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IL-10 Plays Opposing Roles during *Staphylococcus aureus* Systemic and Localized Infections

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IL-10 is a potent anti-inflammatory mediator that plays a crucial role in limiting host immunopathology during bacterial infections by controlling effector T cell activation. *Staphylococcus aureus* has previously been shown to manipulate the IL-10 response as a mechanism of immune evasion during chronic systemic and biofilm models of infection. In the present study, we demonstrate divergent roles for IL-10 depending on the site of infection. During acute systemic *S. aureus* infection, IL-10 plays an important protective role and is required to prevent bacterial dissemination and host morbidity by controlling effector T cells and the associated downstream hyperactivation of inflammatory phagocytes, which are capable of host tissue damage. CD19^+^CD11b^+^ CD5^+^ B1a regulatory cells were shown to rapidly express IL-10 in a TLR2-dependent manner in response to *S. aureus*, and adoptive transfer of B1a cells was protective during acute systemic infection in IL-10–deficient hosts. In contrast, during localized s.c. infection, IL-10 production plays a detrimental role by facilitating bacterial persistence via the same mechanism of controlling proinflammatory T cell responses. Our findings demonstrate that induction of IL-10 has a major influence on disease outcome during acute *S. aureus* infection. Too much IL-10 at one end of the scale may suppress otherwise protective T cell responses, thus facilitating persistence of the bacteria, and at the other end, too little IL-10 may tend toward fatal host-mediated pathology through excessive activation of T cells and associated phagocyte-mediated damage. *The Journal of Immunology*, 2017, 198: 2352–2365.

Immune regulation plays a critical role in protecting the host from the pathology associated with bacterial infection. IL-10, which is produced by various innate and adaptive immune cells, including monocytes, macrophages, T cells, and B cells, is a prototypic immunoregulatory cytokine that is necessary for controlling/regulating the production of proinflammatory cytokines both in vitro and in vivo (1–4). Systemic infection with *Escherichia coli* (5), *Toxoplasma gondii* (6), *Trypansomoa cruzi* (7), and *Plasmodium chabaudi* (8) in IL-10–deficient mice resulted in excessive production of proinflammatory cytokines, including TNF-α and IFN-γ, which contributed to increased host immunopathology, morbidity, and mortality. Neutralization of TNF-α or IFN-γ in these mice attenuated organ damage and reduced mortality (5, 8).

Although immune regulation is critical for protection of the host, certain bacteria can manipulate these immunosuppressive mechanisms to facilitate persistence during infection. Phagocytosis of *Mycobacterium tuberculosis* by macrophages results in IL-10 production, which in turn has the capacity to block phagosome maturation in a STAT3-dependent manner, thereby promoting intracellular bacterial survival (9), in addition to inhibiting a Th1 response, thereby facilitating its persistence in the lung (10). Similarly, *Bordetella pertussis* can promote IL-10 production by both macrophages and dendritic cells (DCs) through expression of the virulence factors filamentous hemagglutinin and adenylate cyclase toxin. This IL-10 then inhibits IL-12p70 production by DCs, and these DCs direct naive T cells toward a regulatory phenotype (11–13). CD25^+^Foxp3^+^ regulatory T cells (Tregs) were expanded in *B. pertussis*–infected mice, and these cells work with IL-10–secreting Tregs in the lungs to subvert bacterial clearance by downregulating Th1 responses (14). Following intranasal challenge with *Streptococcus pneumoniae*, induction of the anti-inflammatory cytokine TGF-β was found to promote local Treg expansion, thereby facilitating bacterial survival within the nasopharyngeal tissue. Immune regulation therefore functions as a double-edged sword, on the one hand, protecting host against potentially fatal excessive proinflammatory responses, whereas on the other hand, it can also benefit the bacterium by dampening down local proinflammatory responses, thus facilitating bacterial persistence.

*Staphylococcus aureus* is an important component of the normal microbiota of most of the healthy human population (15). However, upon invasive entry it is a potentially lethal opportunistic pathogen and is a leading cause of a variety of community-acquired and hospital-acquired bacterial infections. *S. aureus* is
one of the most common causes of bacteria, with a higher mortality than any other bacteria (16). It is also an important cause of other deep-seated infections, including osteomyelitis, septic arthritis, endocarditis, device-related infections, and pneumonia (17). This bacterium has evolved a sophisticated repertoire of immune evasion mechanisms with the capacity to modulate both innate and adaptive arms of the immune response (17, 18). S. aureus has also been shown to manipulate host immunoregulatory mechanisms to facilitate persistence during infection, and IL-10 appears to be at the forefront of this evasion strategy (19).

In a systemic model of chronic S. aureus bloodstream infection, kidney abscesses were still apparent on day 56 post-infection due to impaired T cell responses (20); subsequently, this immunosuppression was attributed to the expansion of myeloid-derived suppressor cells (MDSCs) and to a lesser extent Tregs in the spleens of infected mice compared with uninfected controls (21). Although both IL-10 and TGF-β were produced, immunosuppression was primarily dependent on cell–cell contact that inhibited effector T cell responses (21). Adoptive transfer of MDSCs exacerbated systemic infection in recipient mice following S. aureus i.v. challenge (21). Similarly, during a chronic S. aureus orthopedic biofilm infection, Heim et al. (22, 23) observed an expansion of IL-10–producing MDSCs at the site of infection, which was associated with bacterial persistence. Targeted depletion of these cells resulted in an enhanced proinflammatory response that resulted in increased bacterial clearance (23, 24).

It appears, therefore, that during chronic infection, S. aureus can manipulate host immune-regulatory machinery, in particular MDSCs, to facilitate its own survival. However, depending on the local microenvironment, the mechanism of immunosuppression exerted by these regulatory cells may differ. In the present study, we investigated the importance of IL-10 during acute S. aureus infection in the context of both systemic and local infection. Our data demonstrate that the induction of IL-10 has opposing effects depending on the site of infection, likely due to the distinct cellular microenvironments with which S. aureus engages at these sites. During acute systemic infection, induction of IL-10 favored the host and was required to prevent systemic immunopathology, whereas during acute local infection induction of IL-10 favored persistence of the bacterium. Furthermore, we identified B1a cells as a novel source of IL-10–induced acute systemic infection.

Materials and Methods

Mice

Age- and sex-matched wild-type (WT) C57BL/6, IL-10–reporter (IL-10 GFP) B6.129S6-Ildm1Flv/J (25), and IL-10 knockout (IL-10−/−) mice (6–8 wk old) were housed under specific pathogen-free conditions at the Trinity College Dublin Comparative Medicines unit. Mice were sacrificed at the time points postinfection, mice were sacrificed to assess local and systemic infection levels and associated immune responses. Peritoneal exudate cells were isolated by lavage of the peritoneal cavity with 2 ml of sterile PBS. Erythrocytes were lysed using 0.87% ammonium chloride, and cells were washed and resuspended in complete RPMI 1640 (RPMI 1640 [Biosera], 10% FCS [Biosera], 100 mM l-glutamine [Life Technologies]). 100 µg/ml penicillin/streptomycin [Life Technologies]) for analysis by flow cytometry. The lavage fluid was centrifuged and supernatants were stored at −20˚C for subsequent cytokine analysis. Kidneys, liver, and spleen were homogenized in 3 ml of sterile PBS. Total tissue bacterial burden was established by plating serial dilutions of peritoneal lavage or tissue homogenate on TSA. All plates were incubated for 24 h at 37˚C and CFU were enumerated. Results are expressed as CFU per milliliter.

ELISA

ELISAs for IL-1β, IL-10, IL-17, TNF-α, IL-22, CXCL1, and TGF-β (R&D DuoSet; R&D Systems) were performed on cell culture supernatants, peritoneal lavage fluid, or homogenized whole-skin tissue as per the manufacturer’s instructions.

Flow cytometry

Peritoneal exudate cells were incubated in the presence of PMA, ionomycin, and brefeldin A (Sigma-Aldrich) for 4 h at 37˚C and 5% CO₂. Cells were then incubated with Fc block (1 mg/ml) on ice before surface staining with fluorochrome-conjugated Abs against CD3 (clone 500A2; BD Biosciences), CD206 (clone M1/70; eBioscience), CD11b (clone M1/70; eBioscience), Ly6G (clone HK1.4; BD Biosciences), Ly6C (clone 1A8; BD Biosciences), F4/80 (clone BM8; eBioscience), CD11c (clone 1B1; BD Biosciences), CD19 (clone 1D3; eBioscience), Ly6C (clone HK1.4; eBioscience), CD45 (clone 30-F11; eBioscience), fixable viability dye (eBioscience), CD5 (clone 55-7.3; eBioscience), CD25 (clone PC61.5; eBioscience), and Foxp3 (clone FJK-16s; eBioscience).

For intracellular cytokine staining, cells were fixed and permeabilized using the DakoCytomation IntraStain kit before intracellular staining with fluorochrome-conjugated Abs against IL-17A (clone 17B7; eBioscience), NO synthase 2 (NOS2; clone CXNFT; eBioscience) and IFN-γ (clone XMG1.2; eBioscience). To investigate reactive oxygen species (ROS) activity within phagocytes, dihydrorhodamine 123 assays were performed as previously described (29). For Foxp3 staining, a Foxp3 staining kit (eBioscience) was used. Flow cytometric data were acquired with a BD FACS Canto II (BD Biosciences) and analyzed using FlowJo software (Tree Star). Gates are set on respective fluorescence minus one.
**B1a cell isolation and adoptive transfer or culture**

Purified CD19+CD11b+CD5+ B1a cells were isolated from the peritoneal cavity of naive WT, IL-10−/−, and TLR2−/− mice by FACS sorting using Abs specific to CD19, CD11b, and CD5 and sorted using a Beckman Coulter [DakoCytomation] MoFlo cell sorter. The level of purity of purified B1a cells was >95%. WT B1a cells or PBS were administered by i.p. injection to naive mice (2 × 10^5 cells per mouse). At 12 h posttransfer, mice were challenged by i.p. injection of *S. aureus* Newman (1 × 10^5 CFU). On day 5 postinfection, the peritoneum was lavaged and the kidney, spleen, and liver were isolated to determine local and systemic bacterial burden as previously described.

**In vitro culture of B1a cells**

B1a cells (1 × 10^5) were plated in complete RPMI 1640, without penicillin/streptomycin, and then infected with *S. aureus* at a multiplicity of infection (MOI) of 100. Supernatants were then assessed for IL-10 by ELISA 18 h postinfection.

**Determination of total IgM in the peritoneal fluid**

Total IgM was determined in serial dilutions of peritoneal fluid using an IgM ELISA. Briefly, medium-binding 96-well plates were coated with purified rat anti-mouse IgM (1 µg/ml) (clone II/41; BD Pharmingen). Peritoneal lavage fluid was added. Biotinylated rat anti-mouse IgM (0.5 µg/ml) (Clone R6-60.2; BD Pharmingen) Ab was used as a secondary Ab. Color development was achieved using o-phenylenediamine dihydrochloride (Sigma-Aldrich) in substrate buffer, and OD_{450} was measured using a microplate reader and SoftMax Pro software.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism software. For murine studies, differences between groups were analyzed using an unpaired Student *t* test, Mann–Whitney *U* test, one-way ANOVA with a Tukey comparison posttest, or two-way ANOVA with a Bonferroni correction posttest where appropriate. A *p* value ≤0.05 was considered significant.

**Results**

**IL-10 is rapidly induced both locally and systemically following acute *S. aureus* peritoneal infection**

Using a previously described model of acute systemic infection induced by *S. aureus* peritoneal challenge (30), we investigated the production of IL-10 both locally and systemically. Groups of WT mice received a single i.p. injection of *S. aureus* (1 × 10^8 CFU) or

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**FIGURE 1.** *S. aureus* acute peritoneal infection induces local and systemic IL-10 production. Groups of WT mice received an i.p. injection of *S. aureus* (Newman, 1 × 10^8 CFU). At specific time points postinfection the peritoneal cavity was lavaged and blood was collected to isolate sera. IL-10 concentrations in the peritoneal lavage fluid (A) and the sera (B) were measured by ELISA. IL-10 GFP reporter mice received an i.p. injection of *S. aureus* Newman (1 × 10^8 CFU). At 3 h postinfection, leukocytes were isolated from the peritoneal cavity for FACS analysis, and analysis of IL-10 GFP production by individual subsets was then assessed. The IL-10 GFP expression was primarily detected by a population of CD11b^+ cells comprised of CD11b^CD19^CD5^+ B1a cells, CD11b^Ly6G^Ly6C^- MDSCs, and CD11b^F480^- macrophages (C). Values are expressed as mean absolute numbers of IL-10 GFP^+ cells ± SEM (n = 6–7) (D). Data represent pooled data from three independent experiments, all of which demonstrated a similar trend. Statistical analysis was performed using a two-way ANOVA or one-way ANOVA with a Tukey posttest. *p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.001.
sterile PBS as a control. At specific time points postinfection, peritoneal fluid and sera were collected and IL-10 concentrations were quantified by ELISA. IL-10 was significantly elevated at 3 h postinfection, both locally in the peritoneal cavity (Fig. 1A) and systemically in the blood (Fig. 1B), and had returned to baseline by 24 h postinfection.

To determine the source of the IL-10–producing cells, WT or IL-10 GFP mice were infected with \textit{S. aureus} (1 × 10^8 CFU) or sterile PBS as a control. At 3 h postinfection, ∼2.5% of the total peritoneal cells were IL-10 GFP + as assessed by flow cytometry. Of this IL-10 GFP + population, three distinct subsets of IL-10–producing cells were identified: CD11b intCD19 + cells, CD11b high F480 + macrophages, and a population of CD11b + Ly6g + Ly6c + MDSCs (Fig. 1C). Further analysis of the IL-10 GFP + CD11b intCD19 + population confirmed that these cells were primarily CD5a + CD1d − and thus represented B1a cells, with a smaller population of IL-10–producing CD5a + CD1d − B1b B cells. Considering the absolute numbers of IL-10–producing cells present within the peritoneal cavity at 3 h, we can see that B1a cells represent the primary source of IL-10 within the peritoneal cavity at this early time point (Fig. 1D). No other IL-10–producing subsets of B cells or CD4 + T cells were found at this time point postinfection. Consistent with the ELISA data, by 12 h postinfection the GFP signal within the peritoneal cavity was below the level of detection. We were unable to detect any IL-10 GFP signal in the blood 3 h postinfection likely due to the low concentrations of IL-10 that are present at this site. Furthermore, we did not detect IL-10 production within the spleen or the draining mediastinal lymph nodes at 3 h following \textit{S. aureus} peritoneal challenge (Supplemental Fig. 1).

**IL-10 protects against host morbidity and mortality during \textit{S. aureus}–induced acute peritoneal infection.**

Having established that IL-10 is rapidly produced both locally and systemically during \textit{S. aureus} acute peritoneal infection, we determined the importance of this cytokine in disease outcome. WT and IL-10 −/− mice were infected with \textit{S. aureus} (1 × 10^8 CFU) via i.p. injection. At various time points postinfection, changes in core body temperature were measured as readout of morbidity. In IL-10 −/− mice, core body temperature increased significantly compared with WT mice throughout the first 24 h of infection (Fig. 2A). Using an alternative invasive strain of \textit{S. aureus}, PS80...
(31), at a higher challenge inoculum (5 \times 10^8 CFU) we demonstrated increased mortality in the IL-10^{-/-} mice following S. aureus i.p. challenge (Table I).

Having established the importance of IL-10 in protecting the host from significant morbidity and mortality, we questioned whether IL-10 was important for controlling bacterial clearance in vivo. Groups of WT and IL-10^{-/-} mice were administered S. aureus (1 \times 10^8 CFU) via i.p. injection, and bacterial burden in the blood, peritoneal cavity, kidney, spleen, and liver was assessed. IL-10^{-/-} mice had higher levels of bacterial burden in the blood at 3 h postinfection (Fig. 2B). By day 7 postinfection WT mice had effectively cleared the infection (<1 log CFU remaining at the site of infection and within the organs). However, clearance of infection was impaired in the IL-10^{-/-} mice. On days 3 and 7 postinfection, IL-10^{-/-} mice displayed elevated bacterial burdens in the kidney (Fig. 2C), spleen (Fig. 2D), and liver (Fig. 2E) and a higher bacterial burden locally in the peritoneal cavity on day 7 (Fig. 2F), compared with WT mice. Taken together, we can conclude that IL-10 plays an overall protective role during S. aureus acute systemic peritonitis and prevents bacterial dissemination to peripheral organs.

IL-10 is critical for controlling inflammation during acute S. aureus peritonitis

IL-10 has previously been shown to be critical for constraining excessive inflammatory responses during infection with other organisms (5), and therefore we examined the requirement for IL-10 in controlling both local and systemic immune responses to S. aureus acute peritonitis. WT and IL-10^{-/-} mice were challenged with S. aureus (1 \times 10^8 CFU) via i.p. injection, and local and systemic cytokine and chemokine concentrations were assessed in the peritoneal lavage fluid and sera over time. As expected, in the absence of IL-10, proinflammatory cytokine concentrations were significantly elevated as compared with WT mice both locally within the peritoneal cavity (Fig. 3) and systemically (Fig. 4).

We and others have previously demonstrated important protective roles for IL-17–producing \gamma^\delta T cells and IFN-\gamma–producing Th1 cells during S. aureus infection through the ability of these cytokines to regulate the recruitment and activation of both neutrophils and macrophages, respectively (30, 32). To ascertain whether IL-10 acts upstream to regulate the effects of these T cells, T cell activation and associated phagocytic cell responses were assessed within the peritoneal cavity at specific time points postinfection in WT and IL-10^{-/-} mice. At 3 h postinfection, IL-17 production by \gamma^\delta T cells was significantly increased in the absence of IL-10 (Fig. 5A), and a significant increase in IFN-\gamma production by CD4^+ T cells was observed on day 7 postinfection in the IL-10^{-/-} mice infected with S. aureus compared with WT control mice (Fig. 5B). Similar to the situation in WT mice (30, 32), we did not observe any increased production of IL-17 by CD4^+ T cells or IFN-\gamma production by \gamma^\delta T cells.

**FIGURE 3.** IL-10 deficiency results in excessive proinflammatory cytokine production locally in response to S. aureus–induced acute peritoneal infection. Groups of WT and IL-10^{-/-} mice received an i.p. injection of S. aureus (Newman, 1 \times 10^8 CFU). At the indicated time points after challenge, the peritoneal cavity was lavaged and the concentrations of IL-1\beta (A), TNF-\alpha (B), IL-17 (C), IFN-\gamma (D), IL-22 (E), and CXCL1 (F) were measured by ELISA. Results are expressed as mean \pm SEM (n = 4–5). Data are representative of two individual experiments. Statistical analysis was performed using a two-way ANOVA. *p \leq 0.05, **p \leq 0.005, ***p \leq 0.001.
T cells even in the absence of IL-10 (data not shown). These data demonstrate that in the absence of IL-10, IL-17 production by γδ T cells is exacerbated initially during infection. Additionally, IFN-γ-producing CD4+ T cells, generally only significantly detected in the context of a memory response (32), are prominent in the absence of IL-10.

In the absence of IL-10 there was significantly higher numbers of neutrophils (CD11b+Ly6G+) recruited to the peritoneal cavity at 3 h postinfection when compared with WT mice infected with S. aureus (Fig. 6A). Neutrophil activation as assessed by ROS activity was also elevated at this time point in the IL-10−/− mice (Fig. 6B). At 24 h postinfection, macrophage (CD11b+F480+Ly6G−) recruitment was also significantly increased in IL-10−/− mice, compared with WT mice (Fig. 6C). Moreover, these macrophages expressed significantly higher levels of the activation marker NOS2 (Fig. 6D) at this time point. Taken together, it appears that in the absence of IL-10, overactive γδ T cell and Th1 responses lead to excessive recruitment and activation of phagocytes that are normally required for the clearance of S. aureus, but in this case may be contributing to host pathology and subsequent bacterial dissemination.

The protective role of IL-10 is nonredundant and cannot be compensated for by other immune-regulatory mechanisms

To establish whether IL-10 plays a nonredundant protective role during acute S. aureus peritonitis, TGF-β concentrations and Treg responses were assessed within the peritoneal cavities of WT and IL-10−/− mice following S. aureus challenge. We found that IL-10 deficiency resulted in a slight increase in the concentrations of the anti-inflammatory cytokine TGF-β (Fig. 7A) and significantly elevated levels of CD4+CD25+Foxp3+ Tregs (Fig. 7B) locally within the peritoneal cavity at 12 and 24 h postinfection in IL-10−/− as compared with WT mice. Moreover, these Tregs expressed higher levels of the suppressive marker programmed death protein 1 (PD-1) in the absence of IL-10 (Fig. 7C). These data demonstrate that although Tregs appear to be expanded and express greater levels of PD-1, presumably as a compensatory mechanism in the absence of IL-10, this does not appear to be sufficient to suppress or control effector T cell responses and protect the animals from the significant pathology associated with the IL-10 deficiency.

B1a cells directly activated by S. aureus in a TLR2-dependent mechanism produce IL-10, which in turn controls bacterial dissemination during acute S. aureus peritonitis

Our results indicate that IL-10 produced primarily by peritoneal resident B1a cells contributes to the protection of the host from increased morbidity caused by excessive inflammation and bacterial dissemination during acute peritoneal infection. To determine whether B1a cells produce IL-10 directly in response to S. aureus, CD11b+CD19+CD5+B cells were purified from the peritoneal cavities of naive WT mice and cultured in vitro in the presence of live S. aureus (MOI of 100). After 18 h, IL-10 production was substantially increased as compared with B1a cells cultured in media alone (Fig. 8A). Previous studies have indicated that S. aureus–induced IL-10 production by macrophages is TLR2 dependent (33). To investigate a potential role for TLR2 in B1a cell activation by S. aureus, we cultured naive B1a cells isolated from WT or TLR2−/− mice in the presence of live S. aureus (MOI of 100). B1a cells isolated from TLR2−/− mice produced significantly less IL-10 compared with WT B1a cells in response to S. aureus stimulation (Fig. 8A). This demonstrates that IL-10 production by B1a cells in response to S. aureus is completely dependent on TLR2 engagement.

To confirm a direct protective role for B1a cells in S. aureus infection, we adoptively transferred B1a cells (2 × 105), purified from the peritoneal cavity of naive WT mice, to IL-10–deficient hosts. At 12 h posttransfer the recipient mice were challenged with S. aureus (1 × 109 CFU) and bacterial burden in the peritoneal cavity and peripheral organs was assessed on day 5 postinfection. At 3 h postinfection IL-10 production was shown to be significantly induced in IL-10−/− mice that received WT IL-10–sufficient B1a cells (Supplemental Fig. 2). Although there was no reduction in the levels of S. aureus recovered from the site of infection (peritoneal cavity) in the IL-10−/− mice that received WT B1a cells compared with the PBS treated controls (Fig. 8B),
there was a significant reduction in dissemination of *S. aureus* to the kidney (Fig. 8C) and spleen (Fig. 8D) in the mice that received the B1a cells as compared with the control mice. Importantly, adoptive transfer of IL-10–deficient B1a cells failed to control dissemination (Supplemental Fig. 2). This confirms that IL-10–producing B1a cells are critically important for containing the bacteria locally in the peritoneal cavity, preventing its dissemination to the peripheral organs.

**FIGURE 6.** IL-10 deficiency results in increased migration and activation of neutrophils and macrophages locally in the peritoneal cavity in response to *S. aureus*–induced acute peritoneal infection. Groups of WT and IL-10−/− mice received an i.p. injection of *S. aureus* (Newman, 1 × 10⁸ CFU). At indicated time points after infection, leukocytes were isolated from the peritoneal cavity for FACS analysis to assess the absolute number of neutrophils (CD11b+Ly6G+) present (A) and their production of ROS, detected by rhodamine+ cells post 3 h postinfection (B). At indicated time points after challenge, the absolute numbers of macrophages (CD11b+F480+) present within the peritoneal cavity were also assessed (C), as was their expression of NOS2 at 24 h postinfection (D). Results are expressed as mean ± SEM (n = 4–5). Representative FACS plots are shown for (B) and (D). Data are representative of two individual experiments. Statistical analysis was performed using an unpaired *t* test or two-way ANOVA. *p* ≤ 0.05, **p** ≤ 0.005.
**FIGURE 7.** IL-10 deficiency results in increased concentrations of TGF-β and Tregs locally in the peritoneal cavity in response to *S. aureus*-induced acute peritoneal infection. Groups of WT and IL-10−/− mice received an i.p. injection of *S. aureus* (Newman, 1 × 10⁸ CFU). At the indicated time points after challenge, the peritoneal cavity was lavaged and the concentration of TGF-β (A) was measured by ELISA. At indicated time points after infection, leukocytes were isolated from the peritoneal cavity for FACS analysis to assess the proportion of Tregs (CD4+CD25+Foxp3+) (B) and their expression of PD-1 (C). Results are expressed as mean ± SEM (n = 4–5) with representative FACS plots shown. Data are representative of two individual experiments. Statistical analysis was performed using two-way ANOVA. **p ≤ 0.005, ***p ≤ 0.001.

**IL-10 contributes to disease pathology during acute s.c. *S. aureus* infection by suppressing local proinflammatory cytokine production**

Having observed that IL-10 is critical for constraining inflammation and protecting against bacterial dissemination during acute systemic peritonitis, we wanted to establish whether IL-10 played a similar role during an acute *S. aureus* infection at an alternative localized site.

WT and IL-10−/− mice were challenged with *S. aureus* USA 300 LAC::lux (2 × 10⁷ CFU) via s.c. injection, and at specific time points postinfection lesion sizes, in vivo bioluminescent activity of live bacteria, and local cytokine production were assessed within the skin.

We found that during acute s.c. infection, IL-10−/− mice had reduced lesion sizes as compared with WT mice (Fig. 9A). Additionally, bioluminescent activity at the site of infection (Fig. 9B) was substantially reduced in the IL-10−/− mice as compared with the WT mice at all time points up to day 7, at which point the bioluminescent activity was no longer detectable in the IL-10−/− mice. Similar to the in vivo bioluminescence activity, *S. aureus* CFU recovered from 8-mm punch biopsies were significantly reduced in the IL-10−/− mice as compared with WT mice on day 3 (Fig. 9C). Importantly, we observed no dissemination of *S. aureus* to systemic sites in either the WT or the IL-10−/− mice.

To investigate the mechanism by which IL-10 is facilitating bacterial persistence in the skin, we examined the local immune responses to *S. aureus* at the site of infection. Local tissue cytokine and chemokine concentrations were assessed in the skin at specific time points postinfection. We confirmed that IL-10 was being produced locally in the skin in WT mice following *S. aureus* challenge, and, importantly, concentrations remained elevated throughout the course of the infection (Fig. 10A). Within the skin, populations of CD11b+Gr-1+ MDSCs and CD11b+F4/80+ macrophages were identified as the primary sources of IL-10 during *S. aureus* s.c. infection (Fig. 10B, 10C). IL-10 production was not detected from either CD4+ T cells or B cells in the skin. Furthermore, we did not detect IL-10 production within the draining lymph node on day 3 (Fig. 10B). In the absence of IL-10, concentrations of IL-17, IL-22, and CXCL1 within the skin were significantly elevated, and there was a trend toward an increase in IFN-γ production in the skin of IL-10−/− as compared with WT mice (Fig. 11).

γδ T cells have previously been shown to be critically important for controlling bacterial burden within the skin during *S. aureus* s.c. infection where they direct neutrophil recruitment to the skin site (34). In line with this, we observed a significant increase in γδ T cells locally in the skin at 8 h postinfection in the absence of IL-10 (Fig. 12A). Furthermore, increased neutrophil and macrophage recruitment to the skin lesion was also observed in the IL-10−/− mice compared with the WT mice on day 1 (Fig. 12B). Although an increase in IFN-γ production within the skin was observed at day 7 in the absence of IL-10 (Supplemental Fig. 3B), CD4+ T cells were not identified as a major cell population infiltrating the skin during
**FIGURE 8.** *S. aureus* can directly induce IL-10 production by B1a cells via TLR2 activation, and these B1a cells can protect against bacterial dissemination during *S. aureus*–induced acute peritoneal infection. Peritoneal B1a cells (CD11b−CD19+CD5+) were isolated from the peritoneal cavity of naive WT and TLR2−/− mice and were cultured in vitro with live *S. aureus* (Newman) at an MOI of 100 for 18 h. IL-10 concentrations in the supernatant were then assessed by ELISA (A). Results are expressed as mean ± SEM (n = 3). Groups of IL-10−/− mice received an adoptive transfer of naive IL-10–sufficient B1a (2 × 10^5) cells originating from the peritoneal cavity of WT mice. At 12 h after transfer, mice were challenged with an i.p. injection of *S. aureus* (Newman, 1 × 10^8 CFU). On day 5 after challenge the bacterial burden was assessed in the peritoneal cavity (B), kidneys (C), and spleen (D). Results are expressed as mean ± SEM (n = 9–12). Data represent pooled data from three individual experiments, all of which showed a similar trend. Statistical analysis was performed using an unpaired *t* test or a Mann–Whitney *U* test. **p ≤ 0.005, ***p ≤ 0.001.

During *S. aureus*–induced peritonitis, we demonstrate rapid induction of IL-10 both locally and systemically. This IL-10 production plays a nonredundant role, independent of other regulatory mechanisms in protecting the host from immunopathology and bacterial dissemination by controlling local and systemic proinflammatory responses. In particular, we demonstrate that IL-10 acts upstream of effector T cells, controlling their cytokine responses. We have previously shown that γδ-derived IL-17 and type I immune responses are required at different stages of *S. aureus* peritoneal infection to promote effective bacterial clearance through the ability of these cytokines to regulate downstream phagocyte responses (30, 32). However, it appears that these T cell responses cannot be allowed to proceed unchecked and that effective resolution of infection requires the γδ T cell and CD4+ T cell responses to be tightly controlled so as to avoid host pathology that would ensue from excessive phagocyte activation. In the absence of IL-10, there was a significant expansion of IL-17–producing γδ T cells locally at 3 h postinfection as compared with that observed in WT mice. IL-17 can directly control neutrophil activation (35) and, consistent with this, neutrophils produced greater amounts of ROS in the absence of IL-10. Additionally, IFN-γ–producing Th1 cells were also significantly elevated in IL-10−/− mice on day 7 compared with WT mice. This was associated with significantly increased recruitment of macrophages and greater NOS2 production from the macrophages in the absence of IL-10. It has previously been demonstrated that excessive neutrophil migration to the peritoneal cavity leads to increased bacterial burden and mortality as a consequence of *S. aureus* through its ability to survive intracellularly following i.p. challenge (36); furthermore, we have shown that intracellular survival within phagocytes promotes systemic bacterial dissemination following i.p. challenge with *S. aureus* (31). Additionally, it has been shown that excessive macrophage activation and proinflammatory cytokine production leads to host tissue injury following acute *Citrobacter rodentium* infection (37). Consequently, it is clear that these phagocytic responses and by extension effector T cell responses controlling these phagocytes must be

an *S. aureus* infection in either WT or IL-10−/− mice (Supplemental Fig. 3A). This is consistent with previous studies that have shown that IFN-γR−/− mice had lesion sizes and bacterial burdens similar to those of WT mice following an *S. aureus* skin infection (34). Overall, these results demonstrate that during acute *S. aureus* skin infection, IL-10 is capable of dampening down local proinflammatory responses and in particular γδ T cell responses, which facilitates persistence of the infection.

**Discussion**

The cytokine network plays a pivotal role in orchestrating the inflammatory response to bacterial infections. The balance between pro- and anti-inflammatory cytokines is critical for ensuring that the host elicits an appropriate response to counteract the invading pathogen, while at the same time preventing unnecessary pathology from excessive inflammation. In this study, we provide significant new insights into the role of IL-10 during both localized and systemic acute *S. aureus* infection. To date, *S. aureus* has primarily been shown to manipulate host production of IL-10 for its own advantage to facilitate survival during both chronic systemic and biofilm models of infection (21, 23). Our study demonstrates divergent roles for IL-10 depending on the site of infection. During systemic *S. aureus* acute peritoneal infection, IL-10 protects the host by controlling proinflammatory responses, in particular effector T cell responses, thus preventing excessive inflammation and associated host pathology. However, via the same mechanism of controlling the proinflammatory response, IL-10 can facilitate bacterial persistence during localized s.c. *S. aureus* infection. Furthermore, we identified a novel IL-10–producing cell population within the peritoneal cavity during *S. aureus*–induced peritonitis. We demonstrate that *S. aureus* is capable of directly activating B1a cells through TLR2 engagement, leading to substantial IL-10 production. Importantly, these B1a cells are critical in conferring host protection against systemic *S. aureus* dissemination upon transfer to IL-10–deficient mice during *S. aureus*–induced peritonitis. To our knowledge, this is the first study to report a functionally protective role for B1a cells during *S. aureus*–induced peritonitis.
tightly regulated during bacterial infection so as to con-
strain bacterial dissemination and prevent associated host
immunopathology.

In contrast to the host-protective role of IL-10 during acute
systemic peritoneal infection, IL-10 appears to facilitate bacterial
presence during acute localized s.c. infection. IL-10 production
was sustained locally within the skin tissue up to 7 d after s.c.
challenge with *S. aureus*. In this localized s.c. model of *S. aureus*
infection, MDSCs and macrophages were identified as the main
IL-10 producers. This local induction of IL-10 during our *S.
aureus* s.c. infection was associated with persistence of the
bacteria within the skin. IL-10−/− mice exhibited reduced lesion
size and reduced bacterial burden as evidenced by biolumines-
cent activity at the site of infection compared with WT mice and
had effectively cleared the infection by day 3 as quantified by
CFU. In WT mice, appreciable concentrations of bacteria were
still recoverable from the skin abscess site at this time point.
These results support previous studies, demonstrating that during
an *S. aureus* biofilm infection, IL-10 producing MDSCs prevent
the initiation of an appropriate proinflammatory response re-
quired for bacterial clearance at this localized site of infection,
thereby creating a microenvironment in which it can persist (22,
23). Similar to its role during acute peritonitis, IL-10 appears to
be controlling local effector T cell responses in the skin. In the
absence of IL-10, substantially greater amounts of proin-
flammatory cytokines, specifically IL-1β, IL-22, IL-17, and the
neutrophil chemoattractant CXCL1, were found locally within
the infected tissue compared with WT mice during the course of
infection. γδ T cells have previously been shown to be critical
in the effective clearance of *S. aureus* from the skin through their
ability to produce IL-17 and regulate CXCL1-driven neutrophil
recruitment (34). Similarly, during a murine skin abscess model,
IL-22 expression was previously identified to be independent of
γδ T cells (34), but it was shown to be protective through the
activation of antimicrobial peptide production (38). Inhibition of
either IL-17A or IL-22 alone resulted in significantly larger le-
sion size, providing further evidence that both IL-17A and IL-22
are necessary for local clearance of *S. aureus* during an *S. aureus*
skin infection (38, 39). Consistent with this, we observed a
significant increase in the influx of γδ T cells to the site of
infection in the absence of IL-10. This increased migration of
γδ T cells and elevated production of proinflammatory cyto-
kines, including both IL-17 and IL-22, in the skin resulted in an
increased migration of both neutrophils and macrophages to the
site of infection in the absence of IL-10, which in turn facilitated a
more effective clearance of the infection. Thus, it appears that in the

**FIGURE 9.** IL-10 deficiency results in reduced lesion size and reduced bacterial
burden in response to s.c. challenge with *S.
aureus*. Groups of WT and IL-10−/− mice
received an s.c. infection of *S. aureus*
(USA300 LAC::lux, 2 × 10⁷ CFU). At in-
dicated time points after challenge, the mean
total lesion size (square centimeters) ± SEM
was assessed (A), and representative lesions
for each mouse strain at each time point are
shown (B). Mean total flux (photons per
second per steradian) ± SEM was assessed
(C), representative in vivo bioluminescence
images for each mouse strain at each time
point are shown (D). Bacterial burden in the
skin lesion was quantified on day 3 postin-
fection by 8 mm biopsy punch (E). Results
are expressed as mean ± SEM (n = 4–5).
Data are representative of two individual
experiments. Statistical analysis was per-
formed using a Mann–Whitney U test or
two-way ANOVA. *p ≤ 0.05.
skin once again IL-10 acts upstream to control effector T cells; however, in this case suppression of the T cell response favors the bacterium by impairing the host’s ability to clear the infection. Importantly, during localized s.c. infection, we did not observe any systemic dissemination of bacteria in the absence of IL-10. This is similar to the local chronic biofilm infection model, where the absence of IL-10–producing MDSCs also did not lead to bacterial dissemination (22, 23). Therefore, it appears that during localized S. aureus infection, IL-10 production facilitates bacterial persistence at the site of infection only, but does not promote systemic dissemination.

Taken together, these results demonstrate that the host can tolerate the lack of an IL-10 anti-inflammatory response during local S. aureus infection and in fact removing the brake on the proinflammatory response actually benefits the host by facilitating a more rapid clearance of the bacteria. However, this response cannot be tolerated systemically where a lack of IL-10 leads to excessive proinflammatory responses that are actually detrimental to the host.

Collectively, our current data demonstrate a clear role for IL-10 in controlling T cell and phagocyte activation, which subsequently dictates disease outcome during two contrasting S. aureus infection types. Previously published data have now demonstrated that cellular responses, including Th1, Th17, and γδ T cell responses, are all involved in the host defense against S. aureus infections (30, 32, 34, 40); however, their relative prominence varies depending on whether the infection is local or systemic, the anatomical site of the local infection, and whether the infection is acute or chronic. As the skin is the largest organ of the body and an important barrier against invading bacteria, it comprises a complex immune system involving DCs, keratinocytes, T cells, and regulatory B cells that create a tolerant microenvironment to prevent undesirable inflammation in response to commensal organisms (41, 42). We now show that S. aureus can manipulate this cellular environment to its own advantage, inducing IL-10 production from MDSCs and macrophages, but not B cells, to facilitate its persistence within this niche. In contrast, a systemic infection leads to a more hostile inflammatory host response, as there is a greater impetus on host protection. Unlike the localized...
environment in the skin where immune cells can become tolerogenic due to constant bacterial exposure (43), during a systemic infection the cellular environment is primed for a more robust proinflammatory response to *S. aureus* in this setting.

Our studies have uncovered a previously undocumented immune-regulatory role for B1 cells during acute systemic *S. aureus* peritoneal infection. Following i.p. challenge with *S. aureus*, IL-10 is rapidly produced locally by a population of B1 cells comprising both B1a and B1b subsets. Consistent with previously published studies (23, 44), CD11b+Ly6G+Ly6C+ MDSCs and CD11b+F480+ macrophages were also capable of producing IL-10 at this site; however, the B1a response dominated. Interestingly, CD4+ T cells or Tregs did not appear to produce IL-10 at this site, although they have been shown to be an important source of IL-10 during pulmonary infections (14). Adoptive transfer of IL-10–sufficient B1a cells conferred protection in recipient IL-10−/− mice and prevented bacterial dissemination, suggesting that these IL-10–producing B1a cells are critical for protecting the host from increased morbidity and potentially mortality associated with acute systemic *S. aureus* infection, presumably as a consequence of the ability of these cells to constrain excessive proinflammatory responses. This is consistent with other studies showing peritoneal B1 cells capable of immunosuppression and host protection in response to systemic *Coxiella burnetii* infection (45).

When considering the role of B cells during *S. aureus* infections, focus has almost exclusively been on their function as Ab producers (40). In this study, we report an immune-protective role for IL-10+ B1 cells during *S. aureus* infection. B1 cells represent a regulatory innate-like population highly enriched in peritoneal, pleural cavities and more recently in the skin during inflammation, and they are capable of potent IL-10 production (41, 46). Interestingly, we did not identify any influx of IL-10+ B1 cells from the peritoneal cavity to the skin during s.c. *S. aureus* infection. These IL-10–producing B1a cells have previously been shown to be capable of limiting NK/NKT cell inflammation and macrophage activation following a pulmonary challenge with *Francisella tularensis* (47) and Th1 responses during colitis (48). They have been shown to respond to TLR activation and express B cell receptors that often recognize conserved pathogen patterns (49). B1 cells bridge innate and adaptive immunity by efficiently mounting rapid T cell–independent Ab responses, engaging in phagocytic and antimicrobial activity through the production of IgM (50). We demonstrate that *S. aureus* can directly activate IL-10 production by B1a cells through engagement of TLR2, and that these cells play a protective role in systemic infection. Importantly, the protective effect of B1a cells is independent of IgM, as we found that the levels of IgM were significantly greater in the absence of IL-10; however, this was not sufficient to prevent immunopathology (Supplemental Fig. 4).

It has previously been shown that upon exposure to *S. aureus*, human monocytes/macrophages elicit a robust IL-10 response, which in turn suppresses T cell activation (44). *S. aureus*–induced IL-10 production by monocytes/macrophages is critically TLR2 dependent and, importantly, it appears that *S. aureus*–induced pro- and anti-inflammatory cytokine production by these cells can be uncoupled due to the organism’s capacity to manipulate TLR2 plasticity and engage unique downstream signaling pathways (33). We now demonstrate that IL-10 production by B1a cells is also TLR2 dependent. Interestingly, systemic *S. aureus* infection in TLR2−/− mice also resulted in mortality caused by excessive proinflammatory response and increased bacterial dissemination that was associated with decreased IL-10 production by macrophages (51). This result mirrors that which we observed during systemic infection in IL-10−/− mice suggesting that TLR2-dependent IL-10 production from B1a cells is likely controlling proinflammatory T effector cell responses in this setting.

Overall, this study provides important new insight into the contrasting roles played by IL-10 in response to *S. aureus* at different infection sites. Additionally, we identify a novel subset of IL-10–producing B cells that undergo rapid activation in response to *S. aureus* via TLR2 engagement and are important for protecting the host against significant morbidity and mortality associated with acute systemic *S. aureus* infection. Interestingly, we have found clear contrasting roles for IL-10 during systemic and localized acute *S. aureus* infection. This suggests that the cellular microenvironment at the site of infection can significantly affect the ultimate disease outcome, potentially due to the presence of different cells being present at the infection site and/or the burden of bacteria present. Ultimately, however, *S. aureus* maintains a symbiotic relationship with the host. During systemic infection, IL-10 ensures host and, by extension, bacterial survival, whereas during local infection, IL-10 forgoes host

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**FIGURE 11.** IL-10 deficiency results in increased proinflammatory cytokine production locally in response to s.c. challenge with *S. aureus*. Groups of WT and IL-10−/− mice received an s.c. infection of *S. aureus* (USA300 LAC::lux, 2 × 10^7 CFU). At the indicated time points after challenge, skin biopsies were harvested, homogenized, and the concentrations of IL-1β (A), IL-17 (B), IL-22 (C), and CXCL1 (D) were measured by ELISA. Results are expressed as mean ± SEM (n = 9–10). Data are pooled from two individual experiments. Statistical analysis was performed using two-way ANOVA. *p ≤ 0.05, ***p ≤ 0.001.

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morbidity to facilitate bacterial survival. Taken together, these observations suggest that *S. aureus*–induced immune regulation must be critically important consideration for immunomodulatory therapeutic intervention and vaccination. Disengaging this immunosuppression may prove beneficial during localized s.c. infection; however, in the context of a systemic infection it is clear that uncontrolled effector T cell responses may be detrimental, which has obvious implications for next-generation vaccine design striving to generate effector T cell responses.

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**Disclosures**

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**References**


