Influenza A Virus Exacerbates *Staphylococcus aureus* Pneumonia in Mice by Attenuating Antimicrobial Peptide Production

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Influenza A represents a significant cause of morbidity and mortality worldwide. Bacterial complications of influenza A confer the greatest risk to patients. TH17 pathway inhibition has been implicated as a mechanism by which influenza A alters bacterial host defense. Here we show that preceding influenza causes persistent *Staphylococcus aureus* infection and suppression of TH17 pathway activation in mice. Influenza does not inhibit *S. aureus* binding and uptake by phagocytic cells but instead attenuates *S. aureus* induced TH17 related antimicrobial peptides necessary for bacterial clearance in the lung. Importantly, exogenous lipocalin 2 rescued viral exacerbation of *S. aureus* infection and decreased free iron levels in the bronchoalveolar lavage from mice coinfectected with *S. aureus* and influenza. These findings indicate a novel mechanism by which influenza A inhibits TH17 immunity and increases susceptibility to secondary bacterial pneumonia. Identification of new mechanisms in the pathogenesis of bacterial pneumonia could lead to future therapeutic targets.

**Keywords.** influenza A; *Staphylococcal aureus*; pneumonia; TH17; host defense; coinfection.

Pneumonia is a leading cause of death worldwide, accounting for the highest mortality in children younger than 5 years of age, resulting in approximately 1.4 million deaths in 2010 [1]. In addition, pneumonia ranks 9th overall as a cause of death annually in the United States [2]. Numerous bacterial organisms cause pneumonia, including *Staphylococcus aureus*. The prevalence of *S. aureus* has increased in recent years with the emergence of methicillin-resistant *S. aureus*. MRSA currently accounts for 20%–40% of hospital-acquired and ventilator-acquired pneumonias [3] and 9% of community-acquired pneumonias [4]. A primary cofactor involved in mortality due to community acquired MRSA infection is preceding influenza-like illness [5].

Influenza is a common respiratory illness that affects 5%–20% of the US population yearly and results in approximately 30 000 deaths annually. Although most cases of influenza are not fatal, complications such as bacterial pneumonia can have serious consequences. Increased intensive care admission, mechanical ventilation, and mortality have been described in children and young adults with influenza A and concomitant *S. aureus* infection compared to those with either influenza or *S. aureus* infection alone [6]. Tissue histologic specimens and bacteriologic samples recovered from autopsies performed during the 1918 pandemic reveal that most deaths were likely caused by secondary bacterial pneumonias [7]. Given the significant morbidity and mortality associated with influenza and secondary bacterial pneumonia, the identification of therapeutic immune targets to alleviate this copathogenesis will have substantial clinical benefits.
The role of T cells in host defense against bacterial pneumonia is recently emerging. We have previously demonstrated that mice sequentially infected with influenza A and S. aureus resulted in influenza A-induced attenuation of S. aureus driven T\textsubscript{H}17 pathway activation and increased susceptibility to bacterial pneumonia [8]. However, the breadth and kinetics of influenza A-induced inhibition of the T\textsubscript{H}17 pathway and the explanation for the lung’s increased susceptibility to bacterial infection remains unknown. In addition, the specific mechanism(s) by which the T\textsubscript{H}17 pathway promotes S. aureus clearance has not been previously examined.

**METHODS**

**Mice**

Six to eight week-old male C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). Mice were maintained under pathogen-free conditions and studies performed on age- and sex-matched mice.

**S. aureus Infection**

Methicillin-sensitive S. aureus (MSSA; ATCC 49775) and methicillin-resistant S. aureus (MRSA; USA 300) were used to inoculate mice with either 1 × 10\textsuperscript{8} colony-forming units (CFU) of MSSA or 5 × 10\textsuperscript{7} of MRSA (in 50 µL sterile phosphate-buffered saline [PBS]) by oropharyngeal aspiration.

**Influenza A Infection**

Influenza A PR/8/34 H1N1 and Influenza A CA/07/2009 H1N1 [9, 10] were used to inoculate mice with 100 plaque-forming units (PFU) of influenza (in 40 µL sterile PBS) by oropharyngeal aspiration. Viral burden was determined by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) on lung RNA for viral matrix protein as described [8].

**Analysis of Lung Inflammation**

Mouse lungs were lavaged with 1 mL sterile PBS for inflammatory cell counts. Lung homogenate was used for bacterial colony counting and cytokine analysis by Lincoplex (Millipore, Billerica, MA) or by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). RNA extraction was performed using GraphPad Prism and/or Microsoft Excel software.

**Depletion Antibodies**

BioXcell (West Lebanon, NH) antibodies clones RB6-8C5 (anti-Ly6G/C) and IA8 (anti-Ly6G) and anti-IL-17 were used. Mice received 250 µg (300 µg anti-IL-17) of antibody 24 hours prior to S. aureus challenge.

**Lipocalin 2 Protein**

Lipocalin 2 (Lcn2) recombinant protein purification was performed as described elsewhere [11, 12] with modifications.

**S. aureus Macrophage Binding and Uptake**

S. aureus was labeled with fluorescein isothiocyanate (FITC; Life Technologies). FITC-labeled S. aureus were instilled into mice, and bronchoalveolar lavage (BAL) was performed 24 hours after challenge. BAL cells were stained with fluorescent conjugated antibodies against F4/80, Ly6G, Ly6G/C (GR-1), and Cd11c for flow cytometry. For in vitro studies, alveolar macrophages were isolated from the BAL of naïve, PBS control, and influenza infected mice. Flow cytometry was performed to determine total number of FITC-positive cells and the total number of FITC-positive cells in the presence and absence of interferon γ and/or interferon β.

**Free Iron Quantification**

At indicated time points, mouse lungs were lavaged with 1 mL sterile PBS and Ferrous (Fe\textsuperscript{2+}) ions were measured using an iron assay kit (Abcam, Cambridge MA). Lavage samples were analyzed without assay buffer or iron reducing reagents in order to detect free iron.

**Statistical Analysis**

The data are presented as the mean ± standard error of the mean (SEM). Significance was tested by unpaired t test (for 2 means) or one-way analysis of variance (ANOVA; for multiple data groups) followed by Tukey post hoc test. Data were analyzed using GraphPad Prism and/or Microsoft Excel software.

**RESULTS**

Preceding Influenza A Infection Prolongs S. aureus Pneumonia and Attenuates Acute and Chronic T\textsubscript{H}17 Pathway Activation by S. aureus

We have previously demonstrated that preceding influenza A infection results in increased susceptibility to bacterial pneumonia at 24 and 48 hours following bacterial challenge [8], but the persistence of bacterial colonization remains unknown. We challenged C57BL/6 mice with influenza A PR/8/34 H1N1 for 6 days followed by S. aureus (ATTC 49775) and after 6, 24, 48, 72, 96, and 120 hours, bacterial and viral clearance and lung inflammation were assessed. Preceding influenza infection resulted in decreased clearance of S. aureus in the lung up to 120 hours following bacterial challenge (Figure 1A). There was also significant exacerbation of lung inflammation for up to 120 hours (Supplementary Figure 1A). Histology of lungs coinfectcd with influenza A and S. aureus at 120 hours postbacterial challenge revealed increased inflammation and lung damage...
compared to *S. aureus* alone (Figure 1C). These data show that preceding influenza A infection causes increased inflammation and *S. aureus* burden, as well as bacterial persistence in the lung. We proposed that there would be rapid and persistent attenuation of the Th17 pathway in coinfected mice following bacterial challenge. *S. aureus* induced a more robust Th17 effector cytokine response up to 120 hours following bacterial challenge in mice infected with *S. aureus* alone compared to coinfected mice (Figure 1B, Supplementary Figure 1B and C). Importantly, we examined early Th17 cytokine responses and found that *S. aureus* failed to induce IL-17 (Figure 1D) or interleukin 23 (IL-23; Supplementary Figure 1D) at early time points following challenge in mice previously infected with influenza. Finally, we examined whether 2009 pandemic influenza followed by *S. aureus* challenge would also result in decreased bacterial clearance compared to mice infected with *S. aureus* alone. We challenged C57BL/6 mice with influenza CA/07/2009/A H1N1 for 6 days followed by *S. aureus* (ATTC 49775) and after 24 hours, assessed bacterial clearance. Preceding 2009 H1N1 influenza infection resulted in decreased clearance of *S. aureus* in the lung following bacterial challenge (Figure 1E).
S. aureus induced production of the T\(_{H17}\) cytokine-associated chemokines G-CSF, and KC was inhibited by influenza infection (Supplementary Figure 2). There was no change in IFN-\(\gamma\) production between the groups (Figure 2A), whereas interleukin 10 (IL-10) was increased in co-infected mice compared to those that received S. aureus alone (Figure 2B). Acute TNF-\(\alpha\) production was also reduced in co-infected mice (Figure 2C). Interleukin 6 (IL-6) production was significantly elevated at 6 hours in the mice that received S. aureus only compared to the co-infected mice but remained similar in the coinfected mice at all time points, suggesting that preceding influenza infection does not result in suppression of all cytokines below S. aureus induced levels (Figure 2D). These data confirm that S. aureus induces a T\(_{H17}\) immune response in the lung during bacterial challenge. Further, preceding influenza A infection significantly attenuates the S. aureus-driven T\(_{H17}\) pathway as evidenced by decreased levels of T\(_{H17}\) effector cytokines, T\(_{H17}\) promoting cytokine, and T\(_{H17}\) cytokine-induced chemokines as early as 30 minutes and up to 120 hours following bacterial challenge. Neutrophils Are Not the Primary Mechanism by Which the T\(_{H17}\) Pathway Promotes Bacterial Host Defense

A known function of IL-17A is to enhance inflammation through induction of neutrophil and macrophage chemokines and growth factors [13]. We have published data that suggested the decreased clearance of S. aureus in T\(_{H17}\) pathway knockout mice was not due to a lack of neutrophil recruitment [8]. In order to further investigate the contribution of neutrophils to S. aureus clearance in the lungs, C57BL/6 mice received neutrophil depletion antibodies anti-Ly6G (1A8) or anti-Ly6G/C (RB6-8C5 (RB6)) followed by administration of S. aureus (ATCC 49775) 24 hours later. Bacterial clearance and lung inflammation were assessed 24 hours following bacterial challenge. Mice that received RB6 antibody had severely attenuated clearance of S. aureus, whereas mice that received 1A8 antibody cleared S. aureus similar to control (Figure 3A). Both RB6 antibody and 1A8 antibody resulted in neutrophil depletion in the BAL, whereas only RB6 antibody resulted in macrophage depletion (Figure 3B). Mice that received RB6 or 1A8 antibody prior to bacterial challenge had increased proinflammatory and neutrophil recruitment cytokine production (Figure 3C–F). Histological sections of lungs confirmed suppression of inflammation in the mice that received 1A8 or RB6 (Figure 3G). Interestingly, although the RB6 exacerbated S. aureus infection, there was typical bacterial clearance in the mice that received the 1A8 antibody (neutrophil specific), suggesting that neutrophils are not the mechanism by which the T\(_{H17}\) pathway promotes bacterial host defense.

**Figure 2.** Influenza A alters inflammatory cytokine production following bacterial challenge with *Staphylococcus aureus*. C57BL/6 mice were infected with 100 PFU of influenza A PR/8/34 or vehicle for 6 days, mice were then challenged with 10\(^6\) CFU of *S. aureus* for 6–120 hours. A–D, cytokine concentrations in lung homogenate measured by Lincoplex (\(n=6–7\)). \(* P<.05\) vs. *S. aureus* alone. Significance was tested by unpaired \(t\) test. Abbreviations: CFU, colony-forming units; PFU, plaque-forming units.
Alveolar Macrophage and Neutrophil Binding and Uptake of *S. aureus* Are Not Impaired by Preceding Influenza Infection

It is possible that preceding influenza infection directly attenuates innate immune cell killing of *S. aureus* in the lung (despite increased cell numbers), increasing susceptibility to secondary bacterial pneumonia. To test this, we infected mice with FITC-labeled *S. aureus* for 24 hours and performed flow cytometry. Examination of the *S. aureus* positive macrophages revealed no difference in the total number of FITC-positive cells during co-infection (Figure 4A and 4B). There was a decrease in the...
percentage of *S. aureus* positive neutrophils in influenza co-infected lungs, however, coinfected mice had elevated neutrophil numbers compared to *S. aureus* only infected animals. The total number of *S. aureus* positive neutrophils was not different between groups (data not shown). Further, we isolated alveolar macrophages from influenza infected or control lungs and performed flow cytometry. Macrophages from influenza-infected lungs were able to bind and take up significantly more *S. aureus* than naive macrophages as determined by percent positivity and mean fluorescence (Figure 4C and Supplementary Figure 3A). Finally, interferons have been suggested to inhibit macrophage uptake of bacteria during influenza co-infection [14]. We treated naive alveolar macrophages with IFN-β and/or IFN-γ prior to in vitro flow cytometry. Interferon treatment did not alter macrophage binding and uptake of *S. aureus* (Figure 4E and Supplementary Figure 3B). These data suggest that there is no innate cellular defect in influenza-infected lungs that results in aberrant *S. aureus* clearance.

**Figure 4.** Influenza A infection does not impair alveolar macrophage or neutrophil binding and uptake of *Staphylococcus aureus*. C57BL/6 mice were infected with 100 PFU of influenza A PR/8/34 or vehicle for 6 days, mice were then challenged with $1 \times 10^8$ CFU FITC-labeled *S. aureus* or vehicle for 24 hours. A and B, Total FITC-positive macrophages and neutrophils from *S. aureus* and co-infected lungs by flow cytometry (n = 6–8). C57BL/6 mice were infected with 100 PFU of influenza A PR/8/34 or vehicle for 6 days, macrophages were then collected and suspended with $1 \times 10^7$ CFU FITC-labeled *S. aureus* to allow for phagocytosis. C, Total FITC-positive macrophages from control and influenza-infected lungs as determined by percent positivity and mean fluorescence by flow cytometry (n = 8). Naive alveolar macrophages were stimulated with IFN-β (100 U/mL) and/or IFN-γ (5 ng/mL) prior to stimulation with FITC-labeled *S. aureus*. D, Total FITC-positive cells in lung by flow cytometry (n = 8–17). *P < .05 vs *S. aureus* alone, **P < .05 vs control. Significance was tested by unpaired t test (for 2 means) or one-way ANOVA (for multiple data groups) followed by Tukey post hoc test. Abbreviations: ANOVA, analysis of variance; CFU, colony-forming units; FITC, fluorescein isothiocyanate; IFN, interferon; PFU, plaque-forming units.
**S. aureus** Induces T\(_{H}17\) Pathway Associated Antimicrobial Peptides

Because innate immune cell uptake of **S. aureus** was similar in **S. aureus** and coinfection mice, we examined alternative pathways by which T\(_{H}17\) activation may promote bacterial clearance. To test if the T\(_{H}17\) pathway stimulates antimicrobial peptides (AMPs), C10 airway epithelial cells were stimulated with combinations of IL-17A, IL-22, with or without TNF-α. It has been shown previously that T\(_{H}17\) cytokines and TNF-α have synergistic effects on epithelial cells [15]. We found that TNF-α, IL-17A + TNF-α, and IL-17A + IL-22 + TNF-α treatment synergistically induced Lcn2 expression in C10 cells (Figure 5A). T\(_{H}17\) cytokines induced expression of RegIIIβ (Figure 5B) and CAMP in all groups stimulated with TNF-α (Figure 5C). To determine whether **S. aureus** induces T\(_{H}17\) pathway associated AMPs in vivo, C57BL/6 mice were challenged with influenza A followed by **S. aureus**. **S. aureus** enhanced production of IL-17 and IL-22 associated AMPs: Lcn2, RegIIIβ, CAMP, and S100A8-9 (Figure 5D–H), particularly at acute time points (6, 24 hours). Preceding influenza infection resulted in marked suppression of AMP production. To test whether IL-17 is required for **S. aureus** induction of AMPs in vivo, C57BL/6 mice were challenged with MRSA following the administration of anti-IL-17 antibody. At 24 hours following bacterial challenge, there was overall decreased expression of AMPs (Figure 5I). These data support that **S. aureus** induces T\(_{H}17\) pathway associated AMPs and illustrate a potential mechanism by which the T\(_{H}17\) pathway may promote bacterial immunity in the lung. Further, these findings demonstrate suppression of AMP production by influenza A, a novel mechanism for exacerbation of secondary bacterial pneumonia.

Exogenous Lcn2 Improves **S. aureus** Clearance in the Lung and Rescues Influenza Exacerbation During Coinfection

We proposed that Lcn2 might aid in lung clearance of **S. aureus**. To test this, C57BL/6 mice received Lcn2 or a control protein and 4 hours later were challenged with **S. aureus** (USA 300); 24 hours after bacterial challenge, bacterial burden and lung inflammation were assessed. **S. aureus** clearance was increased in mice that received exogenous Lcn2 compared to control (Figure 6A). There was also decreased lung inflammation (Figure 6B). Next, mice were coininfected with influenza A and MRSA in the presence of Lcn2 or control protein. There was increased bacterial clearance in the mice that received exogenous Lcn2 compared to control (Figure 6C–D). Thus, exogenous Lcn2 rescued **S. aureus** clearance in both mice infected with **S. aureus** alone and coinfected with influenza A. Finally, there were lower levels of ferrous (Fe\(^{2+}\)) ions in the **S. aureus** challenged and coinfected mice that received exogenous Lcn2 compared to control (Figure 6E). These data provide evidence that T\(_{H}17\) pathway induction of AMPs is a likely mechanism used to aid bacterial killing. In addition, these data suggest that Lcn2 depletes iron in the lung microenvironment, inhibiting the growth of **S. aureus**.

**DISCUSSION**

Our findings demonstrate a novel mechanism by which preceding influenza infection impairs immunity against bacterial pneumonia. The data substantiate a crucial role for the T\(_{H}17\) pathway in host defense against **S. aureus** pneumonia. Here we show that preceding influenza in mice causes persistent **S. aureus** infection and suppression of the T\(_{H}17\) pathway. Our study shows that influenza does not inhibit inflammation or **S. aureus** binding and uptake by phagocytic cells. Rather, influenza inhibits **S. aureus** induced T\(_{H}17\) pathway associated AMPs and attenuates bacterial clearance in the lung. Importantly, exogenous Lcn2 improved **S. aureus** clearance and rescued viral exacerbation of infection. In addition, exogenous Lcn2 decreased levels of free iron in BAL fluid. These data indicate a key mechanism by which T\(_{H}17\) pathway activation results in **S. aureus** clearance in the lung. Exogenous AMPs may be useful as a therapeutic target in coinfection.

Multiple mechanisms for increased susceptibility to bacterial infections following viral infection have been investigated [16]. Our current findings demonstrate that the impairment of **S. aureus** clearance and attenuation of the T\(_{H}17\) pathway by preceding influenza A is both an acute and chronic response of the immune system. The early inhibition of IL-17 production likely reflects γδT cell suppression as well as T\(_{H}17\). Mice challenged with **S. aureus** alone induced high levels of IL-17 in the lung that remained elevated following bacterial clearance. Influenza coinfection reduced this T\(_{H}17\) pathway activation throughout the study. Numerous influenza A viruses cause disease in mice and humans. We observed impaired **S. aureus** clearance during coinfection with 2 different strains of murine H1N1 influenza A infections. Different types of influenza viruses may elicit differing responses of the innate immune response and future studies will have to be completed in order to address these differences. Although we observed influenza A attenuation of the **S. aureus**-driven T\(_{H}17\) pathway, there was increased IL-10 in the coinfected mice compared to those that received **S. aureus** alone. Previous studies have shown that influenza infection induces IL-10 [17] and that IL-10 expression suppresses the development of T\(_{H}17\) cytokines during influenza infection [18]. Influenza-induced IL-10 has also been reported to enhance susceptibility to secondary pneumococcal infection [19], although a recent study showed that IFN-γ attenuation of **S. pneumoniae** clearance in mice was IL-10 independent [14]. In that study, IFN-γ levels were elevated in coinfected mice resulting in suppression of macrophage uptake of bacteria. We found no difference in IFN-γ levels in coinfected mice vs **S. aureus** alone, suggesting a minimal role for IFN-γ in the **S. aureus** model. In addition, we observed decreased TNF-α levels induced by

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Figure 5. TH17 cytokines induce antimicrobial peptides in vitro. Influenza A inhibits Staphylococcus aureus induced antimicrobial peptides in vivo. C10 airway epithelial cells were stimulated with combinations of IL-17A, IL-22, and TNF-α (n = 6). A–C, TH17 pathway associated antimicrobial peptide gene expression in cell RNA. *P < .05 vs control, ***P < .05 vs TNF-α. C57BL/6 mice were infected with 100 PFU of influenza A PR/8/34 or vehicle for 6 days, mice were then challenged with 10^6 CFU of S. aureus for 6–120 hours. D–H, TH17 pathway associated antimicrobial peptide gene expression in lung RNA (n = 6–7). C57BL/6 mice received 300 µg of anti-IL-17 antibody 24 hours prior to challenge with 5 × 10^7 CFU of S. aureus for 24 hours. I, TH17 pathway associated antimicrobial peptide gene expression in lung RNA (n = 8). *P < .05 vs control, **P < .05 vs S. aureus alone. Significance was tested by unpaired t test (for 2 means) or 1-way ANOVA (for multiple data groups) followed by Tukey post hoc test. Abbreviations: ANOVA, analysis of variance; CFU, colony-forming units; IL-17A, interleukin 17A; IL-22, interleukin 22; PFU, plaque-forming units; TNF-α, tumor necrosis factor α.
S. aureus in the lungs of coinfected mice. TNF-α production by natural killer (NK) cells has been suggested to be required for S. aureus killing in the influenza coinfection model [20]. It is possible that this suppression of TNF-α in coinfected mice plays a role in impaired host defense independent of the TH17 pathway, although TNF-α is known to synergize with IL-17 in promoting immunity.

IL-17 is known to induce neutrophilia in response to infection. We have previously observed decreased S. aureus clearance in TH17 pathway knockout mice was not solely due to a lack of neutrophil recruitment to the lung [8]. Other groups have suggested influenza-induced defects in innate immune cell uptake of bacteria as a mechanism for viral exacerbation of secondary infection [14]. We investigated the role of neutrophils through antibody depletion. RB6 antibody depletes neutrophils, macrophages, and dendritic cells, whereas 1A8 antibody specifically depletes neutrophils. We observed standard clearance of S. aureus in mice that received the 1A8 antibody, confirming that there are alternate mechanisms by which influenza A inhibits S. aureus immunity. When monocytes were depleted with RB6 antibody, we observed a large increase in bacterial burden, suggesting a role for antigen presenting cells in S. aureus host defense. Decreased phagocytosis of bacteria by neutrophils in the context of influenza and S. pneumoniae infection has been reported [21]. We performed in vitro and in vivo studies to determine if inflammatory cells in the lung were capable of phagocytosis of S. aureus in the presence and absence of preceding influenza infection and found that there was no change in the binding and
uptake of \textit{S. aureus} by macrophages or neutrophils. Further, interferon treatment did not alter macrophage binding and uptake of \textit{S. aureus} in vitro. This mechanism has been suggested in the context of influenza, streptococcal infection [14]. Of note, the assay we used to determine phagocytosis measures both extracellular bound and ingested bacteria. Our data suggest that no innate cellular defect in influenza-infected lungs is present during \textit{S. aureus} coinfection. Future studies will have to be performed in order to differentiate between extracellular bound and ingested \textit{S. aureus} by macrophages.

Because influenza coinfected mice have elevated innate immune cell recruitment compared to \textit{S. aureus} alone and there was no evidence of a defect in bacterial binding and uptake by these cells, we examined the ability of \textit{S. aureus} to induce T\textsubscript{H17} pathway associated AMPs. \textit{S. aureus} drove the expression of several AMPs; however, this was attenuated in influenza coinfected mice. IL-17 and IL-22 can induce production of several AMPs including serum amyloid A, Lcn2, \(\beta\)-defensins, S100 proteins, and RegIII[13, 22–26]. Specifically, Lcn2 can inhibit bacterial growth by sequestering enterobactin and depriving bacteria of iron essential for growth [27]. Although previous studies have shown that Lcn2 is important to host defense against gram-negative and gram-positive bacteria, no prior evidence has shown that Lcn2 aids in immunity against MRSA pneumonia [28–31]. Here we show that IL-17, IL-22, with or without TNF-\(\alpha\) can induce epithelial production of AMPs. Further, \textit{S. aureus} induction of these peptides requires IL-17. Our data suggest that preceding influenza A infection attenuates the T\textsubscript{H17} pathway and subsequent production of AMPs, allowing for a T\textsubscript{H17} dependent mechanism for \textit{S. aureus} clearance. In support of this finding, exogenous Lcn2 rescued \textit{S. aureus} clearance in mice infected with \textit{S. aureus} alone and coinfected with influenza A, confirming the role of AMPs as a mechanism for bacterial killing. Although a prior investigation by Flo et al has shown that Lcn2 deficiency has no effect on bacterial killing of \textit{S. aureus} [28], these experiments were performed in vitro rather than in vivo. They also found that Lcn2 deficiency had no effect on survival of mice infected with methicillin-sensitive \textit{S. aureus} intraperitoneally. This conflicting evidence may be a result of different strains of \textit{S. aureus} or different locations of infection. In our studies, Lcn2 rescued the bacterial clearance of \textit{S. aureus} independent of rescuing the T\textsubscript{H17} pathway (data not shown), suggesting that AMP production occurs downstream of IL-17. Exogenous Lcn2 decreased levels of ferrous ions in the BAL fluid of mice coinfected with \textit{S. aureus} and influenza A, suggesting that Lcn2 treatment results in iron sequestration and inhibits bacterial growth of gram-positive bacteria. These findings are significant given the morbidity and mortality associated with influenza and secondary bacterial pneumonia. These data expand our current knowledge regarding host defense, and future studies may allow for the identification of exogenous AMPs as therapeutic targets. The role of T\textsubscript{H17} immunity is well described in other sites of mucosal host defense, allowing for broader relevance beyond the lung and its epithelium. In addition, influenza A inhibition of T\textsubscript{H17} immunity may be important to a spectrum of pathogens in addition to \textit{S. aureus}. The identification of T\textsubscript{H17} effector mechanisms involved in bacterial clearance provides a novel therapeutic target in viral, bacterial coinfection.

**Supplementary Data**

**Supplementary materials** are available at \textit{The Journal of Infectious Diseases} online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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