free RPMI 1640, 100 ng/ml IL-17, or 300 ng/ml IL-17. Moreover, neutrophils from WT mice were incubated with AG (300 μM) or IL-17 (100 ng/ml) plus AG (300 μM). Neutrophil killing was evaluated by incubating these neutrophils (1 × 10^9/ml) with cecal bacteria (2 × 10^9/ml) at 37°C for 3 h with mild shaking as described in Materials and Methods. The number of replicates ranged between 4 and 15. The results are expressed as the number of viable ingested bacteria (log CFU/ml). *, p < 0.05 compared with bacteria incubated without neutrophils; #, p < 0.05 compared with WT neutrophils incubated with medium plus bacteria; **, p < 0.05 compared with WT neutrophils incubated with IL-17 (100 ng/ml) plus bacteria; and ##, p < 0.05 compared with WT neutrophils incubated with IL-17 (300 ng/ml) plus bacteria (Mann-Whitney U test).
compared with WT mice subjected to the same procedure (Fig. 4, B and C). These data indicate that the absence of IL-17R signaling leads to diminished recruitment of neutrophils to the infection focus in the absence of IL-17R signaling. IL-17R KO mice and their respective WT controls were subjected to sham surgery or NS-CLP sepsis induced by the CLP model. The serum levels of AST (B) and ALT (C) were determined 6 h after sepsis induction. The results are expressed as means ± SEM of 10 animals per experimental group. *, p < 0.05 compared with sham group; #, p < 0.05 compared with WT mice subjected to NS-CLP (ANOVA followed by Bonferroni’s test).

FIGURE 5. Chemokine and cytokine production in the infection focus in the absence of IL-17R signaling. IL-17R KO mice and their respective WT controls were subjected to sham surgery or NS-CLP sepsis induced by the CLP model. TNF-α, MIP-2, and KC concentrations (pg/ml) were quantified in the peritoneal exudate (A, B, and C, respectively) 6 h after CLP as described in Materials and Methods. The results are expressed as means ± SEM of five animals in each group. *, p < 0.05 compared with sham group (ANOVA followed by Bonferroni’s test).
focus and reduced microbicidal activity of the neutrophils with consequent spread of bacteria infection, which contribute to the development of systemic inflammatory response syndrome and organ injury observed in sepsis.

Cytokine and chemokine production at the infection focus in the absence of IL-17R signaling

To determine whether the reduced neutrophil recruitment to the site of infection observed in IL-17R KO mice subjected to NS-CLP is due to reduced production of chemotactic mediators, we measured the levels of chemotactic cytokines and chemokines in the peritoneal cavity of IL-17R KO and WT mice subjected to NS-CLP. Interestingly, as shown in Fig. 5, the peritoneal exudate concentrations of TNF-α, MIP-2, and KC in IL-17R KO mice subjected to NS-CLP were not lowered compared with WT mice 6 h after sepsis induction. Altogether, these data suggest that the reduction in neutrophil recruitment observed in IL-17R KO mice subjected to non-severe infection is not due to reduced production of TNF-α, MIP-2, or KC at the site of infection.

**IL-17R-defective mice subjected to non-severe sepsis have fewer circulating leukocytes**

Considering the literature data showing that IL-17 up-regulates G-CSF and that overexpression of IL-17 causes neutrophilia in mice (32), we determined the number of circulating neutrophils in IL-17R KO mice subjected to sepsis. It was observed that the baseline numbers of blood-circulating leukocytes and neutrophils were similar in naive IL-17R KO and in WT control mice. However, after the induction of non-severe sepsis, IL-17R-deficient mice had fewer circulating neutrophils compared with WT mice (Table I).

**IL-17 induces neutrophil migration to peritoneal cavity and neutrophil chemotaxis**

Reinforcing the importance of IL-17A in neutrophil migration, we demonstrated in Fig. 6A that i.p. injection of IL-17A (3–30 ng/cavity) induces significant neutrophil migration to the peritoneal cavity 6 h after cytokine administration. Furthermore, it was also observed that IL-17 induces neutrophil chemotaxis in the Boyden chamber (Fig. 6B). Therefore, the lack of IL-17R signaling may contribute to diminished neutrophil recruitment and lower host resistance to sepsis.

**Discussion**

Neutrophil recruitment to the site of infection is critical for host defense against invading pathogens, especially extracellular bacteria (1, 45). We have shown that the severity of sepsis induced by CLP (4, 5, 8, 12) or *Staphylococcus aureus* inoculation (6) is closely associated with reduced neutrophil migration to the infection focus. Furthermore, neutrophils from septic patients display a suppressed chemotactic response to iNLP and leukotriene B₄, in contrast to neutrophils from healthy controls (13).

To understand the mechanism involved in neutrophil migration to infection focus and consequently in confining the infection locally, we demonstrated in the present study the critical role of IL-17R signaling. IL-17 has been shown to be one of the major cytokines involved in the recruitment of neutrophils in several experimental models of inflammation (35, 46, 47). Our results show that in IL-17R gene-deficient mice marked diminution of neutrophil migration to the infection focus during NS-CLP-induced sepsis and, as a result, had a significantly lower survival rate. In line

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**Table 1. Number of circulating leukocytes of C57BL/6 (WT) and IL-17R KO mice 6 h after polymicrobial sepsis induced by CLP**

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<thead>
<tr>
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<th>Sham WT</th>
<th>NS-CLP WT</th>
<th>Sham IL-17R KO</th>
<th>NS-CLP IL-17R KO</th>
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<td>Total leukocytes</td>
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<tr>
<td>×10⁶/ml</td>
<td>5.7 ± 0.2</td>
<td>6.16 ± 0.7</td>
<td>4.6 ± 0.95</td>
<td>3.9 ± 0.49⁶</td>
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<td>Neutrophils</td>
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<tr>
<td>×10⁶/ml</td>
<td>2.02 ± 0.15</td>
<td>2.32 ± 0.32</td>
<td>1.64 ± 0.51</td>
<td>1.39 ± 0.20⁶</td>
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<tr>
<td>Mononuclear cells</td>
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<tr>
<td>×10⁶/ml</td>
<td>3.67 ± 0.04</td>
<td>3.84 ± 0.41</td>
<td>2.95 ± 0.43</td>
<td>2.53 ± 0.32⁶</td>
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*Animals C57BL/6 (WT) and IL-17R KO were subjected to sham or NS-CLP. Blood samples were collected 6 h after CLP, and total leukocytes, neutrophils, and mononuclear cells were analyzed, as described in Materials and Methods. The results are expressed as means ± SEM of four animals in each group (sham and NS-CLP).

⁶p < 0.05 compared to NS-CLP WT (analysis of variance, followed by Bonferroni’s test).

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**FIGURE 6.** IL-17 induces neutrophil migration to peritoneal cavity and neutrophil chemotaxis. A, Neutrophils were harvested from the peritoneal cavity 6 h after the i.p. injection of IL-17 (3, 10, and 30 ng/cavity) or its vehicle (saline; control group) in C57BL/6 mice. The results are expressed as the mean ± SEM of five animals per experimental group. *p < 0.05 compared with mice injected with saline (ANOVA, followed by Bonferroni’s test). B, Bone marrow neutrophils from C57BL/6 mice were isolated as described in Materials and Methods. Neutrophil migration in chemotaxis microwell chambers stimulated by 1-h incubation with RPMI 1640 (vehicle; control group) or IL-17 (1–30 ng/ml) was evaluated. *p < 0.05 compared with RPMI 1640 control group (ANOVA followed by Tukey’s test).
with these findings, recent studies demonstrated the importance of IL-17R signaling in host defense against infectious microorganisms, such as *K. pneumoniae*, *E. coli*, *C. albicans*, and *T. gondii* (35, 38–40). On the other hand, this is in contrast to findings from another study which demonstrated that the blockage of IL-17A protected mice from lethality induced by CLP (41). This inconsistency may be explained by the differences in experimental approaches used: we used IL-17R KO-defective mice, whereas the above-mentioned study used neutralizing mAb against only IL-17A. In our experimental conditions, signaling to IL-17A and IL-17F was absent, since both cytokines bind to IL-17R. IL-17F, a member of the IL-17 family, is the isoform that has the highest degree of homology with IL-17A and has a similar spectrum of activity in terms of mobilizing neutrophils (25). Moreover, both IL-17A and IL-17F induce the production of granulopoietic factors (G-CSF and stem cell factor) and CXC chemokines (26, 33, 36, 48, 49).

We found that the loss of IL-17R signaling during non-severe polymicrobial sepsis results in higher numbers of bacteria in the peritoneal cavity and in blood, confirming that neutrophil migration to the infection focus is a crucial event in controlling and overcoming sepsis. The importance of IL-17 against extracellular bacterial infection was first directly proved in the *K. pneumoniae* lung infection model. The authors demonstrated that IL-17R-defective mice showed reduced survival after pulmonary *K. pneumoniae* infection which is associated with increased bacterial number and reduced neutrophil recruitment in the lung (35). Our findings also corroborate another study which demonstrated that impairment of IL-17R signaling results in reduced neutrophil recruitment to the peritoneal cavity after i.p. *T. gondii* infection with consequent increase in mortality (39). It was recently demonstrated that i.p. or intratracheal administration of IL-17 induces significant neutrophil recruitment, confirming that IL-17 induces neutrophil migration (33, 35). Moreover, it seems that IL-17 is also involved in the neutrophil influx into affected organs during systemic inflammation. In fact, in a recent study, it was demonstrated that the neutrophil accumulation in the small intestine observed in TNF-induced shock is reduced by antiserum against IL-17 or deletion of IL-17R (50). These authors also observed that these treatments increase the survival of the TNF-injected mice, which could appear to be in contradiction with our study. However, it is not the case, since in shock in which an infection focus is absent, as observed with LPS or TNF administration, the inhibition of neutrophil migration to the organs has mainly a beneficial effect because it protects the host organs from injury (51–53).

Besides neutrophil migration to the infection focus, microbicidal activity of the migrating cells is fundamental to the containment of the infection (54). In this way, it was observed that neutrophils harvested from IL-17R-defective mice already show reduced microbicidal activity compared with WT neutrophils. Furthermore, the microbicidal activity of WT neutrophils was strongly enhanced by IL-17 treatment. Together these results suggest that IL-17R signaling is critical to the microbicidal activity of neutrophils. The potentiating effect of IL-17 on bacterial killing was blunted in neutrophils from IL-17R-defective mice. It argues against a direct killing effect of the cytokine or contaminant present in the cytokine preparation. Our findings corroborate those of others showing that IL-17 potentiates in vitro neutrophil killing of pneumococcus (55). However, they are in apparent contradiction with the demonstration that IL-17 reduces neutrophil antifungal activity (56). The use of different neutrophil microbial targets may explain the observed differences. Furthermore, our data showed that IL-17 effects are mediated by NO. In accordance, other authors demonstrated that IL-17 induces the expression of inducible NO synthase and NO production in various cell types, including endothelial cells, microglia, and chondrocytes (57–59). In addition, NO is a key mediator of neutrophil microbicidal activity against the majority of pathogens, including Gram-positive and -negative bacteria (2, 3, 60). Additionally, it was recently demonstrated that IL-17 activates macrophage killing of *Bordetella pertussis* (61). Furthermore, IL-17 also enhances the production of antimicrobial substances, such as β-defensins and the acute phase protein 24p3/lipocalin 2, which exhibit potent antibacterial activities (62, 63). Altogether, this study clearly demonstrates the importance of IL-17 in the protective response against infection.

Sepsis is a systemic inflammatory response resulting from the inability of the host to confine the infection locally. Systemic inflammatory response is considered a central deleterious pathogenic event in sepsis. High levels of serum inflammatory cytokines and chemokines are considered important mediators in the development of lethal multiple organ dysfunction syndrome (64, 65). Consistent with the inability of IL-17R-deficient mice subjected to non-severe sepsis to control infection, these mice showed increased systemic levels of TNF-α and of the enzymes AST and ALT, markers of liver injury.

Recent studies demonstrated that IL-17 induces the release of several inflammatory mediators, including chemokines (e.g., KC, MIP-2, and LIX), which induce neutrophil migration (33–35, 37). In an apparent contradiction, we found here that the peritoneal exudate concentrations of TNF-α, MIP-2, and KC in IL-17R-defective mice subjected to NS-CLP were not reduced compared with WT mice subjected to the same procedure. The explanation for these divergent findings is that during the sepsis process, besides IL-17 production, other mediators are produced, such as TNF-α and IL-1β (4, 8), and they also stimulate chemokine production (66–68).

Taking into account that in our study the levels of cytokines and chemokines at the infection site of IL-17R KO mice were not reduced, we decided to investigate why the IL-17R KO mice showed reduced neutrophil migration to the infection focus. Accordingly, we showed that i.p. injection of IL-17 induces neutrophil migration to the peritoneal cavity of mice and, interestingly, we demonstrated a direct effect of IL-17 in which it induced neutrophil chemotaxis in the Boyden chamber. These results suggest that although cytokines and chemokines released by an IL-17R-independent mechanism could contribute to neutrophil migration, IL-17R signaling could be critical to neutrophil recruitment to the infection focus.

The literature data demonstrate that IL-17 also stimulates hematopoiesis, particularly granulopoiesis (69), partly due to the stimulation of G-CSF and the transmembrane form of stem cell factor (70). The present study shows that IL-17R-deficient mice possess less ability to recruit neutrophils into the circulation after the induction of sepsis. In accordance, Tan et al. (71) demonstrated that mice with homozygous deletions of IL-17R have normal circulating numbers of neutrophils, but are much more susceptible to sublethal γ irradiation and show reduced neutrophil recovery. These data suggest that stress-induced granulopoiesis requires IL-17R signaling (71). Furthermore, another study showed that the number of blood neutrophils in IL-17R KO mice is significantly reduced compared with control WT mice after infection with *K. pneumoniae*, demonstrating that these mice have defective granulopoiesis in response to infection (35). Altogether, our results suggest that the reduction in neutrophil recruitment observed in IL-17R KO mice subjected to non-severe sepsis can also be, at least in part, due to the reduced number of circulating neutrophils.

In summary, we demonstrated a critical role of IL-17R signaling in the outcome of sepsis. We showed that IL-17R gene-deficient
mice subjected to non-severe sepsis have reduced neutrophil recruitment to the infection focus, dissemination of infection, and increase in systemic inflammatory response with consequent increase in mortality rate. Finally, it was demonstrated that IL-17 increases neutrophil killing capacity and induces neutrophil migration in vivo and in vitro. Therefore, the data suggest that IL-17 contributes to host protection against sepsis.

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Disclosures

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