Interleukin 15 Mediates Joint Destruction in *Staphylococcus Aureus* Arthritis

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**Background.** *Staphylococcus aureus* arthritis causes severe and rapid joint damage despite antibiotics. Thus, there is a need to identify new treatment targets in addition to antibiotics. Lately, interleukin 15 (IL-15) has been implicated both in osteoclastogenesis and in bacterial clearance—2 important issues in *S. aureus*–induced joint destruction. This has prompted us to investigate the importance of IL-15 in *S. aureus*–induced arthritis.

**Methods.** Toxic shock syndrome toxin-1 producing *S. aureus* was intravenously inoculated in IL-15 knockout and wildtype mice and in wildtype mice treated with anti–IL-15 antibodies (aIL-15ab) or isotype control antibody.

**Results.** Absence of IL-15, either in knockout mice or after treatment with aIL-15ab, significantly reduced weight loss compared with controls during the infection. The severity of synovitis and joint destruction was significantly decreased in IL-15 knockout and aIL-15ab treated mice compared with controls. In IL-15 knockout mice there was a reduced number of osteoclasts in the joints. The host’s ability to clear bacteria was not influenced in the IL-15 knockout mice, but significantly increased after treatment with aIL-15ab.

**Conclusions.** IL-15 is a mediator of joint destruction in *S. aureus*–induced arthritis and contributes to general morbidity, which makes this cytokine an interesting treatment target in addition to conventional antibiotics.
IL-15 has been recognized as an important player in autoimmune diseases such as rheumatoid arthritis (RA), as synovial tissue from RA patients expresses high levels of IL-15 [14–16]. Further, IL-15 has been implicated to be of importance for the development of collagen type II induced arthritis (CIA) in mice [17]. Collectively, these findings have prompted a clinical trial of monoclonal IL-15-blocking antibodies for RA treatment [18, 19].

Lately, new attention has been drawn to IL-15. First, IL-15 plays an important role in early osteoclast differentiation in vitro [20], and lately the importance of IL-15 in receptor activator of nuclear factor kappa-B ligand (RANKL)-dependent osteoclastogenesis in vivo has been observed [21]. Second, Orinska et al [4] described a new role of intracellular IL-15 in a cecal ligation and puncture (CLP) sepsis model, as IL-15 knockout mice displayed an increased bacterial clearance due to enhanced recruitment of neutrophils. Together, these findings suggest that inhibition of IL-15 might be beneficial in S. aureus–induced arthritis, and we hypothesized that absence of IL-15 would be favorable in a mouse model of S. aureus arthritis and sepsis.

Systemic S. aureus–induced arthritis and sepsis was induced in our well-established mouse model where mice are inoculated with the toxic shock syndrome toxin (TSST)-1 producing S. aureus strain LS-1 [22] intravenously. It results in a highly erosive and rapidly progressive joint disease with a close resemblance to the corresponding conditions in humans [23]. In this study, IL-15 knockout and wildtype control mice were used. In addition, wildtype mice were also treated with an anti–IL-15 antibody (aIL-15ab) or isotype control antibody starting at day 3 after S. aureus inoculation. We show that inhibition of IL-15 either by deletion of the IL-15 gene or by the use of aIL-15ab leads to a less destructive arthritis compared with wildtype or control antibody–treated mice, possibly due to reduced cartilage destruction and a reduction in the number of osteoclasts in the joints. General morbidity, bacterial clearance, or mortality were not negatively affected. This is the first report to our knowledge to show that a direct reduction in the number of osteoclasts is accompanied by a reduction in joint destruction in S. aureus arthritis.

MATERIALS AND METHODS

Mice

C57BL/6 IL-15 knockout mice were generated as previously described [24]. Wildtype C57BL/6 mice were obtained from Scanbur (Sollentuna, Sweden). The mice were maintained (10 in each cage) under standard conditions of temperature and light in the animal facility at the Department of Rheumatology and Inflammation Research, University of Gothenburg, Sweden. Permission from the local Animal Research Ethics Committee, in accordance with national animal welfare legislation, was obtained for all the experiments.

Mouse Model of Systemic S. aureus–induced Arthritis

In this study, we used the TSST-1–producing LS-1 strain of S. aureus for infection [22]. Female IL-15 knockout mice (n = 35) and female wildtype mice (n = 32) on a C57BL/6 background were inoculated intravenously in the tail with 0.7–1 × 10^8 S. aureus LS-1 per mouse in a total volume of 200 µL phosphate-buffered saline (PBS). Viable counts were performed to determine the number of bacteria injected. Mice were graded blindly for clinical arthritis severity and frequency. Finger/toe and ankle/wrist joints were inspected, and arthritis was defined as visible erythema and or swelling. To evaluate the intensity of arthritis, a clinical scoring (arthritic index) was carried out using a system where macroscopic inspection yielded a score of 0–3 points for each limb (0, neither swelling nor erythema; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and erythema). The total score was calculated by adding up all the scores within each animal tested.

The overall condition of each mouse was also examined daily by assessing signs of systemic inflammation, that is, weight change, reduced alertness, and ruffled coat. In cases of severe systemic infection, when a mouse was judged too ill to survive another 24 hours, it was culled and defined as death due to sepsis. Kidneys and blood were obtained at days 3, 7, 10, and 14. Limbs for immunohistochemistry staining and histological examination were taken at days 10 and 14.

Treatment of Systemic S. aureus–induced Arthritis with Anti–IL-15 Antibody

C67BL/6 mice (n = 10 in each group) were inoculated intravenously in the tail with 0.8 × 10^8 S. aureus LS-1 per mouse in a total volume of 200 µL PBS. Treatment with aIL-15ab (25 µg/mouse) (Amgen Inc, Seattle, Washington) or isotype control antibody (IgG2a, 25 µg/mouse) (Amgen Inc) started at day 3 after bacterial inoculation. The antibodies were injected intraperitoneally at days 6, 10, and 13 after bacterial inoculation. At day 14, blood, kidneys and paws were obtained for further examination.

Mouse Model of Sepsis

IL-15 knockout (n = 14) and wildtype (n = 19) male and female mice were inoculated with 0.8–1 × 10^8 LS-1 per mouse in a total volume of 200 µL PBS. Viable count was used to check the amount of bacteria injected. Mortality, clinical evaluation of arthritis, and weight change were followed up until day 4 when kidneys and blood were obtained.

Bacterial Load in Kidneys

Kidneys were aseptically dissected, kept on ice, homogenized, serially diluted in PBS, and spread on blood agar plates. The number of colony-forming units (CFUs) per kidney pair was determined after 24 hours of incubation at 37°C.
Cartilage Staining Using Safranin O
The degree of cartilage destruction was studied using Safranin O (Sigma-Aldrich AB, Stockholm, Sweden), which staining intensity is proportional to the proteoglycan content in the cartilage [25]. Joints were fixed, decalcified, and paraffin embedded. Tissue sections from hind paws were cut, deparaffinized, and stained with Weigert Iron hematoxylin prior to Safranin O staining with Fast Green counterstaining. All slides were coded and evaluated with 2 blinded observers. The degree of cartilage destruction of knees, ankles, and toes was evaluated from 0 to 3. Occasionally, one paw was missing in the histological sections or embedded in a way that made it impossible to evaluate the degree of cartilage destruction, and therefore the total score/mouse is divided by the number of evaluated joints.

Histology of Inflamed Joints
Histology of inflamed joints was evaluated from fore- and hind paws cut, deparaffinized, and stained with hematoxylin-eosin (Histolab Products AB, Gothenburg, Sweden). All slides were coded and evaluated with 2 blinded observers. The specimens were evaluated with regard to synovial hyperplasia and cartilage/subchondral bone destruction. The degree of synovitis and destruction yielded a score from 0 to 3 in every joint concerning finger/toes, wrists/ankles, elbows, and knees. Occasionally, one paw was missing in the histological sections or embedded in a way that made it impossible to evaluate the degree of synovitis and bone/cartilage destruction, and therefore the total score/mouse is divided by the number of evaluated joints.

Measurement of Cytokines
Blood was centrifuged at 8000g for 10 minutes. Serum was collected and stored at −20°C for further analysis. Serum protein levels of IL-15 were measured in wildtype mice after S. aureus inoculation using interleukin 15R (IL-15R)/IL-15 complex enzyme-linked immunosorbent assay (ELISA) ready-set-go kit (AH diagnostics, Skärholmen, Sweden) according to the manufacturer’s recommendations and detected on Spectra Max 340PC (Molecular Devices, Sunnyvale, California). In the all-15ab experiment, the serum levels of interleukin 6 (IL-6) were measured by sandwich ELISA (R&D systems, Europe Ltd, Abingdon, UK) according to the manufacturer’s recommendations and detected on Spectra Max 340PC (Molecular Devices). Serum levels of interleukin 10 (IL-10), interleukin 17A (IL-17A), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), IL-6, interleukin 4 (IL-4), and IL-2 in IL-15 knockout experiments were measured using cytometric bead assay mouse Th1/Th2/Th17 cytokine kit (BD Biosciences, Erebodegem, Belgium). The assay was run on FACSCanto II (BD Biosciences).

Statistical Analysis
Statistical analyses were performed using GraphPad Prism software (La Jolla, California). Statistical differences between independent groups were calculated using nonparametric Mann–Whitney U test or Fisher exact probability test. Kaplan–Meier survival plots were prepared and the log-rank test was used for comparison between the 2 survival curves. Cytokine levels were compared between wildtype and IL-15 knockout mice using a mixed model, including days after bacterial inoculation as continuous variable, genotype as fixed variable, and cytokine level as dependent variable. P < .05 was considered statistically significant.

RESULTS
Systemic S. aureus–induced Morbidity and Arthritis Is Ameliorated in IL-15 Knockout Mice Without Affecting the Bacterial Clearance
IL-15 knockout mice lost significantly less weight compared with wildtype mice after intravenous S. aureus inoculation (Figure 1A), whereas no differences could be observed in mortality rate between knockout and wildtype mice (Figure 1B). Clinical severity of arthritis was significantly decreased in the IL-15 knockout mice compared with wildtype mice at days 10 and 14 after intravenous inoculation of S. aureus (Figure 1C). Also, the frequency of arthritis was significantly reduced at day 14 after intravenous inoculation of S. aureus in the IL-15 knockout mice compared with wildtype mice (Figure 1D). The host’s ability to clear bacteria was measured as the CFU/kidney pair at days 10 and 14 after bacterial inoculation, no significant differences were obtained between knockout mice and wildtype mice (Figure 1E).

Cartilage Destruction and the Number of Osteoclasts Are Reduced in IL-15 Knockout Mice
Safranin O staining of cartilage showed significantly reduced cartilage destruction in the IL-15 knockout mice compared with wildtype mice at day 14 after S. aureus inoculation (Figure 2A–C). Cathepsin K staining of the joints revealed a
significantly reduced number of osteoclasts in the IL-15 knockout mice compared with wildtype mice (Figure 2D–G). Sections stained with normal rabbit serum as primary antibody did not show any staining (Figure 2H and I). However, hematoxylin-eosin stained histological sections from days 10 and 14 showed no significant differences in the degree of synovitis or bone/cartilage destruction between IL-15 knockout and wildtype mice, although a clear trend toward a reduction of both synovitis and bone/cartilage destruction was observed in the IL-15 knockout mice (Figure 3A–C).

**Serum Protein Levels of IL-15 Are Elevated in Wildtype Mice Inoculated with *S. aureus***

Serum protein levels of IL-15, measured in wildtype mice, were increased at day 10 after *S. aureus* inoculation compared with noninfected mice (Figure 4A). Serum protein levels of IL-6 were increased in the IL-15 knockout mice compared with wildtype mice at days 3, 10, and 14 (Figure 4B). The serum protein levels of IL-10, IL-17A, TNF-α, IFN-γ, IL-4, and IL-2 did not differ between knockout and wildtype mice (data not shown).
Figure 2. Immunohistochemical staining from mice after induction of systemic *Staphylococcus aureus*–induced arthritis. A, Cartilage Safranin O staining at days 10 and 14 shows the degree of cartilage destruction. B–C, Sections from a Safranin O stained hind paw at day 14 of (B) wildtype mice and (C) interleukin 15 (IL-15) knockout mice (red staining indicates remaining cartilage; scale bar: 0.1 mm, original magnification 5×). D, Osteoclast cathepsin K staining at day 14. E–F, Immunohistochemistry sections from a knee joint of (E) wildtype and (F) IL-15 knockout mice. Black arrows indicate an osteoclast (scale bar: 0.1 mm, original magnification 10×). G, Magnification of osteoclast (original magnification 40×). H–I, Knee joint sections.
Systemic *S. aureus*-induced Morbidity, Arthritis, and Bacterial Load are Decreased in Mice Treated with Anti-IL-15 Antibody

Mice treated with aIL-15ab lost significantly less weight compared with mice treated with isotype control at day 14 after intravenous *S. aureus* inoculation (Figure 5A), whereas no differences could be observed in mortality (Figure 5B). Hema-toxylin-eosin stained histological sections showed that mice treated with aIL-15ab had significantly reduced both severity of synovitis and bone/cartilage destruction compared with mice treated with isotype control antibodies at day 14 after intravenous inoculation of *S. aureus* (Figure 5C). Mice treated with aIL-15ab showed a significantly increased ability to clear bacteria from kidneys compared with mice treated with isotype control antibody (Figure 5D). There were no differences in serum protein levels of IL-6 between mice treated with aIL-15ab and mice treated with isotype control antibody (Figure 5E).

**IL-15 Does not Influence the Outcome of *S. aureus*-induced Sepsis**

In order to investigate the importance of IL-15 during *S. aureus*-induced septic shock, a 10-fold higher (septic) dose of *S. aureus* was intravenously inoculated. A slight increase in weight loss was observed at day 1 in the IL-15 knockout mice compared with wildtype mice (Figure 6A). There were no differences in terms of mortality, severity, or frequency of clinically assessed arthritis at any timepoints (Figure 6B–D). No differences could be observed in bacterial clearance from kidneys between knockout and wildtype mice (Figure 6E).

**DISCUSSION**

This is the first report to our knowledge showing that IL-15 aggravates joint destruction in septic arthritis caused by *S. aureus*. We investigated the importance of IL-15 in systemic *S. aureus*-induced arthritis and sepsis using C67BL/6 wildtype mice and IL-15 knockout mice. IL-15 is a part of the general immune response to the bacteria as the serum protein level of IL-15, measured in wildtype mice, increases during the infection. Mice lacking IL-15 lost significantly less weight throughout systemic *S. aureus*-induced infection. The clinical severity of arthritis was significantly reduced late during infection in...
the IL-15 knockout mice, which was accompanied by a significant reduction in cartilage destruction at day 14. These findings coincided with a significantly reduced number of osteoclasts in the joints compared with wildtype mice. Interestingly, the host’s ability to clear the bacteria or mortality rate was not influenced. Treatment with aIL-15ab significantly reduced weight loss at day 14 after S. aureus inoculation compared with mice treated with isotype control antibody, which also coincided with an increased ability to clear bacteria. Also, the aIL-15-treated mice displayed significantly reduced severity of synovitis and joint destruction at day 14 compared with isotype control–treated mice. In S. aureus–induced sepsis no differences were observed between wildtype and knockout mice with respect to weight loss, mortality, clinical arthritis, and clearance of bacteria in the kidneys.

As IL-15 is absolutely required for the development of NK cells, both IL-15 and interleukin 15Rα (IL-15Rα) knockout mice are considered, and often used, as NK cell–deficient mice [24, 27]. Previous studies using NK cell–depleting antibodies have suggested that NK cells are protective against S. aureus–induced arthritis [28]. However, our data show that the absence of NK cells in the IL-15 knockout animals actually ameliorates the course of infection compared with wildtype mice. NK cells express receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), both essential for osteoclast development, which suggests that NK cells have an important role in the bone remodeling process [29, 30]. Lately, it has also been shown that stimulation of bone marrow cells with a combination of IL-15 and RANKL promotes osteoclast formation in vivo [21], indicating also IL-15 as an important mediator in the osteoclastogenesis. We have observed both reduced cartilage destruction and a reduction in the number of osteoclasts in the joints from IL-15 knockout mice compared with wildtype. Thus, it is possible that the absence of NK cells in the IL-15 knockout mice leads to reduced levels of RANKL and M-CSF and inhibition of osteoclastogenesis. Interestingly, the serum levels of IL-15 increase during the course of infection, as do the severity of cartilage destruction and number of osteoclasts. These findings indicate that the effect of IL-15 on cartilage increases over time. As osteoclasts are of utmost importance for the loss of juxta-articular bone in arthritis [31, 32], it is possible to believe that fewer number of osteoclasts should lead to reduced joint destruction.

Enhanced bacterial clearance has been observed in IL-15 knockout mice in a cecal ligation and puncture (CLP) mast cell–dependent sepsis model [4], because of enhanced recruitment of neutrophils to the site of infection. The suggested mechanism is abrogation of an IL-15–dependent inhibition of chymases produced in mast cells. In hematogenously spread S. aureus infection, the bacteria home and reside particularly in the joints and kidneys [33, 34]. For practical reason the bacterial clearance is measured in kidneys. In line with Orinska et al [4] we found significantly increased bacterial clearance in kidneys in mice treated with aIL-15ab compared with isotype control treatment, whereas the serum levels of IL-6 were similar in both groups. In contrast, there were no detectable differences between IL-15 knockout mice and wildtype mice in terms of bacterial clearance in the kidneys, but we did find enhanced levels of serum IL-6 in the IL-15 knockout mice. One explanation for differences in bacterial clearance in the knockout situation and aIL-15ab treatment could be that in the complete absence of IL-15 in the IL-15 knockout mice, compensatory mechanisms have evolved minimizing the effect on bacterial clearance.

\[\text{Figure 4. Serum cytokine levels of mice after induction of systemic Staphylococcus aureus–induced arthritis. A, Serum levels of interleukin 15R (IL-15R)/interleukin 15 (IL-15) complex in wildtype C57BL/6 mice at baseline, and days 3, 7, and 10 after inoculation of S. aureus. B, Serum levels of interleukin 6 (IL-6) in wildtype and IL-15 knockout mice at the indicated days after S. aureus infection. Horizontal bars indicate median values. Statistical differences for IL-15 levels were calculated using Mann–Whitney U test and for IL-6 levels using a mixed model, including days after bacterial inoculation as continuous variable, genotype as fixed variable and cytokine level as dependent variable. *P<.05 compared with wildtype mice. Squares: wildtype mice; circles: IL-15 knockout mice.}\]
clearance. On the other hand, aIL-15ab treatment was started after bacterial inoculation and does not completely block the action of the cytokine, which creates a different immunological situation. Taken together, our data suggest that the reduced severity of arthritis and decrease in weight loss in the IL-15 knockout mice is not due to increased bacterial clearance, although this could be the mechanism in aIL-15ab treated mice.

As septic shock is the most severe form of S. aureus infection, it is of interest to investigate the outcome of this manifestation in the absence of IL-15. Importantly, when mice were given a septic dose of bacteria, no aggravation of any measured parameter during the infection was noted. Thus, IL-15 has a minor role in S. aureus–induced sepsis.

The role of IL-6 in S. aureus infection is dual [1]. It contributes to increased morbidity and mortality by promoting an overwhelming immune response but is also necessary for bacterial elimination [23, 35]. IL-6 is an important regulator of bone turnover [36] and plays a prominent role in the formation of osteoclasts [37–40]. In addition, IL-6 can induce RANKL expression by stromal cells, which is essential for osteoclastogenesis [41, 42]. Neutralizing antibodies toward IL-6 inhibits osteoclast formation [37]. Thus, it is not surprising that IL-6 contributes to joint destruction in autoimmune

Figure 5. Weight change, arthritis, bacterial persistence, and serum levels of interleukin 6 (IL-6) in wildtype mice treated with anti–interleukin 15 antibody (aIL-15ab) after Staphylococcus aureus–induced arthritis. A, Weight change as a percentage of initial weight and (B) mortality rate. C, Histology of joints from day 14 shows the degree of synovitis and bone/cartilage destruction. D, Bacterial persistence in kidneys and (E) serum levels of IL-6 of wildtype mice treated with aIL-15ab or isotype control antibody at day 14 after bacterial inoculation. In panel A, bars show the mean ± standard error of the mean (SEM). In panels C, D, and E, horizontal bars indicate median values. Statistical differences were calculated using Mann–Whitney U test. Kaplan–Meier survival plots were prepared and the log-rank test was used for comparison between the 2 survival curves. *P < .05; **P < .01 compared with mice treated with isotype control antibody. Squares: isotype control antibody treatment; circles: aIL-15ab treatment.
arthritis [43]. In our study, it is possible that the increased serum levels of IL-6 contribute to decreased morbidity and preserved bacterial clearance in the IL-15 knockout mice, but it is very unlikely that IL-6 contributes to inhibition of joint destruction. Instead, we hypothesize that IL-15 mediates the joint destruction, especially because treatment with aIL-15ab protects the joints during S. aureus infection without influencing the serum levels of IL-6.

In conclusion, this study shows that IL-15 plays an important role in the development of joint destruction during systemic S. aureus arthritis and that IL-15 during S. aureus infection could provide a possible treatment target.

Notes

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