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STAT1 Is Required for Suppression of Type 17 Immunity during Influenza and Bacterial Superinfection

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ABSTRACT
Influenza is an annual, global healthcare concern. Secondary bacterial pneumonia is a severe complication associated with primary influenza virus infection that often results in critical morbidity and mortality. We have identified influenza-induced suppression of antibacterial type 17 immunity as a mechanism for enhanced susceptibility to bacterial superinfection. We have shown that influenza-induced type I IFN impairs type 17 activation. STAT1 is a transcription factor involved in IFN signaling that is shared by types I, II, and III IFN. In this study, we investigated the role of STAT1 signaling during influenza and methicillin-resistant Staphylococcus aureus superinfection. STAT1−/− mice had increased morbidity and airway inflammation compared with control mice during influenza monoinfection. Despite this worsened antiviral response, STAT1−/− mice were protected from superinfection bacterial burden and mortality compared with controls. Type 17 immune activation was increased in lymphocytes in STAT1−/− mice during superinfection. The elevation in type 17 immunity was not related to increased IL-23 production, because type I IFN could inhibit IL-23 expression in a STAT1-independent manner. STAT1−/− APCs were inherently biased toward type 17 polarization compared with control cells. Further, STAT1−/− dendritic cells produced attenuated IL-6 and TNF-α upon heat-killed S. aureus stimulation compared with control. Overall, these data indicate that STAT1 signaling plays a detrimental role in influenza and methicillin-resistant Staphylococcus aureus superinfection by controlling the magnitude of type 17 immune activation. ImmunoHorizons, 2017, 1: 81–91.

INTRODUCTION
Influenza infection remains a leading cause of respiratory illness worldwide. During severe seasons, influenza mortality in the United States estimates range up to 50,000 deaths (1). Secondary bacterial pneumonia complicating primary influenza virus infection is an important cause of morbidity and mortality. It is estimated that nearly all influenza-associated deaths during the 1918 Spanish influenza pandemic were due to secondary bacterial superinfection (2); similarly, it had a major role in morbidity and mortality during the most recent global pandemic that occurred in 2008–2009 (3). In recent years, the role of bacterial superinfection with community-acquired methicillin-resistant Staphylococcus aureus (MRSA) during influenza infection has been appreciated as a significant source of mortality (4–6). A more complete understanding of the immune mechanisms that enhance susceptibility to secondary bacterial pneumonia during influenza infection may yield improved strategies to prevent or treat human disease.
We have previously demonstrated that influenza infection suppresses type 17 immunity via type I IFN, leading to exacerbation of bacterial pneumonia following challenge with Gram-positive and Gram-negative bacteria in mice (7–10). The cytokines classically associated with type 17 immunity are IL-17 and IL-22, which are secreted by T cell subsets and orchestrate inflammation, bacterial clearance, and subsequent airway repair (11, 12). Inhibition of type 17 immunity during influenza and bacterial superinfection occurred via suppression of IL-23, a critical inducer of IL-17 and IL-22 secretion from T cells in the lung (7).

Type I IFN induces transcriptional activity through the STAT pathway. Upon binding of type I IFN-α or -β to the type I IFN receptor (IFNAR), tyrosine kinase–dependent phosphorylation of STAT1 and STAT2 occurs, forming an activated STAT1, STAT2 heterodimer (13). This heterodimer complexes with a third factor, IRF-9, to form the canonical type I IFN–stimulated multimeric transcription factor, IFN-stimulated gene (ISG) factor 3 (ISGF3); this translocates to the nucleus to modulate gene transcription, promoting an antiviral immune state. Of note, type II IFN-γ signals predominantly through formation of activated STAT1 homodimers, whereas type III IFN-α signals via ISGF3 but uses a distinct surface receptor, IFNLR (14). Modulation of STAT1 activity might impact subsequent susceptibility to bacterial superinfection in the lung as a result of its effects on IFN response. To further characterize the contribution of STAT1 toward exacerbation of bacterial pneumonia during influenza infection, we subjected wild-type (WT) and STAT1−/− mice to MRSA pulmonary challenge during primary influenza infection.

**MATERIALS AND METHODS**

**Mice**

Eight-week-old WT C57BL/6 mice were purchased from Taconic Farms. STAT1−/− mice on the C57BL/6 background were obtained from Dr. C. Schindler (Columbia University) and Dr. D. Levy (New York University). Mice were maintained under pathogen-free conditions and were cohoused in the same facility prior to the performed studies. All studies were performed using sex-matched male mice, except where noted. All of the animal studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

**Influenza and bacterial superinfection**

Influenza A/PR/8/34 H1N1 was propagated in chicken eggs, as previously reported (15). Mice were infected with 50 PFU influenza virus (50 µl of sterile PBS) or vehicle by oropharyngeal aspiration (OP). After 6 d of influenza infection, mice were challenged with 5 × 10⁷ CFU MRSA, USA300 (in 50 µl of sterile PBS) or vehicle by OP. MRSA, USA300 was obtained from Dr. A. Prince (Columbia University) and was cultured overnight in modified CCY medium to stationary growth phase. Twenty-four hours after bacterial infection, mouse tissues were harvested for analyses. Mortality studies were performed using a dose of 2.5 × 10⁹ CFU MRSA via OP.

**Analysis of lung infection**

At the indicated time points, mice were subjected to bronchoalveolar lavage with 1 ml of sterile PBS for inflammatory cell counts. Bronchoalveolar lavage fluid (BALF) cells were mounted on slides using a Cytospin (Thermo Fisher) and were stained with PROTOCOL Hema 3 (Fisher Scientific). The cranial lobe of the right lung was homogenized in sterile PBS and used for plating to determine bacterial burden and for cytokine Bio-Plex (Bio-Rad) or LINCOplex (Millipore) analyses. The middle and caudal lobes of the right lung were snap-frozen in liquid nitrogen, followed by homogenization and isolation of whole-lung RNA using an Absolutely RNA Miniprep Kit (Agilent Technologies). RNA analysis was performed using commercially available Assays-on-Demand TaqMan probes and primers (Applied Biosystems).

**Flow cytometry**

At tissue harvest, the left lung was used for flow cytometry analyses. The left lung was digested with collagenase, as previously described (16). Resulting single-cell preparations were stimulated in vitro with PMA (50 ng/ml) and ionomycin (750 ng/ml) for 4 h at 37°C. Cells were stained with Abs against CD4 and γδTCR, fixed and permeabilized, and stained with fluorescent-conjugated Abs against IL-17 and IL-12 (BD Biosciences). Analysis was performed using a FACSARia (BD Biosciences) apparatus.

**Bone marrow dendritic cell culture**

Bone marrow was isolated from the femurs of C57BL/6 and STAT1−/− mice, as previously reported (17). Following 7 d in culture with DMEM containing GM-CSF, the nonadherent cells (bone marrow dendritic cells) were collected and used for assays. Dendritic cells were stimulated overnight with peptidoglycan (PGN) or lipoteichoic acid (LTA) from S. aureus (Sigma-Aldrich) in the presence or absence of IFN-β (10 U/ml) or IFN-γ (10 ng/ml; both from PBL Technology).

**In vitro T cell polarization**

Naïve mouse T cells were isolated from the spleens of OTII mice using CD4+ CD62L+ selection beads (Miltenyi Biotec) (18). Bone marrow dendritic cells from C57BL/6 or STAT1−/− mice were pulsed with heat-killed MRSA or vehicle for 24 h. Following this, naïve OTII T cells were treated with IL-2 (40 U/ml) and OTII OVA peptide (OVA323–339, 5 µM) and were incubated with the bone marrow dendritic cells for 5 d. Cytokine production was assessed using a Bio-Plex assay.

**Statistical analyses**

The data presented in this article represent individual animals (or replicates) with an overlay of the mean ± SEM. Significance was tested by one-way or two-way ANOVA, as appropriate, followed by the Tukey post hoc test. For comparison of two groups, an unpaired t test was used. Mouse survival data were analyzed by the Mann–Whitney U test. All statistical tests were performed using GraphPad Prism software. All studies were repeated a minimum of twice in individual animal cohorts.

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RESULTS

Influenza-induced pulmonary inflammation is enhanced in STAT1−/− mice
It has been previously reported that STAT1−/− mice are deficient in IFN signaling and have altered susceptibility to influenza infection (19). To study secondary bacterial infection in this mouse model, it was necessary to evaluate the impact of STAT1 on influenza monoinfection. To confirm the published phenotype in our mice, WT or STAT1−/− mice were infected with influenza A/PR/8/34 for 7 d. We chose a dose of influenza virus that would induce moderate disease in WT animals due to the expectation that STAT1−/− mice would have a worse disease phenotype. As expected, STAT1−/− mice lost significantly more weight than control animals at day 6 postinfection (Fig. 1A). This increased morbidity did not correlate with a consistent increase in viral titers on day 7, similar to previous findings (Fig. 1B) (19). Next, airway inflammation was assessed by BALF cell analysis. STAT1−/− mice had elevated BALF cell counts and, specifically, neutrophils were increased compared with WT mice, similar to previous reports (Fig. 1C, 1D) (20). Consistent with these findings, the concentration of the proneutrophilic chemokines IL-17 (44.2 ± 22.1 versus 7.7 ± 2.2 pg/ml, p = 0.11) and G-CSF (3527 ± 1534 versus 119.9 pg/ml, p = 0.04) were increased in whole-lung homogenate from STAT1−/− mice compared with WT mice.

Because of the elevated inflammation, we then confirmed that STAT1−/− mice are deficient in known STAT1 signaling. CXCL9 and CXCL10 are well-known type II IFN–induced genes, which are known to signal exclusively through STAT1. STAT1−/− mice lacked production of CXCL9 and CXCL10, confirming our STAT1−/− phenotype during influenza infection (Fig. 2A, 2B).

![Figure 1](https://doi.org/10.4049/immunohorizons.1700030)
Further, we assessed expression of known ISGs. Consistent with protein data, Cxcl10 expression was inhibited in STAT1^{−/−} mice compared with controls (~41-fold) (Fig. 2C). Induction of the type I IFN target gene Mx1 was significantly attenuated in STAT1^{−/−} mice but to a lesser extent (~5-fold) than Cxcl10 (Fig. 2D). Expression of the types I and II ISG Ccl12 was not altered by STAT1 deletion (Fig. 2E). These data indicate that STAT1^{−/−} mice have increased morbidity and airway inflammation compared with WT mice that are unrelated to viral burden, consistent with previous reports, and that ISG responses are differentially impacted.

**FIGURE 2.** STAT1 deletion impairs the influenza-induced ISG response. WT or STAT1^{−/−} mice were infected with influenza A/PR/8/34 for 7 d. The ISG response was measured by Lincoplex on lung homogenate (A and B) (n = 8) or RT-PCR on lung RNA (C−E) (n = 3−15). Naive mice are untreated WT mice. F, influenza infected. *p < 0.05.
STAT1⁻/⁻ mice are protected from influenza and MRSA superinfection

Our previously published data suggested that suppression of type 17 immune activation by IFN is a mechanism by which influenza increases susceptibility to bacterial pneumonia (7). Specifically, we have shown that type I IFN, which signals through STAT1 and STAT2, impairs IL-23 production by dendritic cells, resulting in abrogated type 17 host defense. To examine the role of STAT1 in this pathway, WT or STAT1⁻/⁻ mice were infected with influenza A/PR/8/34 or vehicle for 6 d, followed by MRSA infection for 24 h. Preceding influenza infection resulted in significantly increased MRSA burden in the lung in WT mice, but STAT1⁻/⁻ mice were protected from exacerbation of secondary MRSA pneumonia by influenza infection (Fig. 3A). To then determine whether the protection from bacterial burden was relevant, WT and STAT1⁻/⁻ mice were challenged with a combination of influenza A/PR/8/34 and MRSA known to be lethal in WT mice. As expected, WT mice succumbed to superinfection by 24 h postchallenge; however, STAT1⁻/⁻ mice survived longer, with only 50% mortality at 24 h postinfection (Fig. 3B). This protection was observed in the context of STAT1⁻/⁻ mice having worse morbidity due to influenza infection alone. Similar to influenza monoinfection, STAT1⁻/⁻ superinfected mice had increased BALF cells, predominantly neutrophils, in their lungs compared with WT mice (Fig. 3C, 3D). These data demonstrate that STAT1 is detrimental during influenza and MRSA superinfection, and deletion results in protection from bacterial burden and mortality.

STAT1⁻/⁻ mice have accentuated type 17 immune activation compared with WT mice

Type 17 cytokines are known to induce neutrophilia and promote host defense against S. aureus (7, 9–11). The observation that STAT1⁻/⁻ mice have elevated neutrophilia and improved MRSA clearance during superinfection suggests increased type 17 immune activation. To test this hypothesis, we measured IL-17 and IL-22 gene expression in whole lung from influenza, MRSA, and superinfected mice. Indeed, STAT1⁻/⁻ mice had significantly increased Il17 expression compared with WT mice during influenza monoinfection, MRSA monoinfection, and superinfection (Fig. 4A). Further, STAT1⁻/⁻ mice had increased Il22

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**FIGURE 3. STAT1⁻/⁻ mice are protected from influenza and MRSA superinfection.**

WT or STAT1⁻/⁻ mice were infected with influenza A/PR/8/34 or vehicle for 6 d prior to infection with MRSA (USA300) or vehicle for 24 h. (A) Bacterial burden in the lungs of infected mice (n = 12). (B) Mortality plot for superinfected mice (n = 12 or 13). (C) BALF inflammation (n = 12). (D) Differential BALF cell counts (n = 7 or 8). FS, influenza MRSA superinfected; PMN, polymorphonuclear cells; SA, MRSA infected. *p < 0.05.

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expression during influenza monoinfection and superinfection (Fig. 4B). To better define the impact of STAT1 deletion on type 17 immune activation, we performed flow cytometry on the lungs from mono- and superinfected animals. We have previously shown
that *S. aureus* induces IL-17 production from CD4+ and γδTCR+ cells, which is attenuated by preceding influenza infection (7). Flow cytometry revealed that STAT1−/− mice had increased numbers of CD4+ IL-17+ cells compared with WT mice (Fig. 4C). Further, STAT1−/− mice had increased numbers of γδTCR+ IL-17+ cells during MRSA monoinfection (Fig. 4D). Similarly, STAT1−/− mice displayed increased CD4+ IL-22+ cells (Fig. 4E) and increased γδTCR+ IL-22+ cells (Fig. 4F) compared with WT mice during superinfection. These data confirm a bias toward type 17 immune activation in STAT1−/− mice compared with WT mice and suggest a primary effect on CD4+ T cells.

**Increased type 17 immune activation in STAT1−/− mice is not IL-23 dependent**

Our previous data demonstrated that preceding influenza inhibits *S. aureus*-induced IL-23 production, thus limiting type 17 immunity (7). We hypothesized that STAT1 deletion would result in increased IL-23 production compared with WT mice. Surprisingly, STAT1−/− mice did not display increased expression of IL-23 compared with WT mice in the lung (Fig. 5A). To further investigate the relationship between STAT1 signaling and IL-23 production, bone marrow dendritic cells were isolated from WT and STAT1−/− mice and stimulated with types I and II IFN. As we have previously shown, type I IFN suppressed PGN-induced IL-23 production (Fig. 5B). Type II IFN did not have a similar effect on this pathway. In the absence of STAT1 signaling, type I IFN was still able to inhibit IL-23 production similar to its effect in WT cells. In additional studies using *S. aureus*-derived LTA (which, similar to PGN, is a TLR2 ligand), we confirmed that the effect of type I IFN on IL-23 production by dendritic cells does not require STAT1 (Fig. 5C). Interestingly, type II IFN was able to inhibit IL-23 production in this context in a STAT1-dependent manner. These data indicate that enhanced type 17 immunity in STAT1−/− mice is not due to increased IL-23 production.

**STAT1−/− APCs are biased toward type 17 immune polarization**

Next, we examined whether type 17 immune polarization is inherently enhanced in STAT1−/− mice compared with controls. To do this, we isolated bone marrow dendritic cells from WT and STAT1−/− mice. These cells were pulsed with heat-killed MRSA or vehicle for 24 h and incubated with naive T cells (OVA-specific) from OTII mice in the presence of OVA peptide. Heat-killed MRSA treatment inhibited Ag-specific T cell production of IL-17

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**FIGURE 5. IL-23 production is not impacted by STAT1 deletion.**

WT or STAT1−/− mice were infected with influenza A/PR/8/34 or vehicle for 6 d prior to infection with MRSA (USA300) or vehicle for 24 h. (A) IL-23 gene expression by RT-PCR on lung RNA (*n* = 11 or 12). WT or STAT1−/− bone marrow dendritic cells were stimulated with the Tlr2 ligands PGN or LTA for 24 h in the presence or absence of IFNs. (B and C) IL-23 production by ELISA (*n* = 4–5). F, influenza infected; FS, influenza MRSA superinfected; SA, MRSA infected. *p* < 0.05 versus WT in the absence of IFN.
but increased IL-6 and TNF-α levels compared with control cultures in WT and STAT1−/− mice (Fig. 6). T cells polarized with STAT1−/− dendritic cells produced increased IL-17 compared with T cells polarized with WT dendritic cells in control and MRSA-stimulated settings (Fig. 6A). Interestingly heat-killed MRSA stimulated lower levels of IL-6 and TNF-α in STAT1−/− cultures (Fig. 6B, 6C). Collectively, these data indicate that STAT1−/− dendritic cells favor type 17 polarization of WT naive T cells and have impaired MRSA-mediated induction of proinflammatory cytokines.

DISCUSSION

In this study, we have demonstrated that STAT1 activity is associated with exacerbation of secondary MRSA pneumonia during influenza infection. STAT1−/− mice had decreased bacterial burden and improved survival compared with WT mice during influenza and MRSA superinfection, despite increased morbidity and inflammation due to influenza infection alone. Further, we have shown that this protection from superinfection was associated with increased type 17 immune activation in STAT1−/− mice compared with WT mice. These findings are consistent with our previous studies and further support a critical role for suppression of type 17 immunity in the pathogenesis of influenza and S. aureus superinfection (7–10). In previous studies, impaired secretion of IL-23, which helps to activate and maintain type 17 immunity, was identified as a key mechanism. Interestingly, we found in this study that type I IFN–mediated IL-23 suppression was not dependent on STAT1. Our previous finding that IL-23 production in the lung was dependent on the presence of IFNAR during influenza and
*S. aureus* superinfection suggests that type I IFN regulates IL-23 through alternative signaling mechanism(s) (7). Together, these findings suggest the presence of IFNAR-dependent, STAT1-independent mechanisms for IL-23 suppression. Our finding that bacterial clearance was rescued, despite IL-23 suppression, in the setting of STAT1 deficiency indicates the existence of additional pathways leading to the activation of type 17 immunity independent of IL-23.

STAT1−/− mice demonstrated increased morbidity during primary influenza infection, consistent with previous reports (19–22). In prior studies, the degree of susceptibility varied with the strain of influenza used and the genetic background of the mice. In one study, infection with influenza A/PR/8/34 in C57BL/6 mice, as were used in this study, resulted in increased morbidity and marked granulocytic pulmonary infiltration, which were unrelated to viral burden (20). We report similar findings in this article. That study also demonstrated reduced LD50 to influenza compared with WT mice and demonstrated a bias toward type 2–polarized T cells in the absence of IFN signaling, but it did not study the effects of influenza on type 17 immune responses. The morbidity that we observed during influenza monoinfection was associated with increased inflammatory cells, particularly neutrophils, in BALF, increased lung expression of IL-17 and IL-22, and increased proportions of IL-17+ and IL-22+ T cells in the lung. These findings all suggest increased neutrophilia and inflammation due to enhanced type 17 immune activation, which has previously been shown to be associated with increased lung pathology during primary influenza infection (16). In humans, the role of IL-17 and influenza severity remains unclear. Much of our knowledge on the subject was generated during the most recent H1N1 influenza pandemic, and the results may not be generalizable to the pathogenesis of seasonal influenza infections. In general, those studies have shown that severe pandemic H1N1 influenza was associated with increased peripheral IL-17 but that decreased IL-17 was a risk factor for mortality and more severe disease (23–27). Given its recognized role in the pathogenesis of a spectrum of autoimmune and inflammatory conditions, it is plausible that increased type 17 activity would also be associated with increased inflammatory lung disease during human influenza infections. Although it is tempting to speculate, it is unclear from these studies whether inappropriately decreased IL-17 was a marker of inadequate host immune response or was associated with secondary bacterial infection, either of which could have increased mortality risk.

Although STAT1 deficiency had a detrimental effect on the host response to influenza monoinfection, it had a protective effect when STAT1−/− mice were subsequently challenged with MRSA on day 6 of influenza infection. STAT1−/− mice had significantly decreased lung bacterial burden 24 h following bacterial challenge; the burden of bacteria in the lung was equivalent to that seen following MRSA monoinfection, suggesting complete rescue from the impairment in bacterial clearance from the lung observed during influenza and MRSA superinfection in WT mice. This improvement in bacterial clearance was also associated with a survival benefit, because STAT1−/− mice demonstrated prolonged survival following a combination of influenza and MRSA that was rapidly lethal to WT mice. During superinfection, the total number of inflammatory cells, specifically neutrophils, in the lung was again elevated compared with WT mice. Furthermore, similar to influenza monoinfection, lung expression of IL-17 and IL-22 and the proportion of IL-17+ and IL-22+ T cells in the lung were increased compared with WT mice. This suggests that the improved outcomes during influenza and MRSA superinfection could be due to rescue of type 17 immunity.

The specific effector(s) responsible for these findings remain unclear. Type 17 immunity is generally thought to exert antibacterial effects by the proliferation and recruitment of inflammatory cells, particularly neutrophils, and by the induction of antimicrobial peptides at the mucosal surface. Thus, enhanced inflammatory cell infiltrate present in the lung at the time of bacterial challenge is one possibility: STAT1−/− mice had significant neutrophilia induced by influenza alone, whereas WT mice did not, indicating that a large additional population of phagocytic cells was present in the lung at the time of bacterial challenge. Previous studies demonstrated that neutrophil depletion did not affect *S. aureus* lung bacterial burden (9, 28) and that antimicrobial peptides may have a more significant role (9). Although neutrophils might be dispensable for ultimate *S. aureus* clearance if alternate effector mechanisms remain intact, increased neutrophilia at the time of bacterial challenge could have a significant impact on early bacterial clearance, affecting the overall kinetics of MRSA clearance from the lung. When WT and STAT1−/− mice (n = 8) were subjected to superinfection for 120 h, STAT1−/− mice had complete clearance of MRSA by 120 h, whereas WT mice continued to have elevated bacterial burden (p = 0.04, data not shown). This kinetic response is identical to what was previously described for the clearance of *S. aureus* from the lung during bacterial monoinfection (10). Bacterial clearance was also associated with normalization of neutrophil counts in BALF from STAT1−/− mice, whereas they continued to remain elevated in WT mice, suggesting a primary role for neutrophils in this response. To further test the hypothesis that neutrophils were the primary effector responsible for improved bacterial clearance in STAT1−/− mice, we attempted neutrophil depletion in STAT1−/− mice using 1A8-neutralizing Abs, but we were unable to generate adequate neutrophil depletion. Depletion using RB6-neutralizing Abs, which affect monocyte populations in addition to neutrophils, proved lethal in this model (data not shown).

When we assessed antimicrobial peptide expression in the lung, we found that expression of regenerating islet-derived (REG)3B was significantly increased in the lung in STAT1−/− mice compared with WT mice (n = 8) during influenza monoinfection (p = 0.003), MRSA monoinfection (p = 0.08), and superinfection (p = 0.05) (data not shown). REG3B belongs to the C-type lectin family of antimicrobial peptides, and it mediates antimicrobial activity via binding of bacterial PGN (29). We previously demonstrated that type 17 cytokines induce upregulation of REG3B in mouse respiratory epithelial cells, and influenza and *S. aureus* superinfection decreased expression of REG3B in the lung of WT mice (10). We did not see a similar effect on expression of
lipocalin 2, which we had previously demonstrated to be an important mediator of bacterial clearance in influenza and S. aureus superinfection (10). This finding provides further evidence of the rescue of type 17 immunity in the lung during influenza and MRSA superinfection in the absence of STAT1 activity and suggests a role for antimicrobial peptides in bacterial clearance.

Surprisingly, we found that type I IFN inhibited S. aureus–induced IL-23 production from dendritic cells independently of STAT1. This was unexpected considering our previous finding that IL-23 production by CD11c+ monocytes in the lung was dependent on IFNAR (7). These data suggest an IFNAR-dependent, STAT1-independent mechanism of IL-23 suppression. Other investigators have reported different responses to influenza infection in IFNAR−/− versus STAT1−/− mice, with IFNAR−/− mice showing intact ISG expression, presumably due to signaling redundancy via type III IFN, whereas STAT1−/− mice had no such induction (21). The finding that IL-23 suppression was rescued in IFNAR−/− mice, but not in STAT1−/− mice, is curious; any signaling redundancy mediated by type III IFN would also be abrogated in STAT1−/− deficiency, because signaling occurs for both IFNs via the same transcription factor, ISGF3, containing STAT1. Complicating matters further is the observation that the specific bacterial ligand used (PGN or LTA) had a differential effect on IL-23 suppression in the presence of type II IFN, with WT dendritic cells cultured with type II IFN showing impairment of IL-23 production in the presence of LTA but not PGN. Finally, we found no effect on IL-23 secretion in the presence of type III IFN (data not shown). It is unclear whether the presence of S. aureus–derived PGN in vivo would be sufficient to induce IL-23, despite the concurrent inhibitory effect of LTA in the presence of type II IFN. Other investigators have shown that type II IFN has an important role in influenza and Streptococcus pneumoniae superinfection (30). However, in the setting of the influenza and S. aureus model, we have demonstrated that type II IFN deficiency did not protect mice from impaired bacterial lung clearance (7), suggesting that the primary effect in vivo is mediated by type I IFN.

Despite suppression of dendritic cell–derived IL-23 at levels similar to those seen in WT mice, type 17 immunity was rescued in STAT1−/− mice. Thus, the rescue of type 17 immunity that we observed appeared to be independent of IL-23 activation. The precise pathways for the development, polarization, activation, and maintenance of type 17 immune cells remain incompletely understood. It is known that mediators other than IL-23, including IL-1β, TGF-β, IL-6, and IL-21, also modulate type 17 immune cells (31, 32). Thus, in the setting of STAT1 deficiency, alternate IL-23–independent pathways may play important roles in regulating type 17 immune cell activity. The reason for these observations is unclear and merit further investigation. Naïve APCs from STAT1−/− mice induced higher levels of IL-17 production by WT naïve T cells compared with WT APCs, with or without priming by heat-killed MRSA. T cells from WT and STAT1−/− mice secreted IL-6 and TNF-α following polarization with MRSA-primed dendritic cells, but cytokine levels were significantly lower in STAT1−/− mice. IL-6 and TNF-α are classically associated with type 1 responses. Thus, these results suggest that APCs from STAT1−/− are inherently biased toward type 17 T cell polarization. The inherent bias toward type 17 T cell polarization in the absence of STAT1−/− may contribute to the rescue of type 17 immunity during influenza and MRSA superinfection and obviate the need for IL-23 production.

In summary, we have demonstrated that STAT1 signaling has a protective effect during influenza monoinfection but a detrimental effect during influenza and MRSA superinfection. Diminished antiviral activity due to loss of IFN response during influenza was associated with increased morbidity; however, during pulmonary bacterial challenge this deficiency led to improved bacterial clearance and prolonged survival associated with rescue of type 17 cells, gene transcription, and effectors in the lung. Our data indicate that STAT1 signaling impairs type 17 T cell polarization through effects on APCs. Further studies are required to more fully characterize the mechanisms involved. Ultimately, greater understanding of how IFN responses and type 17 immunity can be modulated may lead to interventions and therapies to reduce morbidity and mortality associated with influenza and influenza and bacterial superinfections.

DISCLOSURES

The authors have no financial conflicts of interest.

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