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Failure To Induce IFN-β Production during Staphylococcus aureus Infection Contributes to Pathogenicity

Amber Kaplan,*† Jun Ma,*† Pierre Kyme,‡ Andrea J. Wolf,* Courtney A. Becker,* Ching Wen Tseng,‡ George Y. Liu,*‡ and David M. Underhill*§

The importance of type I IFNs in the host response to viral infection is well established; however, their role in bacterial infection is not fully understood. Several bacteria (both Gram-positive and -negative) have been shown to induce IFN-β production in myeloid cells, but this IFN-β is not always beneficial to the host. We examined whether Staphylococcus aureus induces IFN-β from myeloid phagocytes, and if so, whether it is helpful or harmful to the host to do so. We found that S. aureus poorly induces IFN-β production compared with other bacteria. S. aureus is highly resistant to degradation in the phagosome because it is resistant to lysozyme. Using a mutant that is more sensitive to lysozyme, we show that phagosomal degradation and release of intracellular S. aureus production in myeloid phagocytes, and if so, whether it is helpful or harmful to the host to do so. We found that S. aureus poorly induces IFN-β production during cutaneous infection (in vitro and in vivo) was protective. Together, the data demonstrate that failure to induce IFN-β production during S. aureus infection contributes to pathogenicity. The Journal of Immunology, 2012, 189: 4537–4545.

Staphylococcus aureus is a Gram-positive bacterium that can be both a commensal and a pathogen. Thirty to 50% of the world’s population is asymptomatically colonized with S. aureus, which mainly inhabits mucosal surfaces (15). However, under certain circumstances, it can become a life-threatening pathogen, often causing soft-tissue infections (16). The pathophysiology associated with serious infection is an area of intense research, especially in the last 10 y as antibiotic-resistant strains (such as methicillin-resistant S. aureus) have become increasingly prevalent (16). Little is known about the role of type I IFNs during S. aureus infection. A few articles have suggested a role for production of type I IFNs by airway epithelial cells in host defense during lung infection. Martin et al. (17) determined that type I IFN signaling is detrimental during S. aureus pneumonia in mice. In contrast, a recent study has shown that type I IFNs protect against S. aureus α-toxin in the lungs (18), and another has determined that stimulation of type I IFNs by treatment with CpG DNA can be protective in a model of posthemorrhage S. aureus pneumonia (19). However, whether innate immune cells such as macrophages and dendritic cells produce type I IFNs in response to S. aureus and how this might affect host defense during infection remains an important question.

During our investigations into how various Gram-positive bacteria induce IFN-β, we noticed that S. aureus induces little of the cytokine in myeloid phagocytes in vitro or during cutaneous infection in vivo. This seemed unusual to us because the related bacterial species, GBS, readily induced IFN-β production from myeloid phagocytes (11). We found that because S. aureus is resistant to degradation in the phagosome, because of modifications to intracellular ligands is essential for induction of IFN-β and inflammatory chemokines downstream of IFN-β. Further, we found that adding exogenous IFN-β during S. aureus infection (in vitro and in vivo) was protective. Together, the data demonstrate that failure to induce IFN-β production during S. aureus infection contributes to pathogenicity.

Materials and Methods

Bacterial strains and culture

Wild type S. aureus strain 113 (WT-SA) and O-acetyltransferase A mutant (ΔOatA-SA) kindly provided by F. Gotz (20). S. aureus Pig1 (kindly provided by D. Leung) (21), and S. aureus Newman were grown in Todd

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Abbreviations used in this article: BMDC, bone marrow-derived dendritic cell; GBS, group B Streptococcus; MOI, multiplicity of infection; ΔOatA-SA, O-acetyltransferase A mutant; PFA, paraformaldehyde; THB, Todd Hewitt broth; WT-SA, wild type S. aureus strain 113; vita-PAMP, pattern-associated molecular patterns found in live but not dead bacteria.

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Hewitt broth (THB) at 37°C with agitation overnight to stationary phase, washed in sterile PBS, then diluted to OD_{600} = 0.4 (determined to be 1 × 10^9 CFU/ml). We generated a bioluminescent strain of *S. aureus* Pig1 (CST22). Isogenic strains harboring Tn4001 lxABCDE KmR (22) were constructed using previously established transduction methods, to introduce the plasmid or the alleles into the CST22 background (23). *S. agalactiae* (GBS) was grown in THB. *Salmonella enterica* serovar Typhimurium and *E. coli* were grown in Luria broth. *L. monocytogenes* was grown in brain-heart infusion broth.

**Mice and murine cell culture**

Casparase-1^−/−^, MyD88^−/−^, and TLR7^−/−^ mice on C57BL/6 background were bred and housed under specific pathogen-free conditions in the Cedars-Sinai Medical Center animal facility. All protocols were reviewed and approved by the Cedars Sinai Institutional Animal Care and Use Committee. C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). TRIF^−/−^ and TLR9^−/−^ mice were provided by B. Beutler (25), and MAVS^−/−^ mice were provided by K. Fitzgerald (26). Conventional bone marrow-derived dendritic cells (BMDC) were made by culturing bone marrow-derived precursors (35) in supplemented RPMI, with 10 ng/ml rGM-CSF (PeproTech) on days 0 and 4. Human cell culture

Monocytes were isolated from fresh human blood. We obtained blood from healthy donors within our hospital, and samples were anonymized before being provided for use and analysis in this study. Blood was layered over lymphocyte separation media (Fisher) and spun at 1000 × g for 25 min with no brake. The interface was removed and washed three times in PBS. Monocytes were negatively selected using a magnetic bead system (Miltenyi Biotech). Isolated monocytes were allowed to differentiate for 7 d into dendritic cells. Growth factors (recombinant human IL-4 and GM-CSF [10 ng/ml each; PeproTech]) were added on days 0 and 4.

**Peptidoglycan purification**

Peptidoglycan was prepared as described previously (27). In brief, overnight cultures of wild type or ΔOatA *S. aureus* were heat killed and washed with acetone. Bacteria were lysed using glass beads and a Turbomix for 10 min at 4°C. Unbroken cells were removed by low-speed centrifugation, and cell-wall fragments were collected by high-speed centrifugation. Washed cell walls were treated with RNase A and DNase I overnight at 37°C followed by overnight treatment with trypsin. The remaining peptidoglycan was extracted for 5 h at 4°C with 10% trichloroacetic acid and washed with acetone.

**S. aureus infection in vitro**

BMDC were plated in 24-well plates (3 × 10^5 cells/well) for RNA or 96-well plates (1 × 10^5 cells/well) for protein measurements 16–24 h before infection. On the day of infection, media was replaced with antibiotic-free RPMI and 400 µg/ml gentamicin was added to cells for 30 min to kill extracellular bacteria. After 30 min, media was replaced with one containing a lower concentration of gentamicin (100 µg/ml), and cells were incubated at 37°C and 5% CO2 for indicated times. Gentamicin-killed bacteria were incubated with shaking at 37°C for 90 min with 100 µg/ml gentamicin and washed in PBS three times. Paraformaldehyde (PFA)-fixed bacteria were incubated with 4% PFA for 10 min at room temperature and washed in PBS three times.

**FIGURE 1.** Lysozyme resistance contributes to the failure of *S. aureus* to induce IFN-β in dendritic cells. (A and B) BMDC were infected with *E. coli*, *S. enterica* serovar Typhimurium (S. Typh), *L. monocytogenes* (L. mon), *Streptococcus agalactiae* (GBS), and three wild type *S. aureus* strains: 113, Pig1, and Newman (New.) (MOI 10). RNA was harvested at 4 h for detection of IFN-β mRNA by RT-PCR (A), and supernatants were harvested at the indicated time points for IFN-β protein bioassay (B). (C) BMDC were infected with WT-SA or ΔOatA-SA (MOI 10). RNA was harvested at the indicated time points for detection of IFN-β mRNA by RT-PCR. (D) BMDC were infected with WT-SA or ΔOatA-SA (at the indicated MOI). RNA was harvested at 4 h for detection of IFN-β mRNA by RT-PCR. (E) BMDC were infected with WT-SA or ΔOatA-SA (MOI 10). Supernatants were harvested at the indicated time points for IFN-β protein assay. (F) BMDC were infected with WT-SA or ΔOatA-SA (MOI 50). Supernatants were harvested at 10 h for IFN-β protein bioassay. All data are shown as mean ± SD.
**Murine whole-blood killing assay**

Murine peripheral whole blood was harvested aseptically via cardiac puncture using a 22-gauge needle to minimize cell lysis and maintain integrity of blood for the duration of the assay. Blood was mixed with PBS or recombinant murine IFN-β. WT-SA were pelleted, washed, and diluted to 10^7 CFU/ml in PBS (without Ca^2+ and Mg^2+), and 25 µl bacteria was immediately mixed with 75 µl blood. One hundred-microliter reactions were performed in sterile, heparinized, 2-ml, round-bottom Eppendorf tubes, in triplicate and incubated at 37°C on a rotary shaker. After 3 h of incubation, 10-fold serial dilutions were plated on THB plates to quantify surviving CFU.

**Cytokine measurement**

IL-1β and TNF-α levels in cell supernatants were measured by ELISA according to the manufacturer’s instructions (BioLegend). IFN-β in cell supernatants was measured via a luciferase 1295-ISRE bioassay (kindly provided by B. Beutler) (11). Cell supernatants were harvested and frozen immediately at −80°C, then later incubated with L929-ISRE cells at a dilution of 1:2 for 8 h. Concentrations of IFN-β were calculated according to a standard curve of luciferase activity from 2-fold serial dilutions of IFN-β.

**Quantitative RT-PCR**

Total RNA was harvested from cells using RNeasy columns (Qiagen) using the manufacturer’s protocol. Total RNA was harvested from skin lesions that were snap frozen in liquid N2 and then stored at −80°C. Skin was then minced on ice, before being ground using an agate mortar and pestle containing liquid N2. Pulverized samples were put through QIA Shredder columns (Qiagen), incubated with proteinase K, and then run through RNeasy columns (Qiagen) per manufacturer’s protocol. Synthesis of cDNA was completed with Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manufacturer’s recommendations and primed with oligo d(T). Quantitative RT-PCR was performed on a Realplex Mastercycler (Eppendorf).

**Experimental model of in vivo S. aureus infection**

Skin infections were performed following an established protocol for generating localized S. aureus s.c. infection (21, 28). In brief, female C57BL/6 mice (7–10 wk old) underwent hair removal at least 24 h before infection (via shaving and use of Nair). Mice were injected s.c. in both flanks with (1 × 10^7 CFU). S. aureus strain 113 (WT or ΔOatA) was used for in vivo RT-PCR. WT-SA and S. aureus strain CST222 (bioluminescent Pig) were used for IFN-β–treated infection studies. Bacterial cultures were washed, diluted, and resuspended in PBS containing 0.5 mg/ml sterile Cytodex beads (GE Healthcare). Fifty units recombinant murine IFN-β was used in WT-SA infections and 250 U IFN-β was used in CST222 infections. Mice were anesthetized with isoflurane and injected with 100 µl on each flank using a 1-ml syringe fitted with a 27-gauge needle. Mice were monitored daily for lesion phenotype and size for WT-SA infections; lesions were harvested after 48 h of infection and surviving CFU quantified. Mice infected with CST222 were monitored using Xenogen IVIS imaging system (Xenogen Corporation, Alameda, CA) to quantify bioluminescence in the lesion (72 h postinfection is shown). Images are grayscale photographs of mice with a color scale overlay that quantifies radiance (photons/s) within a circular region of interest with Living Image software (Xenogen).

**Results**

S. aureus fails to induce IFN-β in dendritic cells

In an attempt to learn more about how Gram-positive bacteria induce type I IFNs, we compared IFN-β production by mouse dendritic cell supernatants after 10 h of infection with S. aureus strain 113 (WT or ΔOatA) was used for in vivo RT-PCR. WT-SA and S. aureus strain CST222 (bioluminescent Pig) were used for IFN-β–treated infection studies. Bacterial cultures were washed, diluted, and resuspended in PBS containing 0.5 mg/ml sterile Cytodex beads (GE Healthcare). Fifty units recombinant murine IFN-β was used in WT-SA infections and 250 U IFN-β was used in CST222 infections. Mice were anesthetized with isoflurane and injected with 100 µl on each flank using a 1-ml syringe fitted with a 27-gauge needle. Mice were monitored daily for lesion phenotype and size for WT-SA infections; lesions were harvested after 48 h of infection and surviving CFU quantified. Mice infected with CST222 were monitored using Xenogen IVIS imaging system (Xenogen Corporation, Alameda, CA) to quantify bioluminescence in the lesion (72 h postinfection is shown). Images are grayscale photographs of mice with a color scale overlay that quantifies radiance (photons/s) within a circular region of interest with Living Image software (Xenogen).
cells infected with several different strains of *S. aureus* or with a panel of bacteria previously reported to induce IFN-β. Although control bacteria induced IFN-β mRNA and protein as expected, none of the *S. aureus* strains did (Fig. 1A, 1B). GBS (11) is related to *S. aureus* and readily induced IFN-β production, so it seemed unusual that none of the *S. aureus* strains did. One major difference between *S. aureus* and many Gram-positive bacteria is that it is highly resistant to degradation by the lysozyme that is produced in myeloid phagocytes (20). *S. aureus* makes an enzyme, peptidoglycan O-acetyltansferase, that structurally modifies its cell-wall peptidoglycan so that it is not recognized by lysozyme (20). We and others have previously observed that degradation of *S. aureus* is an important factor in inducing production of certain cytokines by phagocytes (27, 29, 30). We therefore explored whether degradation could lead to induction of IFN-β during *S. aureus* infection. We infected dendritic cells with a wild type *S. aureus*, SA113 (WT-SA) or an isogenic mutant lacking peptidoglycan OatA (ΔOatA-SA) that is sensitive to lysozyme (20). IFN-β mRNA induction was much higher in cells infected with ΔOatA-SA compared with WT-SA over a wide range of infectious doses (Fig. 1C, 1D). IFN-α did not appear to be induced in response to WT-SA or ΔOatA-SA (data not shown). Infection with ΔOatA-SA also led to greater secretion of IFN-β protein compared with WT-SA (Fig. 1E, 1F). In contrast, ΔOatA-SA and WT-SA stimulate similar levels of TNF-α production particularly at higher infectious doses, consistent with our previous studies (Supplemental Fig. 1A) (27, 29). This observation is not unique to mouse cells, because we also observed greater production of IFN-β by human dendritic cells infected with ΔOatA-SA compared with WT-SA bacteria (Supplemental Fig. 1B). Taken together, the data show that *S. aureus* poorly induces IFN-β production by human and mouse dendritic cells, and that making *S. aureus* more sensitive to lysozyme enhances IFN-β production.

**Mutant *S. aureus* induce IFN-β and IL-1β through independent mechanisms**

A couple publications show that IL-1β release can be dependent on IFN-β in response to certain intracellular bacteria (31, 32). We have previously shown that *S. aureus* poorly activates the inflammasome (and subsequent IL-1β release) in myeloid cells, and that this is also mediated by the resistance of the bacteria to lysozyme (Supplemental Fig. 1C and Ref. 27). We therefore wondered whether the IL-1β effects we have previously reported were simply caused by the IFN-β changes we have now observed. This does not seem to be the case. First, degradation-sensitive *S. aureus* peptidoglycan is sufficient to trigger IL-1β production (Fig. 2A and Ref. 27), whereas peptidoglycan is not sufficient to induce IFN-β (Fig. 2B). Second, adding exogenous IFN-β to dendritic cells has no effect on their ability to produce IL-1β in response to WT-SA or ΔOatA-SA infection (Fig. 2C). Therefore, altered IFN-β production was not a cause of our previously reported change in

**FIGURE 3. IFN-β induced by ΔOatA-SA is MyD88 dependent but TLR dependent.** (A) WT and MyD88−/− BMDC were stimulated with Cpg (5 μg/ml) or infected with ΔOatA-SA (MOI 1). Supernatants were harvested at 10 h for IFN-β protein bioassay. (B) WT and TRIF−/− BMDC were stimulated with LPS (100 ng/ml) or infected with ΔOatA-SA (MOI 1). Supernatants were harvested at 10 h for IFN-β protein bioassay. (C) WT and TLR7−/− BMDC were infected with ΔOatA-SA (MOI 1). Supernatants were harvested at the indicated time points for IFN-β protein bioassay. (D) WT and TLR9−/− BMDC were infected with ΔOatA-SA (MOI 1). Supernatants were harvested at the indicated time points for IFN-β protein bioassay. (E) WT and UNC93B1−/− BMDC were infected with ΔOatA-SA (MOI 1). Supernatants were harvested at the indicated time points for IFN-β protein bioassay. All data are shown as mean ± SD.
IL-1β. We also do not observe that enhanced IFN-β production during infection with ΔOatA-SA is somehow caused by an increase in IL-1β. First, adding exogenous IL-1β had no effect on the ability of the cells to produce IFN-β in response to WT-SA or ΔOatA-SA infection (Fig. 2D). Second, caspase-1–deficient cells showed no defect in IFN-β production, whereas IL-1β was blocked in response to ΔOatA-deficient S. aureus (Fig. 2E, 2F). Taken together, these observations demonstrate that mutant S. aureus induces IFN-β and IL-1β through independent mechanisms.

**IFN-β induction by mutant S. aureus is MyD88 dependent but TLR independent**

We next set out to define how degradation-sensitive *S. aureus* induces IFN-β production. Several TLRs are known to induce IFN-β, including TLR3 and TLR4 (which both use the adaptor TRIF to signal) and TLR7 and TLR9 (which are dependent on MyD88 as an adaptor molecule) (8). We therefore tested the ability of ΔOatA-SA to induce IFN-β in both MyD88- and TRIF-deficient dendritic cells. MyD88 was required for IFN-β secretion in response to infection with ΔOatA-SA; however, TRIF was not (Fig. 3A, 3B). This suggested that TLR7 and/or TLR9 might be involved. TLR7 recognizes single-stranded RNA within endosomes and requires MyD88 to signal, so we infected TLR7-deficient cells with ΔOatA-deficient *S. aureus* to determine whether they were defective for IFN-β production. ΔOatA-SA-induced IFN-β was not dependent on TLR7 (Fig. 3C). TLR9 is another endosomal TLR that recognizes CpG DNA and like TLR7 requires MyD88 to signal. TLR9 was also not required for ΔOatA-SA–induced IFN-β (Fig. 3D). We therefore hypothesized that ΔOatA-SA could stimulate both receptors, and that loss of TLR7 or TLR9 could be compensated for by the other. To test this, we infected UNC93B1-deficient cells with ΔOatA-SA. UNC93B1 is an endoplasmic reticulum membrane protein required for folding and trafficking of all three endosomal TLRs (TLR3, TLR7, and TLR9), and thus UNC93B1-deficient cells fail to express all three receptors (8, 33). Surprisingly, UNC93B1 was not required for IFN-β induced by degradation-sensitive *S. aureus* (Fig. 3E). We also tested whether IFN-β induced by ΔOatA-SA was TLR2 dependent by infecting TLR2-deficient cells, but also found no difference from wild type cells (data not shown). Together, the data show that IFN-β induction in response to ΔOatA-SA is MyD88 dependent yet TLR independent, a situation that is similar to what has recently been observed for group A Streptococcus (*S. pyogenes*) (34).

**IFN-β induction by mutant *S. aureus* requires internalization and degradation of bacteria and intact RNA species but is independent of cytosolic RNA sensors RIG-I and Mda5**

Having observed that TLRs are not involved in IFN-β induction by ΔOatA-SA, we next explored other processes that might be required. In our previous work with these bacteria, we noted that phagocytosis and degradation within phagolysosomes is important for induction of certain inflammatory responses (27, 29). We therefore examined whether these cellular processes are important for induction of IFN-β. When we treated cells with cytochalasin D to block phagocytosis, ΔOatA-SA–induced secretion of IFN-β was blocked (Fig. 4A). Also, when we treated cells with bafilomycin A to prevent acidification of lysosomes, IFN-β production was blocked (Fig. 4B). Together, these results suggested involvement of an internal receptor recognizing ligands exposed in the process of bacterial degradation, but the ligand and the response were different from any we had previously examined.

Recent work by Sander et al. (35) demonstrated the importance of “vita-PAMPs,” pattern-associated molecular patterns that are found in live but not dead bacteria. The study showed that *E. coli* RNA released during phagocytosis could stimulate IFN-β production. The authors showed that RNA vita-PAMPs are present in live bacteria and formaldehyde-preserved bacteria, but are lost in heat- and antibiotic-killed bacteria. Whether a similar mechanism is at work for discrimination between live and dead Gram-positive bacteria is not known. We therefore tested whether IFN-β induced by ΔOatA-deficient *S. aureus* was dependent on the presence of vita-PAMPs by exposing dendritic cells to live, heat-killed, gentamicin-killed, or PFA-fixed WT-SA and ΔOatA-SA. Killing bacteria with heat or gentamicin, conditions that promote degradation of bacterial RNAs (35), abrogated IFN-β production. In contrast, killing bacteria with formaldehyde, a condition that

**FIGURE 4.** IFN-β induced by ΔOatA-SA requires phagocytosis, lysosomal acidification, and intact RNA. (A) BMDC were pretreated with cytochalasin D (CytoD) for 30 min and infected with ΔOatA-SA (MOI 1). Supernatants were harvested at 10 h for IFN-β protein bioassay. (B) BMDC were pretreated with bafilomycin A (BafA) for 30 min and infected with ΔOatA-SA (MOI 10). Supernatants were harvested at 10 h for IFN-β protein bioassay. (C) BMDC were infected with live, heat-killed (HK), gentamicin-killed (GENTA), or PFA-killed WT-SA or ΔOatA-SA (MOI 1). Supernatants were harvested at 18 h for IFN-β protein bioassay. (D) WT and MAVS−/− BMDC were infected with ΔOatA-SA (MOI 10). Supernatants were harvested at the indicated time points for IFN-β protein bioassay. All data are shown as mean ± SD.
preserves bacterial RNAs, maintained IFN-β production (Fig. 4C). Taken together, the data demonstrate that wild type *S. aureus* avoids inducing IFN-β by making itself resistant to degradation within phagosomes and preventing the release of vita-PAMPs. In an effort to identify the receptor that ΔOatA-SA RNA stimulates to induce IFN-β, we explored whether the cytosolic receptors RIG-I or MDA5 might play a role. These receptors recognize RNA in the cytosol leading to IFN-β production, and thus seemed plausible candidates. We infected MAVS-deficient cells (an adaptor required by both RIG-I and MDA5 for signaling) with ΔOatA-SA and compared the amount of IFN-β protein produced to similarly infected wild type cells; however, we saw no decrease in the MAVS-deficient cells (Fig. 4D). RIG-I and MDA5 do not appear to play a role in IFN-β induction by ΔOatA-SA.

**IFN-β stimulates host responses that are protective against *S. aureus***

In different bacterial infection models, IFN-β can either be protective or detrimental to the host (1). Because wild type *S. aureus* is able to avoid production of IFN-β in myeloid cells through synthesis of degradation-resistant peptidoglycan and reduced release of vita-PAMPs, we wondered whether reducing IFN-β production would be beneficial to the bacteria. IFN-β is a potent cytokine known to turn on hundreds of downstream genes (36, 37). We

**FIGURE 5.** IFN-β induces chemokines and enhances phagocyte killing of *S. aureus*. (A and B) BMDC were infected with WT-SA or ΔOatA-SA (MOI 10) and RNA was harvested at the indicated time points. CXCL10 (A) and CCL5 (B) mRNAs were measured by RT-PCR. (C) BMDC were infected (MOI 10) with WT-SA plus 100 U/ml recombinant murine IFN-β or vehicle control (VEH). Cells were lysed after 24 h of infection, and surviving intracellular CFU were plated and counted. (D and E) Murine whole blood was treated with PBS (VEH) or recombinant murine IFN-β (final concentration 10³ U/ml) and mixed with WT-SA (final concentration 1 × 10⁸ CFU/ml). Blood was incubated for 3 h, and surviving bacteria were plated and counted (D). Representative images of PBS- or IFN-β–treated infected whole blood (E).
measured two such genes in dendritic cells infected with ΔOatA-SA compared with WT-SA. Both CXCL10 and CCL5 were highly upregulated in cells infected with ΔOatA-SA (Fig. 5A, 5B). Thus, reduced IFN-β secretion in response to wild type S. aureus also reduces induction of downstream genes, many of which are chemokines important for orchestrating local inflammatory responses including neutrophil recruitment (38).

We also examined whether the presence of IFN-β could directly affect the ability of cells to kill S. aureus. Addition of exogenous IFN-β to dendritic cells infected with WT-SA substantially aided in clearance of bacteria after 24 h (Fig. 5C). Of course, IFN-β acts on many cell types other than dendritic cells, and we therefore wanted to examine the broader effects of IFN-β on cellular responses to S. aureus. We treated fresh murine whole blood with IFN-β and infected with WT-SA. IFN-β–treated whole blood had fewer surviving bacteria after 3 h of infection compared with blood treated with vehicle (Fig. 5D, 5E). Thus, the presence of IFN-β during infection with wild type S. aureus directly activates phagocytes to kill bacteria.

**IFN-β production is suppressed during in vivo S. aureus infection, and adding exogenous IFN-β enhances protection**

Having demonstrated that the presence of IFN-β could enhance bacterial killing in vitro, we next wanted to investigate the role of this cytokine in vivo. Using a s.c. model of S. aureus infection, we measured the induction of IFN-β mRNA in lesions infected with WT-SA or ΔOatA-SA at 4 and 9 h postinfection. ΔOatA-SA lesions had significantly increased IFN-β expression over WT-SA at both time points (Fig. 6A). In addition, CCL5, an important chemokine induced by IFN-β, was also expressed at higher levels in lesions infected with degradation-sensitive S. aureus (Fig. 6B). These data agree with the in vitro studies and confirm that S. aureus is a poor inducer of IFN-β during in vivo infection.

Because S. aureus is a poor inducer of IFN-β during infection, we next explored whether providing exogenous IFN-β could be a viable strategy for enhancing host defense against S. aureus. We infected mice s.c. with WT-SA alone or together with 50 U IFN-β, and assessed CFU in the lesion and lesion size after 48 h. IFN-β treatment significantly reduced the number of S. aureus recovered from lesions after 48 h, indicating that the cytokine is protective in vivo (Fig. 6C). Enhanced bacterial clearance in IFN-β–treated mice was also associated with accelerated formation of necrotic lesions 24 and 48 h postinfection (Supplemental Fig. 2A, 2B), consistent with a more aggressive inflammatory response to the bacteria. WT-SA is a commonly used laboratory strain (SA113), and we therefore wondered whether IFN-β could be used to suppress growth of a clinically important strain as well. We generated a bioluminescent form of S. aureus strain Pig1 (a highly pigmented strain isolated from the skin of a child with atopic dermatitis) that we have confirmed fails to stimulate IFN-β pro-

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**FIGURE 6.** IFN-β production is suppressed during in vivo S. aureus infection, and adding exogenous IFN-β enhances protection. (A and B) Mice were injected s.c. with $1 \times 10^7$ CFU/ml WT-SA or ΔOatA-SA (3 mice/group, 2 lesions/mouse, n = 6 lesions/group). Lesions were harvested and RNA was extracted at the indicated time points for detection of IFN-β (A) and CCL5 (B) mRNA by RT-PCR. (C) Mice were injected s.c. with $1 \times 10^7$ CFU WT-SA with a vehicle control or with 50 U recombinant murine IFN-β (6 mice/group, 2 lesions/mouse, n = 12 lesions/group). Skin lesions were harvested at 48 h postinfection, and surviving bacteria were plated and counted. (D) Mice were injected s.c. with $1 \times 10^7$ CFU of the clinical S. aureus isolate Pig1 (bioluminescent strain CST222) with a vehicle control or with 250 U recombinant murine IFN-β (6 mice/group, 2 lesions/mouse, n = 12 lesions/group). Bioluminescence was quantified at 72 h postinfection. (E) Representative Xenogen images of skin lesions at 72 h. Red bars indicate means. *$p < 0.05$, **$p < 0.01$ (unpaired two-tailed t test).
duction from immune cells (Fig. 1A, B). We infected mice s.c. with S. aureus Pig1 alone or together with 250 U IFN-β and assessed bacterial load in the lesion by Xenogen imaging after 72 h. IFN-β treatment significantly reduced the number of S. aureus in lesions, indicating that the cytokine is protective against a clinical strain of bacteria as well (Fig. 6D, 6E). Together, these data show that S. aureus reduces degradation-induced IFN-β production by phagocytes, resulting in less local inflammatory cell recruitment and enhanced bacterial survival.

**Discussion**

In this study, we have shown that S. aureus is a poor inducer of IFN-β production by host immune cells in vitro and in vivo, and that the failure to stimulate IFN-β production is beneficial to the bacteria. S. aureus avoids host activation of IFN-β in phagocytes through modifications to its cell wall that make it resistant to degradation. This blocks release of molecules from live bacteria that would otherwise be released during killing and degradation in phagosomes, and would stimulate IFN-β production. Using a mouse model of S. aureus skin infection, we show that treatment with IFN-β can promote clearance of bacteria.

The lack of IFN-β induced in myeloid cells by S. aureus is notable with respect to other Gram-positive bacterial species that induce large amounts of IFN-β, including GBS (S. agalactiae) (11), group A Streptococcus (S. pyogenes) (12), and Streptococcus pneumonia (34). Consistent with previous reports, we observed that GBS stimulates IFN-β production in myeloid cells. An important difference between GBS and S. aureus is that it does not modify its cell walls to make it resistant to phagosomal degradation the way that S. aureus does. We hypothesize that this is the reason that GBS stimulates IFN-β production, but S. aureus does not.

We have previously shown that degradation of S. aureus is important for activation of the inflammasome and secretion of IL-1β (27), although this study shows that the mechanisms by which degradation promotes induction of IL-1β and IFN-β are different. Purified, degradation-sensitive peptidoglycan (from ΔOatA-SA) induces IL-1β production, whereas wild type peptidoglycan does not (27). This suggests that the inflammasome is activated by degradation products of peptidoglycan. In contrast, we have observed in this study that IFN-β production is not stimulated by purified peptidoglycans from either wild type or mutant S. aureus, suggesting that the activating ligands for this response are not part of peptidoglycan.

Some recent reports have suggested that IL-1β production and IFN-β production are interrelated (31, 32); however, the two responses appear completely independent of each other in our study of S. aureus. Exogenous addition of either cytokine does not affect the production of the other in response to infection with S. aureus, and IFN-β production is not affected in caspase-1-deficient cells. These findings are consistent with Henry et al.’s study in which IFN-β and IL-1β production are independent of each other in response to extracellular/vacuolar bacteria such as Salmonella, but dependent on each other in response to infection with intracellular bacteria, such as Francisella tularensis and L. monocytogenes (31).

We have previously observed that degradation of S. aureus in phagosomes can lead to exposure of bacterial DNA that triggers TLR9-mediated enhancement of production of classical inflammatory cytokines such as IL-6 and TNF-α (29), and we were thus surprised to discover that TLR9 and other endosomal TLRs do not make significant contributions to induction of IFN-β. Instead, we found that IFN-β production is dependent largely on release of vita-PAMPs as defined by Sander et al. (35). These authors demonstrated that mRNA from live E. coli within phagosomes is able to leak out and leads to activation of inflammatory responses (including IFN-β production). In contrast, mRNAs are rapidly degraded in dead bacteria, and thus are not released when these bacteria are phagocytosed. This led the authors to coin the term “vita-PAMPs” to describe inflammatory ligands associated with live but not dead microbes. Our data are consistent with this concept in that dead S. aureus (even degradation-sensitive S. aureus) do not stimulate IFN-β production unless they have been killed in a manner that preserves mRNA (i.e., fixed with PFA). Thus, we have extended the previous work showing that vita-PAMPs from Gram-negative bacteria can influence inflammatory signaling to now include vita-PAMPs from a Gram-positive organism. Further, we document a strategy by which a live bacterium can prevent exposure of vita-PAMPs and the resulting immune responses. One way in which recognition of E. coli and S. aureus vita-PAMPs appear to be different is in their requirement for MyD88. Most inflammatory cytokines induced by live E. coli are MyD88 independent, whereas we have observed that IFN-β induction by live degradation-sensitive S. aureus is MyD88 dependent, even though this response is TLR independent. Recent work by Gratz et al. (34) has also described a MyD88-dependent, TLR-independent type of IFN-β induction for group A Streptococcus.

We have demonstrated that the ability of S. aureus to avoid inducing IFN-β during infection in vivo is significant for its survival. Upon infection with a degradation-sensitive S. aureus, IFN-β induction led to activation of downstream chemokines, including CXCL10 and CCL5, that are important for orchestration of local inflammatory responses. Further, treating dendritic cells, whole blood, and mice with exogenous IFN-β increased protection against infection with S. aureus. The protective effects of IFN-β during skin infection were seen for two different strains of S. aureus, including a medically relevant clinical isolate, Pig1. The beneficial role of IFN-β during S. aureus infection was previously unrecognized and adds to our understanding of how this important human pathogen is able to subvert the host’s immune defenses.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


