S100A4 Deficiency Is Associated With Efficient Bacterial Clearance and Protects Against Joint Destruction During Staphylococcal Infection

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Background. Efficient host defense mechanisms are crucial for survival in sepsis and septic arthritis. S100 proteins are reported to have proinflammatory and bactericidal properties. The aim of this study was to investigate the role of S100A4 in staphylococcal arthritis.

Methods. S100A4 knockout mice (S100A4KO) and wild-type counterparts (WT) were intravenously and intra-articularly challenged with Staphylococcus aureus strain LS-1. Clinical and morphological signs of arthritis and sepsis, phagocytosis, bone mineral density (BMD), and bone metabolism were then monitored in S100A4 and WT mice.

Results. S100A4KO mice had a lower bacterial load in the kidneys than WT mice (P < .05) but developed more severe clinical signs of arthritis (P < .001) and had higher levels of interleukin 6 and L-selectin (P = .002). S100A4KO mice had fewer morphological signs of synovitis and cartilage/bone destruction following intra-articular instillation of bacteria. S100A4KO mice were protected from loss of BMD and had lower levels of RANKL, MMP3, and MMP9 (P < .05). S100A4 was not bactericidal in vitro.

Conclusions. In staphylococcal infection, S100A4 regulates bacterial clearance as well as systemic and local inflammatory responses.

Invasive staphylococcal infections are severe conditions associated with significant mortality and frequent residual impairments [1]. Staphylococcal infection of the joints can result in rapidly progressing bone destruction and irreversible loss of joint function [1–3]. Our research group has established a murine model of hematogenous septic arthritis, which is a useful tool for the evaluation of host defense mechanisms and staphylococcal virulence factors [2]. However, the problem of staphylococcal arthritis is far from being resolved.

S100 proteins are a family of low-molecular-weight, EF-hand calcium-binding proteins that regulate a network of calcium-dependent and calcium-independent processes [4]. Intracellular S100 proteins regulate mechanisms associated with calcium transport and cell homeostasis such as protein phosphorylation, transcriptional activity, and cytoskeletal rearrangement [5]. S100 proteins are released into the extracellular compartment in response to stress, acting as alarmins and regulating cell proliferation, chemotaxis, and apoptosis [6, 7]. S100A4 is a 101 amino acid protein with a molecular mass of 12 kDa [8]. S100A4 is expressed in the cytoplasm of normal tissue cells, including immune cells such as neutrophils, certain T cells, and activated macrophages [9], while nuclear localization is predominant in tumor cells [10]. Intracellular S100A4 exists as

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a symmetric homodimer that facilitates the binding of its target proteins, which include actin, nonmuscle myosin IIA and IIB, tropomyosin, S100A1, p53, liprin-1β, methionine amino peptidase 2, p37, CCN3, and septins [11]. Extracellular S100A4 interacts with annexin II, RAGE, and heparan sulfate proteoglycans. Through these interactions, S100A4 regulates cell mobility, invasion, and angiogenesis [11]. S100A4 also binds a number of epidermal growth factor (EGF) receptor ligands, thereby inducing EGF/ErbB2 signaling [12].

Accumulation of S100 proteins at sites of inflammation led to the assumption that they may also play a role in regulating the inflammatory response. Indeed, expression of S100 proteins has been shown in synovial tissues and fluid in rheumatoid arthritis (S100A4, S100A8/S100A9) [13, 14], ulcerative colitis (S100A8/S100A9) [14], and psoriasis (S100A7) [15]. Because they are released into the extracellular space, S100 proteins can amplify inflammatory responses [6]. In addition, certain members of the S100 family have bactericidal properties. The S100A8/S100A9 heterodimer inhibits microbial growth by depriving bacteria nutrients through metal chelation [16]. S100A7 can kill both Gram-negative and Gram-positive bacteria with a pH-dependent target specificity [17].

The reasons that prompted us to study the role of S100A4 in staphylococcal infection were as follows: (1) it is expressed in immunocompetent organs, (2) it is implicated in the regulation of inflammation, and (3) it has structural and functional similarities to other S100 proteins that possess bactericidal properties. The results of this study show that S100A4 contributes to bacterial accumulation at sites of infection and mediates bone loss during the course of staphylococcal infection.

**METHODS**

**Mice**

S100A4 knockout mice (S100A4KO) were generated by a germline inactivation of the S100A4 gene (Mts-1) as previously described [18]. S100A4KO mice and their wild-type (WT) counterparts (A/Sn) were bred at the animal facility of the Department of Rheumatology and Inflammation Research, University of Gothenburg. The mice were housed 8–10 animals/cage with a 12-hour light and dark cycle and were fed standard laboratory chow and water ad libitum.

**Bacterial Strains**

*Staphylococcus aureus* strain LS-1, originally isolated from a swollen joint of a spontaneously arthritic New Zealand Black/New Zealand White (NZB/W) mouse [19], was used in all experiments. Before intravenous administration to the mice, the bacteria were thawed, washed in phosphate-buffered saline (PBS), and adjusted to the appropriate concentration. The samples of bacterial mixture were spread on horse blood agar, and the number of bacterial colonies (colony-forming units, CFU) was calculated following 24 hours of incubation. Viable counts were performed in the leftover suspension to confirm the actual number of administered bacteria.

**Staphylococcal Infection**

S100A4KO mice and WT mice, 10- to 11-week-old females, were intravenously injected with *S. aureus* 1.4 × 10⁷ CFU/mouse in 200 mL PBS. Three independent experiments were performed. In experiments 1 and 2 (S100A4KO, n = 21; WT, n = 21), mice were killed on day 11 after staphylococcal inoculations. In experiment 3 (S100A4KO, n = 12; WT, n = 13), mice were killed on day 7. All mice were regularly weighed and examined for signs of arthritis and general appearance. At the termination of each experiment, serum samples were collected and stored at −20°C for serologic analysis, kidneys were removed for bacterial cultures, and the paws were taken for morphological evaluation. The experimental setting was approved by the Animal Experimental Ethical Board at the University of Gothenburg.

Purified peptidoglycan, the major component of *S. aureus* cell wall (kindly provided by Professor Simon Foster, University of Sheffield, United Kingdom), was intravenously injected to WT (A/Sn) mice in escalating doses between 50 and 800 mg/mouse. The paws were taken for morphological evaluation on day 6 after the peptidoglycan injection.

**Induction of Arthritis by Intra-articular Injections**

*S. aureus* was also introduced by a single injection into the knee joints (1 × 10⁷ CFU/knee) of healthy S100A4KO (n = 5) and WT mice (n = 5). In a separate set of experiments, S100A4KO (n = 10) and WT (n = 10) mice were intra-articularly injected with purified peptidoglycan (25 mg/knee). The injected joints were taken for morphological evaluation 3 days after injection.

**Clinical Valuation of Arthritis**

All the mice were inspected individually every second day after bacterial inoculation for signs of arthritis. Arthritis was defined as visible erythema and/or swelling of at least 1 joint. An arbitrary clinical scoring system was used for each limb (0, normal appearance; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and erythema). The arthritic index was constructed by adding the scores of all 4 limbs from each mouse [20].

**Morphological Examination of Joints**

Paws were fixed in 4% formaldehyde, decalcified, and embedded in paraffin. Four-micrometer-thick tissue sections were stained with hematoxylin and eosin. The joint sections were evaluated for synovitis, cartilage, and/or bone destruction by an observer blinded to the identity of the sections. Histological score was constructed as described previously [19]. For
immunohistology, paws were decalcified in 10% EDTA in 0.1M Tris buffer (pH 6.95) for 2 weeks. Five-micrometer-thick paraffin sections were deparaffinized and stained using rabbit antimouse S100A4 antibodies (10 μg/mL) as previously described [21].

**Bactericidal Properties of S100A4**

*S. aureus* (10^7/mL, 10^8/mL, 10^9/mL) was incubated in tryptic soy broth at 37°C with mouse recombinant S100A4 (concentration range, 0–10 mg/mL). Viable counts (number of CFU) were calculated at defined time intervals and compared in S100A4-treated and untreated cultures.

**Phagocytosis**

Heparinized peripheral blood (100 mL) was incubated with fluorescein isothiocyanate (FITC)–labeled *S. aureus* (2 × 10^7 or 1 × 10^7 CFU/sample) at 37°C in a water bath [22]. Control blood samples were incubated with FITC-labeled bacteria on ice. Erythrocytes were lysed and leukocyte surface–attached bacteria were quenched and washed. Cells containing FITC-labeled bacteria were assessed by flow cytometry.

**Bacterial Distribution In Vivo**

FITC-labeled *S. aureus* (5 × 10^7 CFU/mouse in experiment 1 or 1.6 × 10^8 CFU/mouse in experiment 2) was injected intravenously to S100A4KO and WT mice. Two experiments were performed. One hour and 3 hours after inoculation, mice were killed, and spleens, lymph nodes, kidneys, and lungs were removed, processed to cell suspension, and subjected to flow cytometry.

**Bone Mineral Density**

Bone mineral density (BMD) was analyzed in the left femurs of S100A4KO and WT mice using peripheral quantitative computed tomography (pQCT) scan with a Stratec pQCT XCT Research M (Norland) as previously described [23].

**Flow Cytometry**

Cells retrieved from knee synovia and spleens were incubated with Fc Block (2.4G2), followed by incubation with primary antibodies anti-Ly6G (1A8), anti-CD8 (53-6.7), anti-CD4 (GK1.5), anti-B220 (RA3-6B2), anti-CD62L (L-selectin, MEL-14), anti-CD18 (C71/16), anti-CD11b (M1/70) and anti-CD19 (1D3), or isotype controls. All antibodies were from BD Biosciences and analyzed using FlowJo software (FlowJo 887, Tree Star), except anti-CD19 (1D3), which was obtained from eBioscience. Monoclonal antibodies conjugated with phycoerythrin (PE), allophycocyanin (APC), allophycocyanin-H7 (APC-H7), FITC, eFluor450, or biotin were used. Streptavidin conjugated with APC or peridininchlorophyll-protein (PerCp) was used with biotin-labeled antibodies. Between 1 × 10^5 and 5 × 10^5 cells/sample were collected by flow cytometry in a FACScanto II (BD Biosciences) equipped with FACSDiva software and analyzed using FlowJo software. Fluorochrome minus one was used for gating and determination of positive populations.

**Serological Analyses**

The levels of interleukin 6 (IL-6) were measured by a bioassay using cell line B13.29, subclone B9, which is dependent on IL-6 for growth as previously described [24]. Recombinant human IL-6 (NIBSC) was used as a standard. Levels of soluble L-selectin (sL-selectin; CD62L) were measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems) using serum samples diluted 1:1000. Receptor activator of NF-κB ligand (RANKL, dilution 1:25) and osteoprotegerin (OPG, dilution 1:50), matrix metalloproteinases (MMP3 and MMP9, dilution 1:20), and osteopontin (OPN, dilution 1:400) were assessed in serum samples using ELISA (R&D). Serum levels of C-terminal cross-linking telopeptide of type I collagen (CTX-I) and of type II collagen (CTX-II) were determined using ELISA (Immunodiagnostics Systems) in undiluted samples.

**Statistical Analysis**

Statistical analyses were performed with Mann-Whitney U test and Fisher exact test. Values are reported as median and interquartile range (IQR) or as mean ± standard error of the mean (SEM). A P value <.05 was considered significant.

**RESULTS**

**S100A4KO Mice Show More Severe Clinical Signs of Arthritis**

S100A4KO mice (n = 33) and their WT counterparts (n = 34) were inoculated intravenously with *S. aureus* strain LS-1, and their joints were prospectively inspected for signs of arthritis. Clinical signs included swelling, redness, and loss of function. Both the frequency and severity of arthritis were higher in S100A4KO mice compared with their WT counterparts throughout the course of infection (Figure 1A and 1B). On day 7, the median arthritis index reached 3.0 (IQR, 1.5–4.0) in the S100A4KO mice compared with WT mice, which showed a median index of 1.0 (IQR, 0–3.0; P = .006) (Figure 1B). On day 11, the arthritis index continued to increase to 4.0 (IQR, 1.8–5.3) in S100A4KO mice but remained unchanged in WT mice (median 1.0 [IQR: 0.8–2.3]; P = .002) (Figure 1B). Morphological evaluation of the joints on days 7 and 11 showed that the degree of leukocyte infiltration of the synovial tissues and the frequency of erosions was similar in S100A4KO and WT mice (Table 1). S100A4KO mice had higher levels of the cartilage degradation product CTX-II than WT mice (day 11, S100A4KO: median, 24.7 [IQR, 20.5–75.3 pg/mL] vs WT: median, 13.7 [IQR, 3.8–18.6 pg/mL]; P = .02); however, the levels of the bone degradation product CTX-I were similar (S100A4KO: median, 1.0 [IQR: 0.8–2.3] vs WT: median, 0.8 [IQR: 0.1–3.3]; P = .41).
35.7 [IQR, 32.5–39.7 pg/mL] vs WT: median, 34.8 [IQR, 28.6–36.2 pg/mL]; P = not significant).

To evaluate whether staphylococcal products were sufficient for the hematogenous induction of arthritis, WT mice were intravenously injected with increasing doses of peptidoglycans (50–800 mg/mouse) and clinical evaluation of the paws was performed after injection. None of the mice showed any signs of arthritis.

**S100A4KO Mice Show Reduced Bone Loss During Staphylococcal Infection**

Total BMD in the distal metaphyseal region of the femur was higher in S100A4KO mice compared with WT mice on day 7 (median, S100A4KO: 485 mg/cm³ vs WT: 424 mg/cm³; P = .009) and day 11 (median, 432 mg/cm³ vs 373 mg/cm³; P = not significant) after infection. Additionally, loss of total BMD in S100A4KO mice (presented as percentage of uninfected littermates) was less pronounced than that in WT mice (−9.6% vs −19.7%, respectively). Loss of BMD occurred predominantly from the trabecular compartment, from which S100A4KO mice lost 6.8%–7.5% and WT mice lost 15.6%–21.2% of trabecular bone by days 7 and 11 after infection (Figure 1C). The loss of cortical BMD in S100A4KO mice was also low compared with that in WT mice (−1.5% vs −4.3%, respectively) (Figure 1D). Evaluation of bone markers on days 7 and 11 showed that S100A4KO mice had significantly reduced levels of matrix metalloproteinase (MMP3) (E) and MMP9 (F) during staphylococcal infection. Statistical analysis was performed using the Mann–Whitney U test and Fisher exact test. A P value <.05 was considered significant.

**Figure 1.** Frequency (A) and severity (B) of clinical arthritis in S100A4 knockout (S100A4KO; n = 33) and wild-type (WT; n = 34) mice following intravenous inoculation of *Staphylococcus aureus*. The frequency of arthritis is presented as a percentage of the total number of infected mice. The severity of arthritis is represented by the arthritis index constructed by adding the scores from all 4 limbs from each mouse. Reduction in trabecular (C) and cortical (D) bone mineral density (BMD) on days 7 and 11 was more pronounced in WT mice (n = 12) than in S100A4KO mice (n = 14). S100A4KO mice showed significantly reduced levels of matrix metalloproteinase (MMP3) (E) and MMP9 (F) during staphylococcal infection. Statistical analysis was performed using the Mann–Whitney U test and Fisher exact test. A P value <.05 was considered significant.

**S100A4KO Mice Show Increased Levels of Systemic Inflammation**

Following intravenous inoculation of *S. aureus*, both S100A4KO mice (n = 33) and WT mice (n = 34) developed clinical signs of...
infection resulting in subsequent weight loss by day 7 (S100A4KO: median, 22% vs WT: 22%) and day 11 (S100A4KO: median, 25% vs WT: 25%) after infection. Mortality on day 11 reached 14.3% (3 mice died in each group). No significant differences were observed between S100A4KO and WT mice in terms of either weight loss or mortality. Circulating levels of IL-6 and sL-selectin were measured throughout the course of infection to evaluate systemic inflammation. On day 11, S100A4KO mice showed significantly higher levels of both IL-6 (median, 348 pg/mL [IQR, 239–404 pg/mL]) and sL-selectin (median, 2.5 ng/mL [IQR, 1.9–3.4 ng/mL]) than WT mice (IL-6: median, 130 pg/mL [IQR, 95–268 pg/mL]; P = .002; sL-selectin: 1.7 ng/mL [IQR, 1.6–2.1 ng/mL]; P = .015). The levels of MMP3 and MMP9 were 18-fold and 2-fold lower, respectively, in S100A4KO mice compared with WT mice (Figure 1). 

### Table 1. Morphological Evaluation of Joints Following Hematogenous Staphylococcal Infection

<table>
<thead>
<tr>
<th></th>
<th>Synovitis</th>
<th>Bone destruction/erosions</th>
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<tr>
<td></td>
<td>Frequency (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Severity&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Day 7</strong></td>
<td></td>
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<tr>
<td>S100A4KO (n = 11)</td>
<td>43 ± 2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>WT (n = 12)</td>
<td>35 ± 4</td>
<td>1.7 ± 0.2</td>
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<tr>
<td><strong>Day 11</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S100A4KO (n = 18)</td>
<td>53 ± 4</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>WT (n = 18)</td>
<td>54 ± 3</td>
<td>2.3 ± 0.1</td>
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</table>

**NOTE.** Histological score was constructed as described in [19], where 0 points = no damage, 1 point = mild, 2 points = moderate, and 3 points = severe arthritis. S100A4KO, S100A4 knockout mice; WT, wild-type mice.

<sup>a</sup> Frequency represents the proportion of affected joints to all inspected joints (%).

<sup>b</sup> Severity represents the score (mean ± SEM).

### Differential Expression of Adhesion Molecules in S100A4KO and WT Mice

The expression of adhesion molecules on synovial and splenic leukocytes was assessed in both noninfected mice and those given intra-articular injection of *S. aureus* on T lymphocytes (CD4<sup>+</sup> and CD8<sup>+</sup> cells) and granulocytes (Ly6G<sup>+</sup> cells) of S100A4KO and WT mice.

Intra-articular injection of peptidoglycan (25 mg/knee) induced similar morphological signs of synovitis in both S100A4KO (arthritis index: median, 2.5) and WT mice (arthritis index, median, 2.1). No difference in erosivity was found between S100A4KO (5 of 10) and WT (6 of 10) mice.

### Table 2. Changes in Bone Metabolism Markers During the Course of Staphylococcal Infection

<table>
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<tr>
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<th>Staphylococcus aureus infection</th>
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<tr>
<td></td>
<td>Day 7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RANKL, ng/mL</td>
<td>S100A4KO</td>
</tr>
<tr>
<td></td>
<td>WT</td>
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<tr>
<td>OPG, ng/mL</td>
<td>S100A4KO</td>
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<tr>
<td>RANKL/OPG ratio</td>
<td>S100A4KO</td>
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<td></td>
<td>WT</td>
</tr>
<tr>
<td>Osteopontin, ng/mL</td>
<td>S100A4KO</td>
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<tr>
<td></td>
<td>WT</td>
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</tbody>
</table>

**NOTE.** Values were reported as mean ± SEM. OPG, osteoprotegerin; S100A4KO, S100A4 knockout mice; WT, wild-type mice.

<sup>a</sup> Day 7: S100A4KO mice, n = 11; WT mice, n = 12.

<sup>b</sup> Day 11: S100A4KO mice, n = 18; WT mice, n = 17.

<sup>c</sup> P = .04.

<sup>d</sup> P = .03.
and CD8+ lymphocyte populations were significantly reduced in the spleens (Figure 3B, numbers 2 and 3) and synovia (Figure 3B, numbers 5 and 6) of S100A4KO mice. The expression of integrins (CD11b and CD18) was similar between noninfected and infected S100A4KO and WT mice.

S100A4KO Mice Have Reduced Phagocytic Capacity Supporting Efficient Clearance of Bacteria

Comparison of the staphylococcal load in the kidneys on days 7 and 11 showed that S100A4KO mice (n = 22) had consistently lower bacterial counts than WT mice (n = 23) (Figure 4A). The in vivo distribution of staphylococci was studied in 2 independent experiments (n = 6/group) following intravenous inoculation of FITC-labeled bacteria. One hour after bacterial inoculation, S100A4KO mice had somewhat lower bacterial accumulation in lungs (4.5% vs. 6.4%) and significantly higher bacterial accumulation in kidneys (2.17% vs. 0.37%; P = .015) compared with WT mice. The bacterial counts in spleens and lymph nodes were similar.

Phagocytosis of FITC-labeled bacteria in vitro was studied in 3 independent experiments. Peripheral blood leukocytes from S100A4KO (n = 10) and WT (n = 9) mice had similar phagocytic capacity at bacterial concentrations of 2 × 10^6 CFU. The phagocytic capacity was significantly reduced in S100A4KO mice at higher bacterial concentrations (1 × 10^7 CFU) (Figure 4B).

To assess whether S100A4 affected bacterial growth, S. aureus at concentrations of 10^2, 10^3, and 10^4 CFU/mL was cultured with increasing concentrations of recombinant mouse S100A4 protein (0–10 µg/mL). Bacterial growth was measured after 1, 3, 5, and 8 hours. Bacterial counts in the S100A4-treated cultures were similar to those in the nontreated cultures at all time points tested (data not shown), suggesting that S100A4 has no direct effect on bacterial growth in vitro.

DISCUSSION

In this study, we showed that S100A4 deficiency leads to the efficient elimination of S. aureus during hematogenous infection. Indeed, injected fluorescent-labeled staphylococci passed through lungs, spleen, and lymphoid nodes of S100A4KO mice and accumulated in the kidneys 1 hour after intravenous inoculation, followed by more efficient elimination of bacteria from the kidneys during the later stages of infection. Interestingly, the phagocytic capacity of S100A4KO leukocytes was impaired, especially in the presence of high concentrations of bacteria. This may potentially prolong bacterial circulation and their exposure to bactericidal peptides and facilitate bacterial accumulation in the kidneys. In vitro experiments using recombinant S100A4 molecules showed no direct bactericidal effect of this protein on staphylococci. Therefore, S100A4KO mice had an unimpaired bactericidal capacity. Attachment of bacteria to cell surfaces protects them from host bactericidal proteins and serves as an important virulence mechanism [25, 26]. Thus, impaired phagocytosis in S100A4KO mice may reduce virulence by favoring bacterial killing in circulation.

S100A4KO mice were significantly protected from both leukocyte influx into the joint cavity and cartilage and bone destruction compared with WT mice. This was observed in experiments using both systemic (intravenous) and intra-articular bacterial challenge. Clinical signs of severe arthritis, including swelling and redness, observed in S100A4KO mice were not consistent with morphological findings. This suggests that inflammatory responses in S100A4KO mice are efficient during the initial stages of inflammation, such as vasodilation.
and formation of extracellular edema, but that leukocyte recruitment and migration may be weakened.

Both phagocytosis and leukocyte migration require recognition of bacteria by adhesion molecules [27]. Lymphocytes from the spleens and synovia of S100A4KO mice showed reduced expression of L-selectin compared with those in WT mice. This difference was seen in noninfected mice but was accentuated during infection. L-selectin is required for recruitment of leukocytes to sites of inflammation. Inhibition of both P-selectin and L-selectin in mice results in increased S. aureus accumulation in the kidneys [28], while macrophages lacking the expression of β-integrins show defective phagocytosis [29]. During sepsis, rapid shedding of L-selectin results in increased levels of sL-selectin [30]. Following bacterial challenge S100A4KO mice had higher levels of sL-selectin in blood circulation. This is consistent with our results showing higher levels of sL-selectin in S100A4KO mice in parallel with severe systemic inflammation.

S100A4KO mice also had low levels of MMP3 and MMP9 during S. aureus infection. MMPs play an important role in host immune responses and antibacterial defense [31]. MMP3 deficiency results in impaired granulocyte migration and low

Figure 3. The expression pattern of leukocyte adhesion molecules was assessed in the spleens and synovial tissues from noninfected mice (NI) and after intra-articular challenge with Staphylococcus aureus (1 × 10⁶ colony-forming units/joint). Single-cell cultures of spleen (A) and synovial tissues (B) from S100A4 knockout (S100A4KO; n = 6) and wild-type (WT; n = 10) mice were stained for integrins (CD18⁺ and CD11b⁺) and L-selectin (CD62L⁻) and analyzed by flow cytometry on granulocyte (Ly6G⁺) and T lymphocyte (CD4⁺ and CD8⁺) populations. Statistical analysis was performed using the Mann–Whitney U test. A P value <.05 was considered significant.
bacterial clearance from the colon [32], and MMP9 deficiency decreases the severity of staphylococcal arthritis [33]. Low MMP levels in S100A4KO mice may be partly responsible for the discrepancy between the clinically overt arthritis seen in S100A4KO mice and the relatively moderate morphological findings. Additionally, the degree of bone loss in S100A4KO mice was less than that in WT mice, although both S100A4KO and WT mice showed reduced BMD during infection. We have recently shown that staphylococcal infection causes a pronounced reduction in BMD as early as 3 days after bacterial inoculation [3]. In the present study, infected S100A4KO mice also had low levels of RANKL. Neutralization of circulating RANKL blocks osteoclast maturation and reduces bone loss during staphylococcal infection [34], suggesting that this may be a possible explanation for bone protection in S100A4KO mice.

Our results demonstrate an important role for S100A4 in host defense during Staphylococcus aureus infection. A lack of S100A4 results in mobilization of systemic inflammation, more efficient bacterial clearance, and increased protection against joint destruction caused by staphylococcal arthritis.

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**References**