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The immunology of influenza virus-associated bacterial pneumonia

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Abstract

Infection with influenza virus has been a significant cause of morbidity and mortality for more than a hundred years. Severe disease and increased mortality often results from bacterial super-infection of patients with influenza virus infection. Preceding influenza infection alters the host's innate and adaptive immune responses, allowing increased susceptibility to secondary bacterial pneumonia. Recent advances in the field have helped to define how influenza alters the immune response to bacteria through the dysregulation of phagocytes, antimicrobial peptides, and lymphocytes. Viral-induced interferons play a key role in altering the phenotype of the immune response. Potential genetic modifiers of disease will help to define additional immunologic mechanisms that predispose to viral, bacterial super-infection with the overarching goal of developing effective therapeutic strategies to prevent and treat disease.

Introduction

Infection with influenza virus is a significant cause of morbidity and mortality throughout the world. Severe disease and increased mortality can often result from bacterial super-infection primarily with the Gram-positive organisms, *Staphylococcus aureus* or *Streptococcus pneumoniae*. This review discusses the recent advances in our understanding of the immunological mechanisms by which influenza A virus infection increases the susceptibility to secondary bacterial pneumonia and how this might inform future strategies to prevent or treat this lethal combination.

Seasonal influenza infection occurs annually, and baseline immunity to seasonal influenza infections exists within communities due to prior exposure. Influenza pandemics occur when

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a new, highly pathogenic virus strain emerges for which there is no immunity within the human population. Pandemic viruses spread easily from person to person across a wide geographic area, affecting a large proportion of the population. During the 1918 pandemic of influenza A virus H1N1, more than 50 million people died from influenza and bacterial super-infection [1]. During the 2009 pandemic of influenza A virus H1N1, 25–50% of hospitalized virus-infected patients were super-infected with bacterial pneumonia [2–7], and super-infection was associated with higher morbidity and mortality [2,6,8–10]. Vulnerability to secondary bacterial infection peaks at approximately one week post-influenza infection (Figure 1). Influenza virus infection facilitates secondary bacterial infection through multiple immunological mechanisms. Preceding influenza virus infection leads to the dysregulation of both innate and adaptive immune responses, predisposing the host to secondary bacterial infection. Understanding the immune mechanisms that predispose patients with influenza virus infection to bacterial super-infection is paramount to preventing deaths in future influenza virus pandemics.

Influenza-induced defects in innate immunity against bacteria

Alveolar macrophages reside in the normal airway and are the first cells to defend against bacteria. Additionally, macrophages and neutrophils are recruited to the airways by cytokines and chemokines in order to ingest and kill bacteria. Preceding influenza infection causes dysregulation of both macrophages and neutrophils, limiting the ability to defend against subsequent bacterial infection (Figure 2).

Alterations in phagocyte quantity

Recent work has investigated how preceding influenza virus infection affects the number of macrophages and neutrophils available in the airway to fight bacterial infection. It has been shown that influenza virus infection resulted in the loss of 90% of mouse resident alveolar macrophages by one week post-infection, with the remaining 10% of macrophages displaying a necrotic phenotype [11]. Cell death was thought to be related to a secondary necrotic process due to an increased number of damaged macrophages (measured by distorted nuclei and an increase in the number of cytoplasmic vacuoles) in the airspace of influenza virus-infected mice compared with mock-infected mice. Recruited inflammatory monocytes replaced the resident cells during bacterial super-infection. This alteration of innate cells in the influenza virus-infected lung resulted in an early defect in *S. pneumoniae* uptake at 3 hours post-bacterial challenge. The alveolar macrophage population was fully replaced by two weeks post viral infection and early innate host defense to *S. pneumonia* was restored.

Earlier work has demonstrated that influenza virus super-infection with *S. aureus* or *S. pneumoniae* resulted in enhanced neutrophilic inflammation in the lungs at time points mimicking human susceptibility to co-infection. More recent studies have confirmed these data. Mice infected with *S. aureus* six days after administration of influenza virus had higher numbers of neutrophils in bronchoalveolar lavage fluid 24 hours later compared with mice that received bacteria alone [12]. Mice infected with *S. pneumoniae* seven days after receiving influenza virus also had higher numbers of neutrophils in the bronchoalveolar lavage and lung tissue and higher bacterial load in the lung compared to mice that received

bacteria alone [13]. In these studies, higher numbers of neutrophils recruited to the airways correlated with increased mortality. However, neutrophil depletion (using an anti-Ly6G-specific antibody) showed that neutrophils did not have a significant effect on *S. pneumoniae* burden or morbidity, assessed by body weight loss, during super-infection, suggesting a neutrophil-independent mechanism for pathogenesis [13].

A potential mechanism by which Panton-Valentine leukocidin (PVL)-producing USA 300 clonotype *S. aureus* may be taking advantage of neutrophil recruitment during co-infection is through the lysis of neutrophils resulting in the release of proteases. PVL is a pore-forming exotoxin produced by certain strains of *S. aureus*. The PVL-producing USA 300 clonotype of *S. aureus* (also known as methicillin-resistant *S. aureus*; MRSA) has emerged globally in recent years as an important human pathogen [14]. Human neutrophils exposed to influenza virus for three hours and subsequently incubated with media from cultured MRSA showed an increased rate of neutrophil cell death compared to neutrophils exposed to MRSA alone. In addition, there was an upregulation of the cell surface marker CD11b, indicating neutrophil activation, in the neutrophils exposed to both influenza virus and MRSA compared to either pathogen alone [15]. Influenza and bacterial super-infection with MRSA could potentially increase neutrophil recruitment to the lung and augment PVL-related damage to neutrophils.

Other studies using a pre-clinical murine model of bacterial superinfection have shown suppression of neutrophil recruitment in response to bacterial infection following the resolution of influenza infection. Mice challenged with *S. pneumoniae* at both two and six weeks post-infection with murine adapted influenza virus (PR8) had substantially reduced numbers of neutrophils in bronchoalveolar lavage fluid 24 hours after bacterial infection compared with mice that received bacteria alone. At these time points, influenza virus was undetectable in the lungs, and pre-bacterial challenge lung cellularity and cytokine levels had returned to the pre-influenza infection levels. The decreased recruitment of neutrophils was linked to a sustained desensitization of macrophages to Toll-like receptor (TLR) ligands [16], suggesting that influenza virus induced a prolonged refractory state of the innate immune response. Consistent with this, nuclear translocation of the p65 subunit of nuclear factor-κB (NF-κB) was inhibited in macrophages, but not in airway epithelial cells, in response to a TLR5 agonist (flagellin). There was further evidence that TLR2 and TLR4, in addition to TLR5, were similarly affected by influenza virus infection. In summary, the level of neutrophil recruitment after bacterial super-infection was dependent on the timing of bacterial challenge relative to influenza infection. At days 3 – 7 days post-influenza infection, enhanced neutrophil recruitment was seen with secondary bacterial challenge. At weeks 2 – 6 post-influenza infection, bacterial super-infection resulted in lower numbers of neutrophils recruited to the co-infected airways, which was associated with sustained desensitization of alveolar macrophages to bacterial toll-like receptor ligands. Despite increased numbers of neutrophils during influenza complicated by bacterial super-infection, mouse models in which neutrophils have been depleted show no difference in bacterial burden compared to those with no neutrophil depletion, suggesting that dysregulation of phagocyte function or phagocyte-independent mechanisms play a more important role during influenza and bacterial super-infection.

Alterations in phagocyte function

Ongoing investigation has explored the function of both macrophages and neutrophils in the airway during influenza and bacterial super-infection. Recent work has demonstrated that influenza virus does not induce a defect in *S. aureus* uptake by alveolar macrophages *ex vivo* or *in vivo* 24 hours after challenge, suggesting a phagocyte-independent mechanism for pathogenesis [17]. Somewhat contrary to these findings, other investigators have demonstrated functional impairment of both macrophages and neutrophils associated with a decreased generation of intracellular reactive oxygen species (ROS) [18]. Oxidative burst is a process by which nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) produces ROS, toxic compounds used to kills bacteria within phagocytes. NADPH oxidase-deficient mice, gp91(phox −/−), failed to clear *S. aureus* sufficiently from the lungs, both with and without preceding influenza virus infection. In addition, mosaic gp91(phox +/−) mice, that have both $gp91^{phox}$ WT and deficient neutrophils, also failed to clear *S. aureus* from the lungs, both with and without preceding influenza virus infection. These data suggest a functional defect in phagocytes during super-infection. In further support of a role for reactive oxygen species (ROS), overexpression of granulocyte/macrophage colony-stimulating factor resulted in an elevated production of ROS by macrophages and decreased morbidity, mortality and bacterial burden in influenza virus-*S. aureus* super-infection [19]. Although different laboratories have demonstrated disparate findings regarding phagocyte function during co-infection, these differences can be explained by timing of infection, timing of phagocyte analysis, virus and bacteria dosing, and different strains of virus and bacteria.

In addition to phagocytosis and ROS-dependent killing, neutrophils can kill bacteria by extruding neutrophil extracellular traps (NETs) [20,21]. The cytotoxic effects of NETs are related to their antimicrobial protein components such as histones, defensins and myeloperoxidase. It has been reported that NETs entangle with alveolar-capillary surfaces of the lungs during influenza virus infection, potentially causing lung damage [22]. Recent work has shown that influenza virus infection that is complicated by secondary *S. pneumoniae* was associated with increased NET generation [23]. However, despite the high levels of NETs released in super-infection, they did not participate in bacterial killing *ex vivo*. In fact, they may promote increased pulmonary pathology owing to the release of neutrophil-associated cytotoxic proteins. Extracellular histones released by NETs are known to contribute to inflammation during sepsis [24]. In summary, current evidence supports the concept that defects in macrophage and neutrophil function are induced by primary influenza virus infection in certain contexts.

Antimicrobial peptides

In addition to phagocyte-dependent mechanisms enhancing susceptibility to secondary bacterial infections, there are phagocyte-independent mechanisms of pathogenesis. Recent work has shown that preceding influenza virus infection suppressed the production of interleukin-17 (IL-17)- and IL-22-associated antimicrobial peptides (such as lipocalin 2, CAMP, REG3B, S100A8 and S100A9) in mouse lungs in response to secondary *S. aureus* challenge [17]. Furthermore, exogenous supplementation of the antimicrobial peptide lipocalin 2 improved *S. aureus* clearance and reduced inflammation in super-infected mice.

These data suggest an additional innate defect in the setting of influenza virus infection that increases susceptibility and severity of bacterial super-infection. Regulation of antimicrobial peptide production during super-infection in humans has not been evaluated.

Antiviral interferons

Seminal work has identified the antiviral IFN pathway as an important mechanism by which influenza virus suppresses extracellular pathogen host defense. Studies have investigated both type I IFNs (IFNα and IFNβ) and type II IFN (IFNγ) and their respective roles in influenza virus and bacterial super-infection. Mice deficient in type I IFN receptor 1 (*Ifnar1−/−* mice) that were infected with influenza virus and then, 5 days later, challenged with *S. pneumoniae* were protected from secondary bacterial infection. Of note, *Ifnar1−/−* and wild-type mice infected only with influenza virus showed similar lung viral burden or weight loss at days 5 or 7 post-infection. However, wild-type mice had attenuated production of CXCL1 and CXCL2 during super-infection compared with mice infected with *S. pneumoniae* alone, which was thought to be a potential mechanism for increased sensitivity to bacterial pneumonia [25]. More recently, the protein lysine methyltransferase Setdb2 was characterized as an interferon-stimulating gene and found to regulate CXCL1 expression and recruitment of neutrophils. Setdb2$^{GT/GT}$ mice with influenza super-infected with *S. penumoniae* 5 days later demonstrated increased CXCL1, increased neutrophil recruitment, and lower bacterial burden compared to wild-type mice [26].

Influenza virus-induced type I IFNs have also been shown to inhibit IL-23 production, which is required for the generation of type 17 immunity and for effective clearance of secondary *S. aureus* pneumonia [12]. Indeed, influenza virus-infected mice that were challenged (6 days later) with *S. aureus* had reduced numbers of IL-17-producing γδT cells and IL-17-producing CD4$^+$ T cells compared with mice challenged with bacteria alone [12]. Similar results were found for *S. pneumoniae* super-infection [27]. Moreover, adoptive transfer of *Ifnar1−/−* γδT cells into wild-type mice reduced their susceptibility to secondary *S. pneumoniae* infection [27]. Interestingly, mice that were colonized with *S. pneumoniae* and subsequently infected with influenza virus showed synergistic stimulation of type I IFNs and this diminished the recruitment of macrophages through decreased levels of CC-chemokine ligand 2 (CCL2; also known as MCP1) production, resulting in increased bacterial colonization [28]. Therefore, type I IFN signalling potentially interferes with both neutrophil recruitment and IL-17 responses, suggesting a mechanism for decreased bacterial clearance. However, the question arises - is type I IFN production sufficient for exacerbation of secondary bacterial infection? Poly I:C and TLR7 ligands used to induce type I IFNs in the absence of virus exacerbated both *S. aureus* and *S. pneumoniae* burden in the lungs [29]. However, studies in our laboratory using exogenous IFNβ did not show increased susceptibility to *S. aureus* infection (Robinson & Alcorn, unpublished observations). So, although type I IFNs are probably necessary for exacerbation of secondary bacterial infection, it remains unclear whether they are sufficient.

Additional work has been done to identify the role of IFNγ in the immunopathogenesis of super-infection. It has been shown that mice deficient in IFNγ-mediated signalling were protected from influenza virus-*S. pneumoniae* super-infection. Influenza virus-induced IFNγ

production was shown to inhibit phagocytosis of bacteria by macrophages and direct application of IFNγ downregulated the expression of the class A scavenger receptor MARCO (macrophage receptor with collagenous structure) on macrophages [30]. More recent work has shown that IL-13 downregulates the production of IFNγ at early time points (3 days post-influenza), when susceptibility to super-infection with *S. aureus* was reduced. At later time points during influenza infection (7 days post-influenza), mice had low IL-13 levels and elevated IFNγ levels, but were more susceptible to *S. aureus* super-infection. Blocking of the decoy receptor IL-13Rα2 resulted in reduced bacterial burden when given to mice during the period of increased susceptibility (6 days post-influenza) to bacterial super-infection, consistent with increased levels of IL-13[31]. Conversely, another investigation has shown that *Ifng*$^{-/-}$ mice were susceptible to influenza virus-*S. aureus* super-infection, similar to wild-type mice [12]. This difference may be explained by the use of different strains of bacteria, different doses of influenza and/or bacteria or different timing of infection. In summary, IFNs are highly expressed during viral infections and likely have an important role in the inhibition of bacterial host defense by influenza virus, but the molecular mechanisms involved warrant further exploration.

Influenza-induced defects in lymphocytes and antibacterial host defense

Peak susceptibility to secondary bacterial pneumonia occurs approximately one week into influenza infection. At this time, innate γδ T cells, NK cells, innate lymphoid cells, and adaptive αβ T cells have migrated to the airways as part of the immune response to viral infection. Preceding influenza infection alters the immune response of these T cells when challenged with secondary bacterial infection (Figure 3).

Type 17 immunity

Both γδ T cells and T helper 17 cells are part of the type 17 immune response, releasing IL-17 and IL-22 in response to pathogens. Prior infection with influenza virus attenuates *S. aureus*-induced (or *S. pneumoniae*-induced) IL-17 production by T_H17 cells and γδ T cells in mouse lungs [12] and transfer of exogenous γδ T cells improves the clearance of bacteria following super-infection [27]. Compared with wild-type mice, *Il22*$^{-/-}$ mice display increased bacterial burden and increased mortality upon *S. pneumoniae* super-infection [32]. Manipulation of this pathway during influenza virus infection by addition of the type 17-inducing cytokines IL-1β or IL-23, rescued IL-17 and IL-22 production following bacterial challenge and improved *S. aureus* clearance [12,33]. In outbred mice, the ability to generate IL-1β in response to secondary *S. aureus* infection was correlated with decreased morbidity [34]. IL-27, known to inhibit the development of Type 17 cells through the induction of STAT1, has recently been shown to play a role in influenza and bacterial super-infection. Mice deficient in IL-27 signaling (IL-27R) were less susceptible to secondary *S. pneumoniae* and *S. aureus* during influenza infection [35] (Robinson & Alcorn, unpublished data). In summary these data indicate an important role for the suppression of type 17 immunity as a mechanism of secondary bacterial co-infection susceptibility and severity.

Natural Killer, T regulatory, and Innate Lymphoid Cells

Additional work has indicated that influenza virus infection may attenuate natural killer (NK) cell responses to *S. aureus* in the lungs [36]. Compared with control mice, suppression of NK cells in influenza virus-infected mice resulted in reduced tumor necrosis factor (TNF) production and increased *S. aureus* burden during bacterial super-infection. NK cell-derived TNF led to the induction of alveolar macrophage activity against *S. aureus*. Macrophages were otherwise attenuated by NK cells through cell to cell interactions but could be activated by exposure to exogenous TNF or IL-15 (which recruits NK cells). Furthermore, recent work has shown that the loss of CD200R (cd200r −/− mice) from antigen presenting cells rescued secondary *S. pneumonia* exacerbation during influenza infection. NK cells were increased in these mice compared to wild-type [37].

A possible role for regulatory T (T_{Reg}) cells in susceptibility to secondary bacterial pneumonia has also emerged, as production of the anti-inflammatory cytokine IL-10 has been shown to be increased during influenza virus-*S. pneumoniae* super-infection [38]. In addition, neutralization of IL-10 with specific antibody decreased *S. pneumoniae* growth and mortality in mice [39]. Although T_{Reg} cells are known to produce IL-10 during influenza virus infection [40], it is not clear whether T_{Reg} cells are responsible for the IL-10 production during super-infection. Interestingly, treatment of super-infected mice with an indoleamine 2,3-dioxygenase (IDO) inhibitor resulted in decreased IL-10 production and improved *S. pneumoniae* clearance [41].

Finally, a potential role for innate lymphoid cells (ILCs) in super-infection has been examined. ILCs expressing ST2 (also known as IL-1RL1) termed ILC2s have been found to accumulate in lung tissue following influenza virus infection, and depletion of ILC2s or blockade of ST2 resulted in loss of integrity of the airway epithelium [42]. *Il1rl1*$^{-/-}$ mice have deficient ILC2 function, and influenza virus-*S. pneumoniae* super-infection in these mice resulted in increased bacterial burden compared with wild-type mice. Increased inflammation is suggested by elevated levels of IL-6, IL-1β, CXCL1, IL-10 and IL-33 in *Il1rl1*$^{-/-}$ mice compared with wild-type mice [43]. It is possible that ILC2s protect against denudation of the airway epithelium during influenza virus infection, allowing for a more severe secondary bacterial infection. Although ST2 only played a limited role in the development of bacterial super-infection in this investigation, the secondary bacterial challenge was performed at 14 days post-influenza infection, well outside the typical window of susceptibility to secondary bacterial pneumonia.

Together, these data suggest NK cells, T_{Reg} cells and ILCs may potentially play a role in host defense against secondary bacterial infection during influenza infection. Overall, it remains likely that dysregulated inflammation is the key to increased susceptibility to secondary bacterial pneumonia.

Genetic susceptibility to infection

Over the past decade, human and mouse genetic studies have identified pathways that are required for host immunity to *S. pneumoniae* and *S. aureus*, which may provide clues to the pathways that are perturbed by influenza virus infection. Patients with mutations that affect

TLR/IL-1 receptor signaling, such as in the adaptor proteins myeloid differentiation primary-response gene 88 (MYD88) and IL-1R-associated kinase 4 (IRAK4), have increased susceptibility to pyogenic infections, including meningitis, sepsis and abscesses due to *S. pneumoniae* and *S. aureus* [44]. Interestingly, these patients can mount pathogen-specific T and B cell responses but are unable to make glycan-specific antibody responses to pneumococcus. In mouse models, *MyD88* is essential for innate immunity to *S. pneumoniae* [45]. Consistent with a defect in innate immunity, patients that have mutations in NF-κB essential modulator (*NEMO*) and *IKBA*, which result in impaired NF-κB signalling downstream of TLRs and cytokine receptors, also suffer from pyogenic infections with *S. pneumoniae* and *S. aureus*. These patients also have impaired immunity against mycobacterial disease.

S. aureus pneumonia is also a complication of hyper IgE syndrome (HIES) which is due to mutations in signal transducer and activator of transcription 3 (*STAT3*) [46]. Patients with HIES lack bacteria-specific T_H17 cells and have reduced STAT3 signalling in non-myeloid cells. Supernatants from cultures of activated T cells from these patients failed to mediate *S. aureus*-induced antimicrobial activity, including chemokines and antimicrobial peptides, in skin keratinocytes and lung epithelial cells [47]. These data suggest a link between defective T_H17 cell responses and abnormal mucosal immunity against *S. aureus* in humans. It has recently been shown that the acute phase response to *S. pneumoniae* is regulated by hepatic expression of STAT3 and RELA and this could explain in part the increased susceptibility of HIES patients to infection. In addition, MRSA can be cleared from the lungs in the absence of T cells, B cells or NK cells but clearance was attenuated in the absence of STAT3 expression in the lung epithelium [48]. One downstream regulator of this response was REG3γ, a STAT3-dependent soluble C-type lectin that can bind *S. aureus* [48]. Thus the susceptibility of HIES patients to *S. aureus* pulmonary infection could represent defects in STAT3 signalling in both the myeloid and non-myeloid compartment. Although there is currently no evidence that the aforementioned genetic mutations have a role in influenza virus-bacteria super-infection, we speculate that they may influence super-infection in addition to bacterial infection alone. Consistent with this hypothesis, *MyD88* is known to be indispensable for host defense against primary influenza virus infection [49] and NEMO-deficient patients are susceptible to other severe viruses such as adenovirus, herpes simplex virus and cytomegalovirus [44]. STAT3 is a negative regulator of type I IFNs [50], and as mentioned, type I IFNs are known to inhibit IL-23 and subsequent *S. aureus*-induction of type 17 immunity, allowing for enhanced susceptibility to secondary bacterial pneumonia [12].

Therapeutic opportunities

Various approaches for preventing and treating influenza and secondary bacterial pneumonia have been tested in mice. A wide range of antibiotics and anti-inflammatories have recently been investigated. The steroid dexamethasone was shown to limit inflammation during influenza virus-*S. pneumoniae* super-infection, but did not improve morbidity or the outcome of disease [13]. Furthermore, early dexamethasone treatment during influenza virus infection worsened viral burden [51]. However, the combination of dexamethasone with ampicillin or azithromycin improved *S. pneumoniae* clearance,

decreased lung pathology and improved survival in these two studies. Ampicillin treatment alone resulted in increased neutrophil recruitment and mortality in a TLR2-dependent manner, likely due to excessive *S. pneumoniae* lysis [52]. Azithromycin alone reduced *S. pneumonia* burden, but did not improve lung injury [13,52]. Linezolid, an antibiotic that is active against MRSA by binding to the 50S ribosomal subunit and inhibiting protein synthesis, has also been shown to reduce secondary bacterial pneumonia in experimental models by attenuating IFNγ expression [53]. Mice treated with recombinant IFNγ prior to bacterial challenge with *S. pneumoniae* seven days post-influenza virus infection showed partially reversed protective effects of linezolid. Thus, some antibiotic approaches may work through immune modulation in addition to their direct antimicrobial properties. Triple therapy with linezolid, clindamycin and vancomycin decreased inflammatory cytokine production, *S. aureus* burden, and morbidity during influenza virus super-infection [54]. These data suggest that antibiotic or steroid therapies that limit inflammation may be of some benefit during influenza and secondary bacterial co-infection.

However, as we understand more about the immunological susceptibility to bacterial super-infection, new potential immunotherapeutic strategies may become available. Therapies aimed at inhibiting chemokines, chemokine receptors, or TLRs warrant investigation. Recently, small molecule inhibitor of CCR2 (the receptor for CCL2) was evaluated in influenza virus-*S. pneumoniae* infection [55]. Inhibition of CCR2 decreased lung injury, morbidity and mortality in mice without impacting bacterial burden or later antibody responses to influenza virus. However, attempts to limit neutrophil recruitment with neutralizing antibody specific for CXCL2 during super-infection provided no improvement [13]. A TLR4 agonistic monoclonal antibody, UT12, has also been investigated during influenza virus-*S. pneumoniae* super-infection. Mice received UT12 prior to both influenza virus administration and *S. pneumoniae* bacterial challenge two days post-influenza infection. In this case, UT12 hastened macrophage recruitment induced by C-JUN N-terminal kinase (JNK) and NF-κB pathway-dependent CCL2 production [56]. While we continue to investigate the potential of developing targeted prophylaxis, rapid clinical recognition of bacterial pneumonia complicating influenza infection and rapid implementation of therapeutic antibiotics by clinicians remains the most prudent strategies to reduce post-influenza bacterial pneumonia.

Conclusions

In recent years, there has been renewed interest in understanding the immunologic mechanisms of influenza virus complicated by bacterial super-infection. The role of phagocytes remains a critical area of investigation. Resident alveolar macrophages may be depleted by influenza infection, allowing for enhanced susceptibility to bacterial super-infection. Enhanced neutrophilia has been demonstrated during the window of susceptibility to bacterial super-infection, although neutrophil depletion does not alter the outcome of mortality or bacterial burden. Preceding influenza may alter the function of phagocytes related to a decreased generation of intracellular reactive oxygen species, although different laboratories continue to find inconsistencies in the data supporting the dysregulation of phagocyte-mediated clearance of bacteria. This may be a result of the fine details of experimental models. Antimicrobial peptides have recently been shown to play a key role in

the pathogenesis of viral, bacterial super-infection. The mechanisms by which virus-induced interferons interfere with normal antibacterial defense remains an active area of research. Both Type I (αβ) and Type II (γ) interferons have been show to play a key role, with Type I IFN interfering with both neutrophil recruitment and Type 17 immunity, and Type II IFN interfering with the expression of the scavenger receptor MARCO. Although interferons are necessary for the development of influenza, bacterial super-infection, more investigation needs to be done to determine the sufficiency of interferons in the observed disease pathogenesis. The role of T cells, both innate and adaptive, has also emerged as a mechanism for susceptibility. The altered T cell phenotypes that develop during resolving influenza infection likely impact innate antibacterial immunity against secondary bacterial infection. As additional genetic mutations and genetic modifiers of human disease are discovered, we will likely uncover additional immunologic mechanisms that predispose to both viral and bacterial infections. Finally, the challenge remains to develop effective therapeutic approaches that will limit acute lung injury during viral infection, while preserving bacterial clearance pathways. Basic science approaches to dissect the molecular and cellular pathways of influenza and complicating bacterial super-infection will inform novel clinical strategies to strategically target disease pathogenesis.

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Highlights

- Influenza complicated by bacterial infection increases morbidity and mortality
- Preceding influenza causes defects in antibacterial phagocyte function
- Preceding influenza attenuates antibacterial antimicrobial peptide production
- Preceding influenza causes defects in lymphocyte-mediated immunity
- Genetic mutations can predispose to both viral and bacterial infections

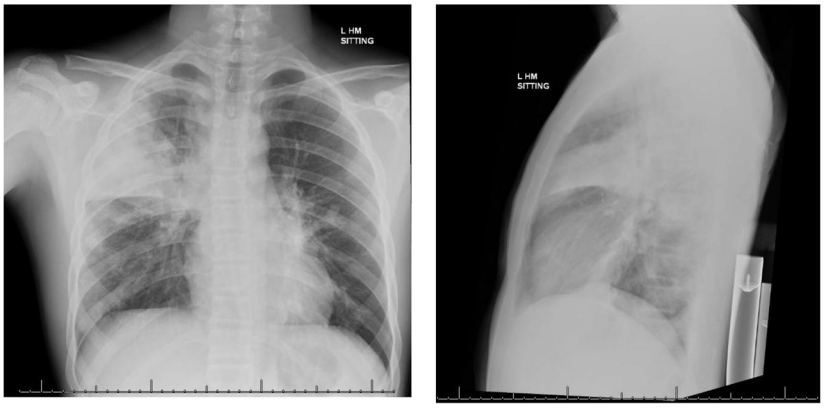

Figure 1.

Chest radiographs of a child with influenza A H1N1 super-infected with methicillin-resistant *Staphylococcus aureus*. The window of vulnerability to secondary bacterial super-infection typically occurs typically one week post-influenza.

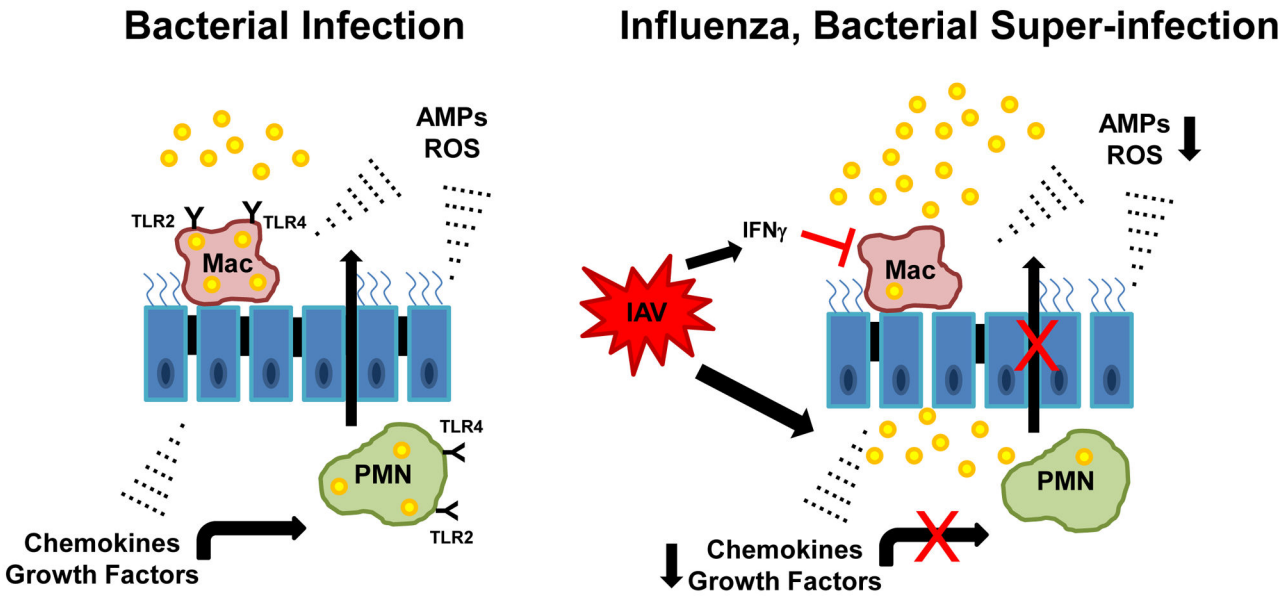

Figure 2.

Preceding influenza attenuates innate host defense against secondary bacterial infection. In the context of bacterial infection alone, alveolar macrophages (Mac) recognize pathogens via pattern recognition receptors initiating an inflammatory cascade. Cytokines, antimicrobial peptides (AMPs), and reactive oxygen species (ROS) are generated by macrophages, recruited neutrophils, and the lung epithelium resulting in pathogen clearance. Preceding influenza (IAV) results in impaired macrophage and neutrophil killing of bacteria and decreased extracellular mediators (AMPs).

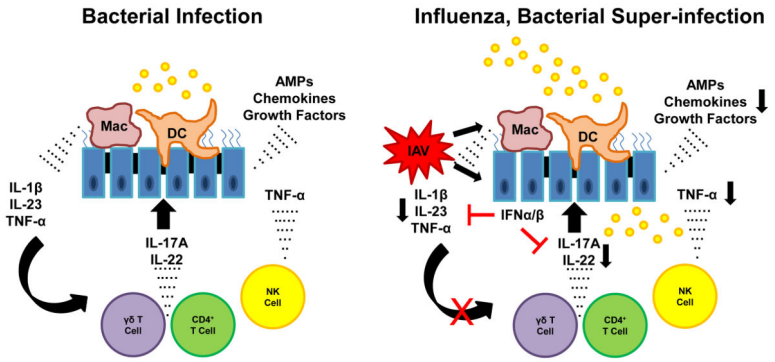

Figure 3.

Influenza infection results in inhibition of Type 17 immunity in the lung. Bacterial infection induces robust IL-17, IL-22, and TNF-α production in the lung. This process mediates inflammation and antimicrobial host defense. In the context of preceding influenza (IAV), type I IFNs inhibit Type 17 immunity by attenuating IL-1β and IL-23 production by macrophages and dendritic cells. IL-17A and IL-22 production by γδ and CD4+ T cells is markedly reduced resulting in impaired host defense against bacterial challenge.