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Critical Role of Signaling Through IL-1 Receptor for Development of Arthritis and Sepsis During Staphylococcus aureus Infection

Olof H. Hultgren, Lena Svensson, and Andrej Tarkowski

IL-1R-deficient mice (IL-1R<sup>−/−</sup>) and their wild-type controls (IL-1R<sup>+/+</sup>) were i.v. inoculated with 1 × 10<sup>7</sup> or 10<sup>6</sup> *Staphylococcus aureus* per mouse to mimic bacterial sepsis and septic arthritis. The disease outcome was severely worsened in the IL-1R<sup>−/−</sup> mice as compared with IL-1R<sup>+/+</sup> mice. Indeed, 3 days after inoculation of 10<sup>7</sup> *S. aureus* per mouse 84% of IL-1R<sup>−/−</sup> mice displayed clinical signs of septicemia as compared with none of the IL-1R<sup>+/+</sup> mice. On day 9 after inoculation with 10<sup>6</sup> *S. aureus* per mouse 75% of the IL-1R<sup>−/−</sup> mice were dead as compared with none of the IL-1R<sup>+/+</sup> mice. Also, the number of staphylococci in circulation was 25- to 30-fold increased in IL-1R<sup>−/−</sup> mice as compared with IL-1R<sup>+/+</sup> mice, the most probable reason for the outcome. The frequency and severity of septic arthritis were significantly increased in IL-1R<sup>−/−</sup> mice, as compared with IL-1R<sup>+/+</sup> mice, following i.v. inoculation of staphylococci. This was probably due to an increased accumulation of bacteria in the joints of IL-1R<sup>−/−</sup> mice as compared with their wild-type controls. Interestingly, while serum levels of IL-18 in IL-1R<sup>−/−</sup> mice were significantly lower than in IL-1R<sup>+/+</sup> mice 24 h after inoculation of *S. aureus*, both IL-18 and IL-1β were significantly increased in IL-1R<sup>−/−</sup> vs IL-1R<sup>+/+</sup> mice 4 days after the bacterial inoculation. In conclusion, IL-1R signaling plays a crucial role in host protection during systemic *S. aureus* infection as seen by the fatal outcome of *S. aureus* sepsis and arthritis in IL-1R-deficient mice. *The Journal of Immunology*, 2002, 168: 5207–5212.

Sepsic arthritis is a highly destructive joint disease with high mortality. *Staphylococcus aureus* is the causative organism in ~40% of adult cases and the common route of bacterial spreading is via the bloodstream (1). A mouse model has been established in which staphylococci are i.v. inoculated to trigger sepsis and septic arthritis (2). The pathogenesis of murine *S. aureus* sepsis and septic arthritis involves bacterial as well as host factors. The expression of certain bacterial virulence factors, e.g., capsular carbohydrates, adhesins, and regulatory genes, make the bacteria more resistant to phagocytic functions, and the increased bacterial survival results in an increased joint inflammation as well as increased risk of death (3). The host response must meet the demand of an effective clearance of bacteria.

One of the first steps for the innate immune system in recognizing staphylococci is via the Toll-like receptors (TLRs) (4), e.g., TLR2 and TLR4, recognizing peptidoglycan and lipoteichoic acids, respectively (5). The TLRs share an intracellular (IC) signal pathway with at least two cytokine receptors, IL-1R (6) and IL-18R (7). The receptor signal is conducted in sequence via a myeloid differentiation marker (MyD88), IL-1R-associated kinase, TNFR-associated factor 6, and TGF-β-activated kinase, leading to nuclear translocation of NF-κB (4, 8). Following activation of NF-κB several genes with proinflammatory end products are transcribed. The importance of this IC signal pathway for protection against staphylococcal infection was recently shown using MyD88-deficient mice (9).

While IL-18 was previously shown to protect against joint inflammation but aggravate septicemia during *S. aureus* arthritis (10), the importance of IL-1αβ signaling during septic arthritis is hitherto unknown. The results from a model of noninfectious arthritis show that blockade of IL-1β or IL-1α delays disease onset and ameliorates already established collagen-induced arthritis (11). Furthermore, IL-1R antagonist-deficient mice develop spontaneous arthritis resembling rheumatoid arthritis (12), altogether indicating arthritogenic properties of IL-1R signaling in inflammatory joint diseases. IL-1R is responsible for signal transduction of IL-1αβ (13), while the type 2 receptor is lacking a cytoplasmic tail and is therefore not capable of signal transmission but functions rather as a decoy receptor (14).

Using IL-1R-deficient mice and their wild-type controls, we present evidence for the major importance of IL-1R signaling in *S. aureus* infection in controlling staphylococcal growth and subsequently protecting the host against septic death and septic arthritis.

Materials and Methods

**Mice, bacteria, and inoculation procedure**

Age- and sex-matched male or female 6- to 15-wk-old C57BL/6 mice, intact or defective with respect to the IL-1R type I gene, were used throughout the study. Procedure of IL-1RI gene disruption has been described in detail elsewhere (15). IL-1R-deficient mice (B6.129S7-Il1r<sup>tm1Imx</sup>) as well as their wild-type controls (C57BL/6J) were obtained from The Jackson Laboratory (Bar Harbor, ME). At the time of experiments, IL-1R-deficient mice had undergone five backcrosses to the C57BL/6 background. Mice were kept and bred at the animal facility of the Göteborg University (Göteborg, Sweden). They were kept under standard conditions for at least 2 wk prior to experimentation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 Abbreviations used in this paper: TLR, Toll-like receptor; IC, intracellular; rm, recombinant murine.

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conditions of temperature and light and were fed standard laboratory chow and water ad libitum.

*S. aureus* strain LS-1 was originally isolated from a spontaneously arthritic New Zealand Black/White mouse (16). Before each experiment, bacteria were cultured on blood agar (5% human erythrocytes) for 24 h and then reincubated on blood agar for another 24 h. A bacterial solution was prepared in PBS at a concentration of 5 × 10^6 or 5 × 10^5 bacteria/ml. Two hundred microliters of the solution were injected into one of the tail veins on day 0. Viable counts were used to check the concentration of bacteria injected.

**Evaluation of arthritis and sepsis**

All mice were bled and monitored individually. Limbs were inspected at regular intervals. Arthritis was defined as visible erythema and/or swelling of at least one joint. To evaluate the severity of arthritis we used a clinical scoring in which macroscopic inspection yields a score of 0–3 for each paw (0, normal; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and occasionally ankylosis), resulting in an arthritis index ranging from 0 to 12 for each mouse. Arthritic index was calculated by a summation of arthritis scores from all mice in the experimental group divided by the number of animals in each group.

The severity of sepsis was judged clinically modified from a previous description (17) where coat appearance (0–1), posture or movements (0–1), and temperature (0–1) yielded a severity score of maximally 3 per mouse. Temperature score was obtained when the mice were severely ill and cold as judged by hand.

Weight was regularly checked with 0.1-g accuracy.

**Determination of staphylococcal load ex vivo**

Bacterial samples from talocrural and radiocarpal joints were obtained using cotton sticks. The bacterial presence was defined if 15 or more CFU per joint were found. Both kidneys were removed aseptically, placed on ice, homogenized, and diluted in 10 ml PBS. Viable counts were done to examine bacterial concentration. To check the staphylococcal load in blood, mice were bled and viable counts were used to titrate the bacterial concentration.

Colonies were tested for catalase (using 18% hydrogen peroxide) and coagulase (using rabbit EDTA-plasma; BD Microbiology Systems, Sparks, MD) reactivity.

**Hematological analyses**

Mice were bled from the tail into heparinized tubes. Total leukocyte counts were determined in a hematocytometer (Sysmex KX-21; TOA Medical Electronics, Kobe, Japan). Blood smears were prepared and stained by the May-Grunewald-Giemsa method for differential counts.

**In vitro assays of cytokine production, phagocytosis, and IC killing**

Spleen cells were passed through a nylon mesh, and erythrocytes were depleted by NH_4Cl lysis. Splenocytes were cultured at 2 × 10^6 cells/ml in Iscove’s medium supplemented with 10% FCS, 5 × 10^{-3} M 2-ME, 2 mM t-glutamine, and 50 μg/ml gentamicin and incubated with 1 × 10^7/ml formalin-killed *S. aureus* LS-1 strain. Supernatants from cell cultures were collected after 24 and 48 h.

Intraperitoneal macrophages were extracted, adjusted to 2 × 10^6 cells/ml, and incubated in a 24-well plate (Nunc, Roskilde, Denmark) according to a previously detailed procedure (18, 19). Adherent macrophages were incubated with 500 μl of *S. aureus* at a concentration of 5 × 10^7 bacteria/ml for 50 min and subsequently washed three times in Iscove’s medium. The IC content of live *S. aureus* was then checked at three time intervals: 0, 4, and 24 h. The content at the 0-h interval is regarded as a measure of the phagocytosis, and the two later time intervals are regarded as a measure of the efficacy of the IC killing capacity contra-IC growth of bacteria (18).

**Cytokine reagents and analyses**

Recombinant murine (rm)IL-1β (R&D Systems, Minneapolis, MN) was reconstituted in accordance with the manufacturer’s instructions, then further diluted in PBS to desired concentration.

IL-6 was measured using the murine hybridoma cell line B13.29. subclone B9, selected for its IL-6 dependency (20). For the assay, B9 cells were harvested by centrifugation, and after one wash in IL-6-free medium they were seeded at 5 × 10^3 cells/well into microtiter plates (Nunc) in the presence of serum samples and grown for 68 h before adding [H]^Hthyminidine to study proliferative responses to IL-6. After 4 h, the cultures were harvested onto filters and counted in a beta counter. Mouse IL-6 (Genzyme, Cambridge, MA) was used as a standard. The serum samples and standards were all set up in triplicate.

TNF and IL-1β were measured using Quantikine mouse immunoassays (R&D Systems). IL-18 was measured at a quantitative test kit for mouse IL-18 (MBL, Nagoya, Japan). All the assays were performed in accordance with the manufacturer’s instructions.

**Statistics**

Categorical data were analyzed using Fisher’s exact test, and unpaired Student t test was used for statistical analyses of weight changes. All other data were analyzed using the nonparametric Mann-Whitney U test. A value of *p* ≤ 0.05 was regarded as statistically significant. Results are presented as mean ± SEM (n = number of mice).

**Results**

**Severe sepsis and decreased survival in IL-1R-deficient mice during *S. aureus* infection**

IL-1R-deficient mice and their wild-type controls were i.v. inoculated with 1 × 10^7 *S. aureus* per mouse. The obvious clinical signs of sepsis in IL-1R knockout mice were seen on the third day after inoculation of bacteria, when a majority of the IL-1R-deficient mice displayed sepsis as compared with none of the wild-type mice (Fig. 1). As expected, the severity of sepsis differed significantly between groups (1.2 ± 0.2 vs 0 ± 0; *p* < 0.0001) at the same time point. The IL-1R-deficient mice started to die at day 4. In a first experiment, three of 15 IL-1R/−/− were dead at day 4 as compared with none of the controls (0 of 17; NS). The survivors were severely ill and were sacrificed at day 4 to obtain organ samples. The weight was checked daily and, interestingly, 24 h after the inoculation of 1 × 10^7 *S. aureus* the wild-type mice displayed significantly more weight loss as compared with the IL-1R-deficient animals. This finding was repeated in a second experiment with the 1 × 10^7 dose (data not shown). However, at days 2 and 3 after the inoculation of 1 × 10^7 *S. aureus* no differences in weight loss were recorded between groups, though the general appearance as measured by sepsis score was highly different at day 3.

These results show that the susceptibility of the IL-1R/−/− mice to *S. aureus* infection is increased. To titrate how high the susceptibility is, IL-1R/−/− and IL-1R+/+ were inoculated with 1 × 10^6 *S. aureus* per mouse. As in the case of high dose experiment, wild-type mice responded to infection with an initially more pronounced weight loss as compared with IL-1R knockouts (Fig. 2).

**FIGURE 1.** Septicemia in 25 IL-1R-deficient mice and 25 wild-type mice following i.v. inoculation of 1 × 10^7 *S. aureus* per mouse. Data are pooled from two experiments. Value of *p* is calculated using Fisher’s exact test.
With this lowered bacterial dose, signs of septicemia were not evident in any of the groups at day 3. However, at day 7 after the inoculation of *S. aureus*, the IL-1R-deficient mice had lost significantly more weight as compared with wild-type animals, and three of 12 IL-1R−/− were dead as compared with none of the controls. The experiment was finished 2 days later at day 9. At this time point, only 25% (3 of 12) of the IL-1R−/− mice were alive, whereas no mortality was seen in the wild-type mice (Fig. 3).

**IL-1R-deficient mice display increased frequency and severity of *S. aureus* arthritis**

At day 3 after inoculation of 1 × 10⁷ *S. aureus*, 36% of the wild-type mice (*n = 25*) displayed septic arthritis as compared with 68% of IL-1R-deficient mice (*n = 25*; *p < 0.05*). Similarly, the severity of arthritis at day 3 was more pronounced in IL-1R-deficient animals as compared with wild-type mice (Fig. 4). Due to severe septicemia in the IL-1R-deficient group, experiments were finished at days 3–4. To be able to follow the development of arthritis for a longer time, a markedly reduced inoculum size (10⁶ per mouse) was given i.v. to 12 IL-1R−/− mice and 15 IL-1R+/+ control animals. Again, at day 3, differences were seen between groups, IL-1R−/− mice displaying somewhat increased frequency of septic arthritis as compared with IL-1R+/+ mice (42 vs 7%; *p = 0.06*). At day 7, 45% (four of nine) of the IL-1R-deficient mice displayed arthritis as compared with 7% (1 of 15) of wild-type mice (*p < 0.05*). The severity of arthritis was significantly increased in IL-1R−/− vs IL-1R+/+ mice at days 3 and 7 also after inoculation of a lower dose of staphylococci (Fig. 4). The experiment was finished at day 9 after inoculation of 1 × 10⁶ *S. aureus* per mouse, a time point when one of three IL-1R-deficient mice displayed arthritis as compared with 2 of 15 wild-type animals.

The state of IL-1R deficiency is not beneficial to host with regard to survival or septic arthritis. There are at least two hypothetical possibilities for this finding. 1) IL-1R−/− mice accumulate live *S. aureus* in tissues as compared with wild-type mice, or 2) IL-1R signaling down-regulates inflammation during *S. aureus* arthritis, directly or indirectly. To check the first possibility we examined the bacterial load in tissues of IL-1R−/− and IL-1R+/+ mice after inoculation of 1 × 10⁷ *S. aureus* per mouse. The load of *S. aureus* was checked in blood, kidneys, and joints after i.v. inoculation of 1 × 10⁷ *S. aureus* per mouse. While very few staphylococci were found in blood 4 h after inoculation of bacteria in both IL-1R−/− and IL-1R+/+ groups, increasingly more *S. aureus* were found in the blood of IL-1R−/− as compared with wild-type mice 48 and 72 h after bacterial inoculation (Fig. 5A). Indeed, the increase of *S. aureus* in the circulation of IL-1R−/− mice was ~100-fold between the time points 4 and 72 h. Such an increase was absent in wild-type mice. The bacterial load in kidneys was checked at day 4 after inoculation of bacteria. IL-1R-deficient mice displayed a higher load (3.1 ± 1.1 × 10⁸ CFU)
as compared with wild-type mice (4.9 ± 1.2 × 10^7 (NS)). However, at day 4, three IL-1R^−/− mice of 15 had died, probably the most sick ones carrying the highest bacterial load.

The bacterial presence in joints was examined also at day 4 after bacterial inoculation. Staphylococci were significantly more prevalent in IL-1R-deficient mice as compared with their controls (Fig. 5B). Furthermore, five of the seven IL-1R^−/− mice with presence of *S. aureus* displayed positive culture in two or more of the four joints checked. The only wild-type mouse with presence of staphylococci displayed one positive joint.

Because IL-1β is a potent mediator of neutrophil recruitment from bone marrow into blood (21), we checked whether IL-1R signaling influences the neutrophil counts in circulation in response to *S. aureus* infection. While no difference in neutrophil counts was recorded between IL-1R intact or IL-1 defect mice before inoculation of staphylococci, blood of IL-1R-deficient mice displayed significantly fewer neutrophils 4 and 24 h after the inoculation of bacteria (Fig. 6).

Next, we analyzed the phagocytic properties as a function of IL-1R expression. The phagocytosis did not differ between IL-1R^−/− and IL-1R^+/− macrophages preincubated with or without 100 pg/ml rmIL-1β. However, the addition of rmIL-1β at 1 ng/ml to the cultures significantly increased phagocytosis by wild-type macrophages as compared with IL-1R-deficient macrophages (3973 ± 673 S. aureus per 10^5 cells vs 2257 ± 110; p < 0.05). Expression of IL-1R did not affect the IC killing ability of macrophages.

**IL-1R signaling increases production of TNF and IL-6 in vitro and controls production of IL-18 in vivo in response to *S. aureus***

Next we analyzed the importance of IL-1R signaling for production of cytokines in vivo in response to *S. aureus* arthritis and in vitro to formalin-killed *S. aureus*. While TNF and IL-6 are produced in response to *S. aureus*, the role of IL-1R signaling for production of these cytokines during staphylococcal infection has not been previously analyzed. The serum concentration of these two cytokines was assayed in IL-1R intact/defect mice 4 h, 24 h, and 4 days after inoculation of *S. aureus*. The importance of IL-1R signaling for the in vivo production of IL-6 was seen already at 4 h after bacterial inoculation. Later, differences in IL-6 production due to functional IL-1R were diminished and overcome (Table I). In contrast, IL-1R deficiency did not affect TNF production during staphylococcal infection (Table I). In vitro, IL-6 was severely decreased in absence of IL-1R signaling (Table II). Spleen cells, defect with respect to IL-1R, stimulated with formalin-killed *S. aureus* cell walls produced significantly less TNF and IL-6 as compared with spleen cells with an intact IL-1R (Table II). The production of IL-1β was not influenced by the deficiency of IL-1R as measured by serum concentration at 4 or 24 h after bacterial inoculation or, in vitro, in response to *S. aureus* cell walls. However, 4 days after inoculation of *S. aureus* the serum concentration of IL-1β was significantly increased in IL-1R-deficient mice as compared with wild-type mice (Table I).

Measuring the serum concentration of IL-18 24 h after bacterial inoculation, the IL-1R-deficient mice displayed a decreased production as compared with wild-type mice, indicating that IL-1R signaling is important for optimal production of IL-18. Interestingly, 4 days after inoculation of *S. aureus* IL-18 production was significantly increased in the IL-1R-deficient mice as compared with wild-type controls (Table I). IL-18 was not detected in vitro in response to formalin-killed *S. aureus*. 
Inflammatory cytokines such as TNF, IL-6, IL-1 due to, e.g., a nonoptimal recruitment of neutrophils and a diminished IL-1R-decient mice, as compared with IL-1R intact controls, is the reason for the severe clinical outcome of septic arthritis in IL-1R-decient mice. IL-18 found in IL-1R-decient mice as compared with wild-type controls could thus contribute to the severe septicemia of S. aureus infection. The high serum levels of IL-18 as shown to be a detrimental mediator in sepsis development of septicemia (10). The high serum levels of IL-18 found in IL-1R-deficient mice as compared with wild-type controls could thus contribute to the severe septicemia of S. aureus infection. The severe bacteremia in IL-1R-deficient mice is present in parallel with obvious clinical signs of sepsis. The severe condition of IL-1R knockout mice might be caused directly via staphylococcal products or indirectly via host mediators, e.g., cytokines, highly induced by high staphylococcal load. IL-18 was previously shown to be a detrimental mediator in S. aureus infection concerning development of septicemia (10). The high serum levels of IL-18 found in IL-1R-deficient mice as compared with wild-type controls could thus contribute to the severe septicemia of S. aureus infection. The protective effects mediated by IL-1R signaling with respect to development of sepsis and septic death are greater as compared with protection mediated by TNF/lymphotixin α (29). Indeed, we have previously demonstrated that, while being susceptible to S. aureus infection, TNF/lymphotixin α double-mutant mice or TNF single-deficient mice were protected against septic arthritis. This is not the case for IL-1R-deficient mice. The increased frequency and severity of septic arthritis in IL-1R−/− mice is present in parallel with obvious clinical signs of sepsis. The severe condition of IL-1R knockout mice might be caused directly via staphylococcal products or indirectly via host mediators, e.g., cytokines, highly induced by high staphylococcal load.

### Table I. Serum concentrations of cytokines after i.v. inoculation of S. aureus to IL-1R+/+ and IL-1R−/− mice

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>IL-1R+/+</th>
<th>p</th>
<th>IL-1R−/−</th>
<th>4</th>
<th>IL-1R+/+</th>
<th>p</th>
<th>IL-1R−/−</th>
<th>24</th>
<th>IL-1R+/+</th>
<th>p</th>
<th>IL-1R−/−</th>
<th>96</th>
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<tr>
<td>TNF</td>
<td>8 ± 3</td>
<td>NS</td>
<td>16 ± 6</td>
<td>16 ± 5</td>
<td>25 ± 7</td>
<td>59 ± 13</td>
<td>NS</td>
<td>97 ± 40</td>
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<td></td>
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</tr>
<tr>
<td>IL-6</td>
<td>140 ± 23</td>
<td>0.002 30 ± 15</td>
<td>8100 ± 2100</td>
<td>0.06</td>
<td>2800 ± 610</td>
<td>2100 ± 1200</td>
<td>NS</td>
<td>3300 ± 990</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IL-1β</td>
<td>6 ± 2</td>
<td>NS</td>
<td>10 ± 1</td>
<td>16 ± 2</td>
<td>17 ± 4</td>
<td>54 ± 9</td>
<td>0.002</td>
<td>159 ± 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>316 ± 33</td>
<td>0.01</td>
<td>173 ± 25</td>
<td>190 ± 28</td>
<td>0.001</td>
<td>512 ± 55</td>
<td></td>
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</tr>
</tbody>
</table>

* Mice, intact or defective with respect to IL-1R gene, were inoculated i.v. with 1 × 10⁸ S. aureus. Blood samples were obtained from 11 IL-1R+/+ and 10 IL-1R−/− at 4 and 24 h after inoculation of staphylococci. The serum levels of cytokines at 96 h after inoculation of S. aureus were checked in 12 IL-1R-deficient and 16 wild-type mice sacrificed at this time point. Values are expressed as picograms per milliliter (mean ± SEM). Values of p are calculated using the Mann-Whitney U test. NA, Not analyzed.

### Table II. Supernatant concentrations of cytokines following in vitro stimulation with formalin-killed S. aureus

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>IL-1R+/+</th>
<th>p</th>
<th>IL-1R−/−</th>
<th>24</th>
<th>IL-1R+/+</th>
<th>p</th>
<th>IL-1R−/−</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>182 ± 20</td>
<td>&lt;0.05</td>
<td>84 ± 12</td>
<td>220 ± 29</td>
<td>&lt;0.05</td>
<td>89 ± 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2100 ± 750</td>
<td>&lt;0.05</td>
<td>250 ± 190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>7 ± 1</td>
<td>NS</td>
<td>7 ± 1</td>
<td>9 ± 2</td>
<td>NS</td>
<td>9 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
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</tr>
</tbody>
</table>

* Spleen cells from noninfected mice, intact or defective with respect to IL-1R gene, were stimulated in vitro using 1 × 10⁷ formalin-killed S. aureus. Detection limit is 25 pg/ml. Values are obtained from three mice in each group and expressed as picograms per milliliter (mean ± SEM). Values of p are calculated using the Mann-Whitney U test. NA, Not analyzed; ND, not detected.
mice, as compared with IL-1R−/− controls, is most probably explained by staphylococci being densely expressed in the joints of the former group. The high staphylococcal load in joints is probably leading to recruitment of phagocytic cells and local (i.e., intra-articular) induction of proinflammatory cytokines. In analogy to IL-1β, IL-18 is known to promote joint inflammation in noninfectious arthritis (30–32). As shown by serum analyses, high amounts of IL-18 are produced in IL-1R−/− mice only a few days following bacterial inoculation. It is possible that the high IL-18 production in IL-1R-deficient mice, compared with the controls, increases the joint manifestations via recruitment of inflammatory cells and their production of reactive oxygen intermediates.

The regulation of IL-18 production was recently reviewed (8). Because its promoter region contains a NF-κB recognition sequence that is downstream of IL-1R signaling, it is not very surprising that IL-18 production is influenced by an IL-1R knockout situation. However, the impact of IL-1R signaling on IL-18 production has not been previously described. Evidently, IL-18 production is directly or indirectly dependent on a functional IL-1R, as seen by higher serum levels of this cytokine 24 h after the inoculation of bacteria in wild-type mice as compared with IL-1R-deficient ones. The significantly increased IL-1β/IL-18 serum levels at 4 days after inoculation of staphylococci in IL-1R−/− vs IL-1R+/+ mice could simply be due to an increased staphylococcal load, in the former mice, constituting a stronger stimulus for cytokine production. Alternatively, IL-1R signaling may inhibit excessive production of IL-1β and IL-18 during prolonged infection/inflammation via a presently unknown pathway.

The results obtained in this study clearly show high susceptibility in severe infection outcome in response to S. aureus in an IL-1R-deficient condition. The results are corresponding to the susceptibility for S. aureus infection described for mice with a MyD88 deficiency and are more pronounced as compared with the susceptibility effects of a TLR2 or IL-18 deficiency. In conclusion, IL-1R signaling is protective in S. aureus arthritis against septicemia and subsequent septic death as well as against development of septic arthritis, due to critical importance of this molecule in controlling accumulation of S. aureus in vivo.

Acknowledgments

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