Postinfluenza Bacterial Pneumonia: Host Defenses Gone Awry

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Influenza is a common respiratory pathogen causing both seasonal and pandemic disease. Influenza infection predisposes the host to secondary bacterial infection of the respiratory tract, which is a major cause of both morbidity and mortality in flu-related disease. In this review, we will discuss innate and adaptive antiviral responses during influenza infection, and review how these responses modulate protective immunity against secondary bacterial pathogens of the lung. Specific emphasis will be placed on implications of bacterial superinfection and mechanisms involved.

Introduction

INFLUENZA VIRUS IS A COMMON pathogen of the respiratory tract, infecting up to 20% of the U.S. population annually. Most individuals infected with influenza recover without serious sequela. However, influenza causes over 200,000 hospitalizations and 36,000 deaths per year, with many of these deaths attributable to secondary bacterial infection of the lung. Influenza virus is a zoonotic disease that can be transmitted between animals, including swine, birds, porcine, dogs, horses, and humans (van der Meer and others 2010). The ability of this virus to infect many different species and to jump from species to species makes influenza a formidable pathogen. Immunity to influenza is short lived, due largely to antigenic drift (mutations introduced into the viral genome during replication) and antigenic shift (the swapping of whole gene segments between 2 different influenza strains) (Hussell and Williams 2004). The rare episode of antigenic shift only occurs in the event that 2 different influenza strains infect the same cell at the same time, allowing for reassortment of genes from the different strains into the new particle (van der Meer and others 2010). Antigenic shift is thought to account for many of the major influenza pandemics, including the 1918 H1N1 Spanish flu pandemic, which resulted in exceedingly high rates of both morbidity and mortality (Cunha 2004).

Influenza, a member of the Orthomyxoviridae family, is a single-stranded RNA virus with a segmented genome. There are several different types of influenza virus (eg, Influenza A, B, and C), which are classified based on composition of antigenic core proteins. The virus is composed of 11 proteins whose genome consists of 8 gene segments: hemagglutinin (HA), neuraminidase (NA), matrix protein, nucleo-protein, polymerase A, polymerase B1 and 2, and the nonstructural proteins (van der Meer and others 2010). Previous work has identified 16 subtypes of HA and 9 subtypes of NA in circulation (McCullers 2006). These glycoproteins are important in mediating influenza infectivity. Hemagglutinin initiates virus binding to sialic acid receptors on the surface of host cells, promoting fusion of virus and host cellular membranes (Bhatia and Kast 2007). By comparison, NA is required for the release of newly synthesized viruses, allowing particles to freely invade and infect new cellular targets (Tamura and Kurata 2004).

In recent years, the threat of several influenza pandemics has loomed on the horizon. For instance, the highly pathogenic avian influenza H5N1 caused over 380 human deaths with an overall fatality rate of over 60% (Maines and others 2008). In April 2009, a novel influenza A virus was identified in Mexico and Southern California, referred to as the 2009 pandemic H1N1 swine flu. Initial reports indicate that the 2009 H1N1 is the result of antigenic shift or reassortment of 2 or 3 different viruses, resulting in a combination of genes not previously reported in humans. It is speculated that the resulting 2009 H1N1 virus is a combination of the Eurasian H1N1 swine influenza, the H1N2 swine virus, and possibly the trH3N2 swine flu (van der Meer and others 2010). The 2009 H1N1 influenza strain tended to cause disease in younger and less immunocompromised subjects than its seasonal counterparts (Chang and others 2010). Additionally, the 2009 H1N1 influenza virus is capable of replicating in human lung tissue, a feature that is shared with other highly pathogenic influenza strains, but normally absent in seasonal influenza viruses (Zhang and others 2010). Although known to be a highly pathogenic strain with mortality as high as 20%, the role of bacterial coinfections contributing to the increased mortality associated with the 2009 H1N1 influenza is uncertain (Jain and others 2009; Louie and others 2009).

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This review will focus on synergism between influenza and bacterial pathogens, with a particular emphasis on potential mechanisms that contribute to enhanced susceptibility to secondary bacterial infection.

Host Defense Responses of the Respiratory Tract

The lung has evolved a sophisticated armamentarium of mechanical and immune host defense mechanisms to protect against microbial invasion. Physical barriers include the production of mucus at the mucosal surface and a robust mucociliary escalator to remove microbes and cellular debris (Jakab 1981). In addition to mechanical defenses, control of viral and bacterial infections of the respiratory tract requires both the innate and the adaptive arms of the immune response (Takeuchi and Akira 2010).

Pathogen recognition receptors and signaling pathways in influenza infection

Infectious insults trigger immediate responses from the immune system via nonspecific recognition of pathogens, resulting in secretion of effector molecules that limit the spread of pathogens. Pattern recognition receptors (PRRs) are germline-encoded receptors capable of detecting pathogens by recognizing evolutionarily conserved microbe-specific structures, referred to as pathogen-associated molecular patterns (PAMPs). There are 4 major classes of PRR families identified to date: toll-like receptors (TLRs), C-type lectin receptors, retinoic acid-inducible gene (RIG)-I-like receptors, and NOD-like receptors (Takeuchi and Akira 2010). The 2 most important PRRs in influenza host recognition are the TLRs and RIG-I-like receptors (RLRs).

TLRs are expressed on both hematopoietic and structural cells, and are activated by PAMPs that are components of viruses, bacteria, fungi, and parasites (Medzhitov 2001). These PRRs primarily recognize exogenous pathogens found at the plasma membrane or internalized within endolysosome of cells. In particular, the influenza virus is recognized through TLR3, TLR7 (and perhaps TLR8 in humans), and TLR9 (McGill and others 2009). In contrast, bacterial components typically activate TLR2, TLR4, TLR5, and TLR9 (Takeuchi and Akira 2010). However, TLRs required for bacterial recognition can also participate in immune responses to influenza. For instance, TLR4 has been shown to modulate the pathogenesis of H5N1 avian influenza virus (Imai and others 2008). The majority of TLRs require recruitment of the adaptor molecule MyD88, leading to activation of NF-κB and the mitogen-activated kinase (MAPK) cascade, culminating in expression of a variety of proinflammatory cytokines, chemokines, and adhesion molecules (McCullers 2006). TLR3 and MyD88-independent signaling of TLR4 requires the adaptor TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF), which results in the induction of type I IFNs and expression of IFN-inducible genes (Takeuchi and Akira 2010).

Viral recognition and signaling through the RLR family results in the induction of type I IFN response and expression of proinflammatory cytokines (Takeuchi and Akira 2010). The family member RIG-I and melanoma differentiation-associated gene 5 (MDA5) are localized to the cytoplasm of the cell and recognize short (up to 1 kb) negative strain viruses and longer (>2 kb) positive strain viruses, respectively (Ehrhardt and others 2010). Another family member, LGP2, may function as a regulator of RIG-I and MDA5 function by sequestering dsRNA or inhibiting RIG-I conformational changes (Satoh and others 2010). Unlike other PRRs, both the activation and deactivation of RIG-I and MDA5 are mediated through ubiquitin ligases (Takeuchi and Akira 2010). It is uncertain what the relative contributions of TLRs and the RLR family are to the generation of innate responses in influenza infection. However, it has been suggested that RIG-I mediates both a type I IFN and proinflammatory cytokine response in epithelial cells, whereas TLR3 expressed primarily by hematopoietic cells mediates the elaboration of proinflammatory cytokines but not type I IFNs (Le Goffic and others 2007).

Role of innate immunity in lung host defense

The first line of defense against an invading pathogen is the innate immune system composed of leukocytes, such as macrophages, neutrophils, and natural killer (NK) cells, and structural cells, such as airway and alveolar epithelial cells (AECs). Immediately upon microbial recognition, the innate immune cells mount a non-antigen-dependent response to sequester and destroy pathogens. In the lung, the initial phagocytic cell to come in contact and respond to invading microbes is the alveolar macrophage (AM) (Broug-Holub and others 1997). The vigor to which AMs internalize both influenza and extracellular bacteria is key to initial containment of infection. In the setting of overwhelming infection or functional impairment of the AM, recruited neutrophils, exudate macrophages, and structural cells (such as epithelial cells) are activated and aid in the eradication of pathogens either by phagocytosis and killing or by secreting cellular mediators. While polymorphonuclear leukocytes (PMN) are required for effective clearance of most extracellular bacterial pathogens, the role of neutrophils in host defense responses in influenza infection is controversial. In fact, most studies suggest that PMN are either not required or even potentiate lung injury during severe influenza pneumonia (Deng and Standiford 2005; Wareing and others 2007; McGill and others 2009; Seki and others 2010). NK cells participate in antibacterial and antiviral immunity by producing chemotactic and activating cytokines, including IFN-γ and tumor necrosis factor (TNF)-α, and perforin-mediated lysis of infected cells to limit viral spread (McGill and others 2009). Finally, γδ T cells respond early in infection by producing cytokines such as IFN-γ and interleukin 17 (IL-17) required for effective antibacterial immunity.

Role of adaptive immunity in host defense response

As influenza propagates, the adaptive immune response is initiated and specific immunity is generated. The adaptive response is primarily initiated by dendritic cells (DCs) present within the lung airway and interstitium, which drive the clonal expansion and differentiation of virus-specific T and B cells (Tamura and Kurata 2004; McGill and others 2009). In addition to antigen presentation, DCs also initiate the production of proinflammatory cytokines, chemokines, and type I IFNs (IFN-α and IFN-β) (McGill and others 2009). During the early adaptive phase (days 5–7) in naïve animals, T cells mediate a rapid decline in influenza viral titer,

whereas in the later phase (day 7 onwards) eradication is more B cell dependent (Tamura and Kurata 2004). Clearance of influenza A infection from the lung requires killing of virally infected cells by antigen-specific $CD8^+$ cytotoxic T cells (McCullers 2006; McMurry and others 2008). Viral entry into epithelial cells is mediated by binding of HA localized in the viral envelope to sialic acid residues at the cell surface. As a site for influenza viral replication, AECs are susceptible to $CD8^+$ T-cell-mediated apoptosis via recognition of virus peptide/major histocompatibility complex (MHC) I complex or other death receptors such as Fas/FasL and TNF-related apoptosis-inducing ligand (TRAIL)/death receptor 5 (Brincks and others 2008). In addition to apoptosis mediated by CD8 T cells, recruited exudates macrophage also cause TRAIL-induced epithelial apoptosis (Herold and others 2008). Increasing evidence suggests that the activation of $CD8^+$ antigen-specific T cells is complex and the magnitude and duration of the CLT response is regulated by cytokine and costimulatory signals (McGill and others 2010). B cells provide protective immunity through the secretion of IgM early after primary infection, followed later by IgG and IgA (Tamura and Kurata 2004). Both IgA and IgM at the mucosal surface provide protection during the initial stages of influenza infection, whereas IgG neutralizes newly replicating viruses once infection is already established (McMurry and others 2008).

Synergism Between Influenza Viral Infections and Bacterial Pneumonia

The link between influenza and bacterial pneumonia has been recognized for many years, as the French physician R.T.H. Laennec described the markedly increased prevalence of bacterial pneumonia following an epidemic of influenza ("la grippe") in 1803 (McCullers 2006). This association came into heightened awareness following the 1918 Spanish flu pandemic, where an estimated 40–50 million persons died worldwide, and over 50% had evidence of concomitant *Streptococcus pneumoniae* respiratory infection (Jakab 1981). Subsequent studies have confirmed a positive correlation between increased morbidity and mortality during influenza epidemics and pandemics and the increase in secondary *S. pneumoniae* infection (McNamee and Harmsen 2006). A causative relation has been established experimentally in humans, as the administration of low dose of H1N1 influenza to healthy subjects results in increased upper airway colonization with pneumococcus (Wadowsky and others 1995). While most research has focused on the predisposing effects of influenza virus on subsequent bacteria pneumonia, other respiratory viruses, including respiratory syncytial virus (RSV), measles virus, human parainfluenza viruses, adenovirus, and rhinoviruses, can also predispose to secondary bacterial infection. In addition to pneumococcus, several other bacteria have been found in patients already virally infected, including *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Mycoplasma pneumoniae* (McCullers 2006).

Animal Models of Postinfluenza Bacterial Pneumonia

Due to the challenge of mechanistic studies examining pathophysiological effects of pneumotropic influenza virus in humans, animal models have been employed. Most studies have been done in mouse models using a murine attenuated strain of influenza A H1N1 termed A/PR/8/34 (McCullers and Rehg 2002; van der Sluijs and others 2004; McNamee and Harmsen 2006; Smith and others 2007; Sun and Metzger 2008; Shahangian and others 2009; Stegemann and others 2009). Although using a murine model is advantageous because of the numerous reagents available, influenza infection in mice is not transmitted from animal to animal; therefore, other animal models such as the guinea pig, which transmit from animal to animal, may indeed be more physiologically relevant (Lowen and others 2006). However, the time course between murine influenza models and human influenza infections is similar. In humans, the influenza virus rapidly proliferated in the lung epithelial cells, reaching a peak viral titer in lung 3–5 days after inoculation. Most mild to moderate cases of influenza resolve by day 7, which corresponds to a decrease in viral titers and an absence of recoverable infectious viruses (Jakab 1981). Although influenza viral antigen is not detected in the host by 15–18 days postinfection, recent evidence suggests that low levels of influenza antigen may persist in the host for extended periods of time (McGill and others 2009). Typical clinical presentation of bacterial pneumonia postinfluenza generally develops after the first week of primary viral infection. In murine models, enhanced susceptibility to secondary bacterial challenge is observed as early as 3 days postinfluenza, but appears to most prominent by 7 days postviral infection (LeVine and others 2001; Smith and others 2007; Sun and Metzger 2008; Stegemann and others 2009; Small and others 2010). Innate antibacterial responses continue to be depressed for up to 14 days (McCullers and Rehg 2002; van der Sluijs and others 2004; Speshock and others 2007). In humans, *S. pneumoniae* is the bacteria most associated with pneumonia following influenza infections (McNamee and Harmsen 2006). For that reason, this pathogen has been employed most commonly in animal models (LeVine and others 2001; McCullers and Rehg 2002; van der Sluijs and others 2004; McNamee and Harmsen 2006; Smith and others 2007; Sun and Metzger 2008; Stegemann and others 2009).

Mechanisms of Enhanced Susceptibility to Bacterial Pneumonia Postinfluenza Infection

There have been several mechanisms proposed to explain the increase in susceptibility to bacterial pneumonia following influenza infection of the respiratory tract (van der Sluijs and others 2004). Previous work has shown that influenza infects epithelial cells and causes virus-induced tissue damage (Ramphal and others 1979). Thus, one potential mechanism of enhanced susceptibility to subsequent bacterial infections could be due to damage to the respiratory epithelium or exposure of new binding partners to which bacteria can more readily adhere (Plotkowski and others 1986; McCullers and Rehg 2002; Avadhanula and others 2006). Influenza causes the activation of both the innate and the adaptive immune response and the production of a variety of different cellular mediators (de Jong and others 2006). Consequently, the production of a variety of cytokines and chemokines could affect the recruitment and function of cells in response to a secondary bacterial infection (LeVine and others 2001; McNamee and Harmsen 2006; Smith and others 2007; Sun and Metzger 2008; Shahangian and others 2009;

Stegemann and others 2009; Small and others 2010). Finally, the dysregulation of cellular mediators themselves could skew the immune response, rendering the host more permissive to subsequent bacterial infections (LeVine and others 2001; Seki and others 2004; McNamee and Harmsen 2006; Sun and Metzger 2008; Shahangian and others 2009; Small and others 2010).

Altered bacterial adherence to respiratory epithelium after viral infection

Influenza virus has particular tropism for respiratory epithelium. Viral infection of respiratory epithelial cells results in phenotypic changes in cells occurring as early as 24h postinfection, alterations that promote the adherence of bacterial pathogens to these cells (Fig. 1) (Ramphal and others 1979). For example, there is enhanced binding of radiolabeled bacteria to tracheal explants postinfluenza infection *ex vivo*, as compared to control noninfected tracheas (Plotkowski and others 1986). This effect is not unique to influenza, as infection with RSV or human papilloma virus has been shown to enhance the ability of *H. influenzae* and *S. pneumoniae* to adhere to both primary respiratory epithelial cells and immortalized cell lines (Avadhanula and others 2006). Influenza can alter the cellular characteristics by exposing or modifying surface molecules and cell receptors on host cells so that *S. pneumoniae* and other bacteria can more readily adhere (McCullers and Rehg 2002). Electron microscopy studies of evolving influenza respiratory infection indicate denuding of both ciliated and nonciliated epithelial cells, resulting in exposure of basement membrane and replacement of damaged epithelium by basal progenitor cells (Plotkowski and others 1986). Bacterial adherence is enhanced by binding to exposed basement membrane components such as fibronectin, and by expression of a unique repertoire of glycoproteins by basal progenitors (Fig. 1). Moreover, influenza neuramidase can enzymatically modify carbohydrate moieties at the epithelial cell surface, which can facilitate the binding of bacteria to these new or altered glycoproteins. Finally, inflammatory cytokines produced during influenza infection induce the upregulation of platelet-activating factor receptor (PAFR), a receptor *S. pneumoniae* utilizes to invade respiratory epithelium (Cundell and others 1995). Mice deficient in PAFR have significantly reduced bacterial outgrowth in their lungs, diminished dissemination of the infection, and prolonged survival after influenza infection followed by a subsequent bacterial infection (van der Sluijs and others 2006). The role of PAFR remains somewhat controversial, as others have found no effect of antibody-mediated neutralization of PAFR on susceptibility to bacterial superinfection in influenza-infected mice (McCullers and Rehg 2002).

Along with effects on adherence properties of respiratory epithelium, influenza can also impair the vital function of the mucociliary transport system. Using an *ex vivo* tracheal explant system, Pittet and others (2010) found no effect of influenza infection on binding of *S. pneumoniae*, but did observe a substantial reduction of mucociliary velocity in virally infected tracheas as compared to their noninfected counterpart. Thus, alterations in mechanical clearance of bacteria from the airway may represent an additional means by which influenza and possibly other viruses predispose the host to secondary bacterial infection.

Impaired leukocyte recruitment and/or activation

Studies examining effects of influenza infection on leukocyte recruitment and/or activation during secondary bacterial challenge have produced conflicting results (Table 1). Most studies suggest that while early recruitment of neutrophils in flu-infected mice postsecondary bacterial challenge is reduced, later neutrophil accumulation (24h postbacteria) is uninhibited or even increased (LeVine and others 2001; van der Sluijs and others 2004; Smith and others 2007; Shahangian and others 2009). Interpretation of findings at later time points (eg, 24h) is often confounded by marked differences in lung bacterial burden. Despite elevated numbers of neutrophils recruited to the airways, there are defects in the function of these cells, including decreased phagocytic activity, myeloperoxidase production, respiratory burst, and lysozyme secretion (LeVine and others 2001; Seki and others 2004; McNamee and Harmsen 2006). Studies also suggest increased apoptosis and reduced survival in neutrophils coinfected with influenza virus and *S. pneumoniae* (Engelich and others 2001). The presence of apoptotic neutrophils within the airspace is of relevance due to the immune suppressive environment generated by the uptake of apoptotic cells by AM and other phagocytes present in the lung. In addition to functional neutrophil defects, more robust binding of bacteria to receptors on epithelial cells can shield microbes from neutrophil-mediated killing (McNamee and Harmsen 2006).

Infection with influenza results in important changes in both recruited exudate macrophage and resident AM function. Influenza stimulates increased accumulation of exudate macrophages within the lung, which occurs in a CCR2-dependent fashion (Herold and others 2008). These cells express TRAIL, which induces apoptosis and breakdown of alveolar epithelium, culminating in lung injury. AM phagocytic function is also altered during the evolution of flu. Early in infection (day 3 postinfluenza), AM phagocytic capacity is enhanced, whereas at later time points the ability of these cells to ingest extracellular bacteria is markedly diminished (Hashimoto and others 2007; Sun and Metzger 2008). Impaired AM phagocytic function is temporally associated with the cytokine-mediated downregulation of the scavenger receptor MARCO (Sun and Metzger 2008). Diminished AM effector responses have also been causally linked with reduced accumulation and production of TNF-α by NK cells in animals coinfected with both influenza and bacteria (Small and others 2010).

Dysregulated cytokine responses

Dysregulated cytokine and chemokine production during primary influenza infection can both promote deleterious tissue injury and impair innate responses required for effective clearance of bacterial pathogens (Fig. 2). Enhanced production of proinflammatory cytokines, such as TNF-α, IL-6, IL-1β, and chemokines, have been detected in lungs of animals and humans infected with influenza (LeVine and others 2001; Speshock and others 2007), which can results in vigorous inflammatory cell influx, alveolar epithelial injury, and high permeability pulmonary edema (referred to as the cytokine storm). However, some, but not all, studies suggest an inadequate proinflammatory cytokine/chemokine response in flu-infected mice when secondarily challenged
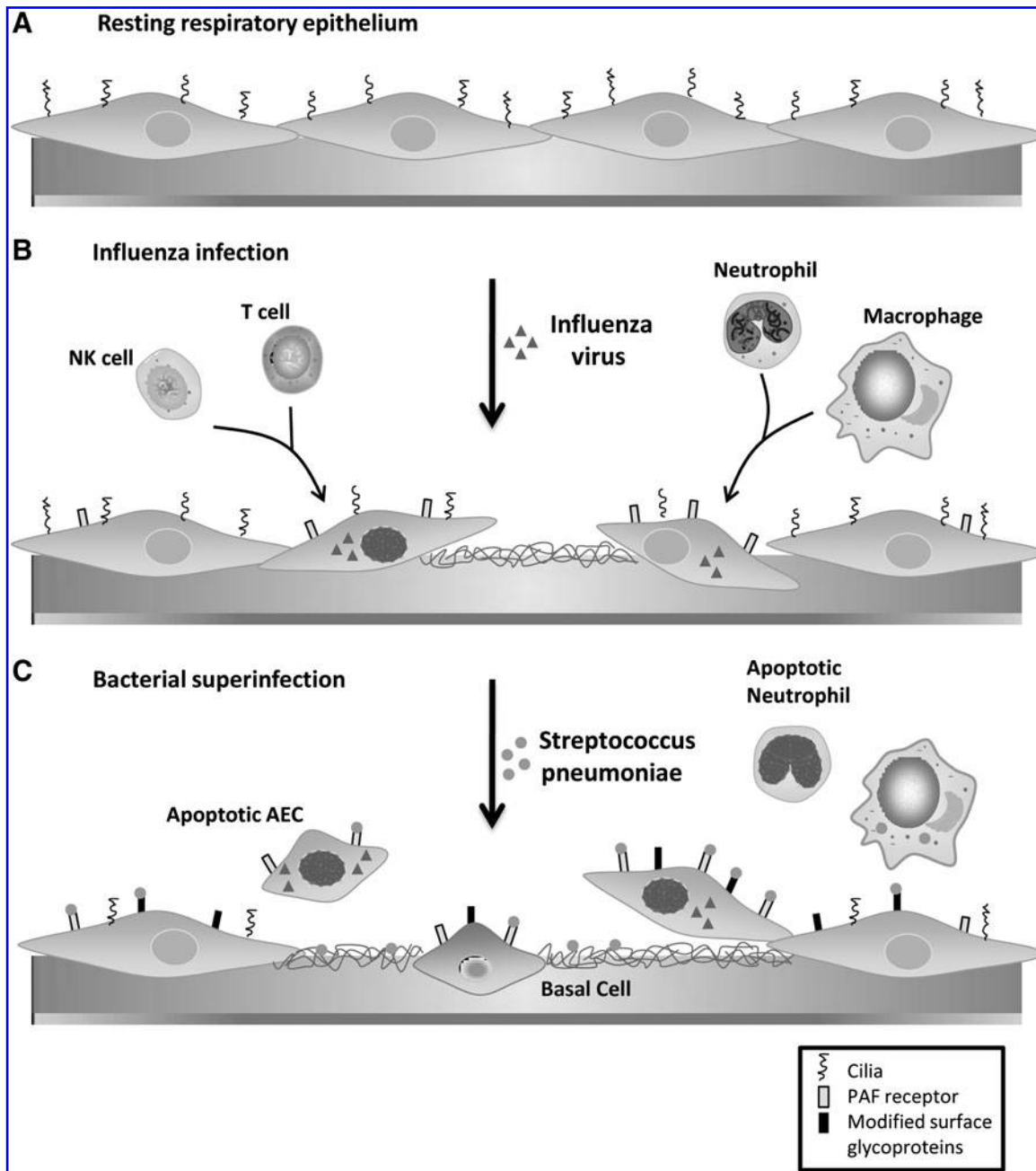
FIG. 1. Effects of influenza virus on respiratory epithelium promoting bacterial adherence. In a normal resting respiratory epithelium (**A**), airway and AECs provide a protective barrier between the airway/airspace and the lung interstitium. Airway epithelial cells express cilia, which aid in the proximal movement of cellular debris and other secreted substances from the distal airway and airspace. (**B**) Influenza virus (*triangle*) infects epithelial cells by binding of the viral hemagglutinin to the sialic acid residues on the host cells, resulting in the endocytosis of virus. Recruited T cells, exudate macrophages, and neutrophils can control influenza infection, but may also cause collateral damage leading to apoptosis of respiratory epithelium, exposure of the basement membrane components, and the appearance of basal progenitor cells to replace damaged epithelium. Proinflammatory cytokines produced in response to influenza induce upregulation of platelet-activating factor (PAF) receptors on airway and alveolar epithelial cells (*black rectangle*). During bacterial superinfection (**C**), *Streptococcus pneumoniae* (*circle*) and other bacterial species bind to PAF receptors and other glycoproteins (*gray rectangle*) made accessible or modified on epithelium, basement membrane, or basal progenitor cells after influenza infection. Additionally, influenza can promote loss of ciliary number and/or function, leading to decreased mucociliary transport capacity, further contributing to bacterial superinfection.

with a bacterial pathogen (Fig. 2). For example, macrophages from flu-infected mice produce diminished amount of neutrophil activating chemokines (mocrophage inflammatory protein 2 [MIP-2] and keratinocyte-derived chemokine [KC]) in response to PAMP stimulation (Shahangian and others 2009). Moreover, defects in AM production of IL-15 has been shown to contribute to reduced accumulation of NK cells and NK-derived TNF-α production, resulting in inadequate reciprocal activation of AM phagocytic activity (Small and others 2010).

TABLE 1. CHANGES IN EFFECTOR CELL FUNCTION
IN POSTINFLUENZA BACTERIAL PNEUMONIA

Cellular effector	Change in function

Alveolar macrophage	↓ Phagocytosis
	↓ Scavenger receptor
	  expression
	↕ Cytokine/chemokine
	  expression

Neutrophil	↑ Recruitment ↓ Phagocytosis ↓ Killing ↓ Respiratory burst ↑ Apoptosis
Natural killer cell	↓ Recruitment ↓ TNF production
Airway/alveolar epithelial cell	↑ PAF receptor ↑ Apoptosis ↑ Bacterial adhesion

Abbreviations: PAF, platelet-activating factor; TNF, tumor necrosis factor.

Mechanisms contributing to reduced effector responses of innate cells have not been fully delineated. One possible contributing factor is the disproportionate release/activity of immunosuppressive cytokines such as IL-10. Concurrent with the release of inflammatory cytokines, flu also stimulates the production IL-10 (Speshock and others 2007). The antiinflammatory properties of IL-10 curtail the magnitude of inflammatory responses in both pulmonary and systemic inflammation, including influenza (Standiford and others 1995; McKinstry and others 2009). Conversely, enhanced IL-10 bioactivity can also mediate deactivation of leukocytes and impairs innate immunity in several disease states such as sepsis (Steinhauser and others 1999). Van der Sluijs and associates identified IL-10 as a major mediator of influenza-induced suppression of antibacterial host defense, as treatment of flu-infected mice with anti-IL-10 monoclonal antibody reversed defects in clearance of *S. pneumoniae* and substantially improved survival (van der Sluijs and others 2004). Others, however, have been unable to confirm improved outcomes after dual infection in IL-10$^{-/-}$ mice. Another cytokine with immunosuppressive properties is transforming growth factor (TGF)-β. Latent TGF-β is synthesized as a preprocessed protein (latent TGF-β) that requires cleavage to its biologically active form. Interestingly, the neuramidase glycoprotein of influenza has been shown to activate latent TGF-β to its active form, which may function to inhibit antibacterial immunity (Schultz-Cherry and Hinshaw 1996). In total, establishing a definitive role for IL-10 and TGF-β requires further study.

IFNs, including IFN-γ and type I IFNs, represent key cytokine mediators of antiviral immunity. However, there is compelling evidence that both type I and type II IFNs can paradoxically suppress host responses to secondary bacterial challenge. Sun and Metger described the novel observation that T cell depletion reversed the flu-induced impairment in *S. pneumoniae* clearance (Sun and Metzger 2008). Importantly, a similar phenotype was observed in the dual
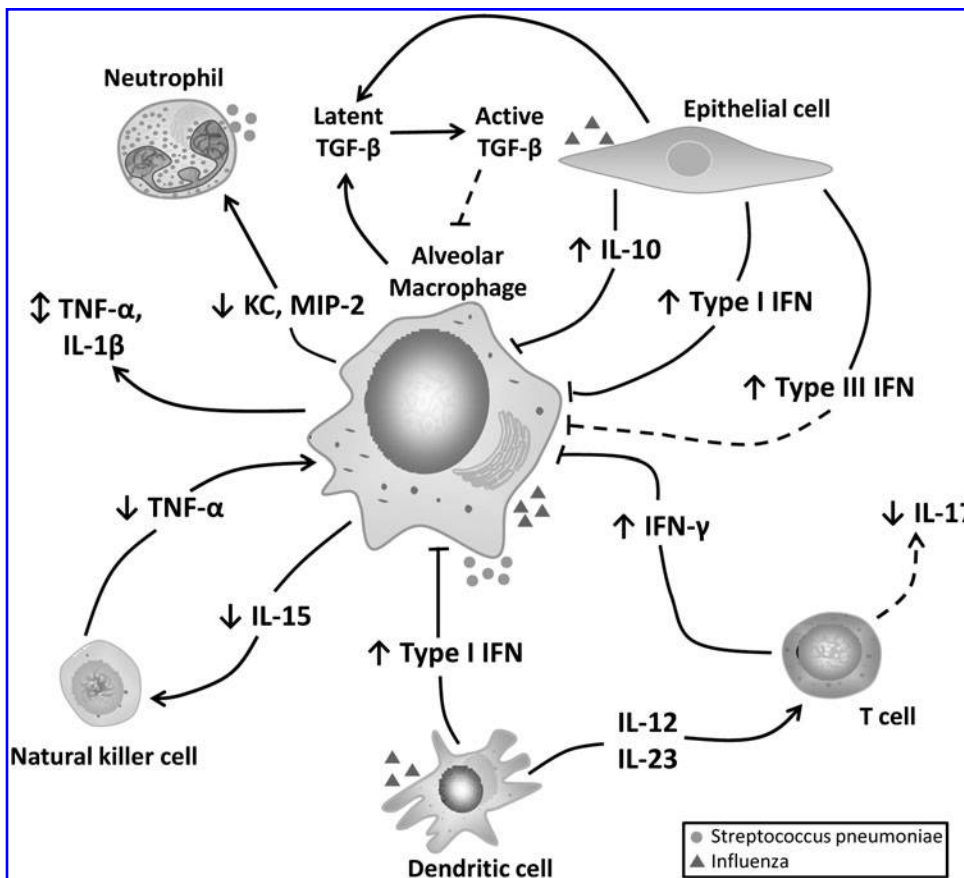

FIG. 2. Dysregulation of cytokines and chemokines during primary influenza infection followed by secondary bacterial superinfection. Schematic showing the complex interactions between cytokines and chemokines that activate (*arrow*) or inhibit (*block*) cellular effectors important in host defense against bacterial pathogens. Established pathways are illustrated by solid lines, whereas speculative pathways are shown in *dashed lines*.

infection model in mice deficient in IFN-γ (IFN-$\gamma^{-/-}$) or mice treated with anti-IFN-γ neutralizing antibody. Incubation of AM with recombinant IFN-γ *in vitro* suppressed bacterial phagocytosis, which was coincident with reduced cell surface expression of MARCO.

Type I IFNs play a critical role in the innate resistance to influenza viral infection and the induction of the adaptive immunity effector response by regulating the transcription of genes related to the protection and establishment of influenza infections in uninfected cells (Lindenmann and others 1957). To combat type I IFNs, the influenza A virus genome contains a potent IFN antagonist, the NS1 protein, which not only inhibits type I IFN expression from infected cells, but also suppresses DC maturation resulting in diminished CD8 T cell responses (Fernandez-Sesma 2007). Some, but not all, studies suggest that mice deficient in the common type I IFN receptor (IFNR$^{-/-}$) display impaired host response to influenza (Price and others 2000). Somewhat surprisingly, influenza-infected IFNR$^{-/-}$ mice were found to be considerably more resistance to secondary pulmonary challenge with *S. pneumoniae* than their wild-type counterparts (Shahangian and others 2009). In this model, IFNR$^{-/-}$ mice produced elevated amounts of the CXC chemokines KC and MIP-2, which correlated with increased neutrophil recruitment to the lungs (Shahangian and others 2009). The mechanism by which endogenous type I IFNs selectively suppress chemokine production has not been defined, but others have shown that type I IFNs can inhibit TLR signaling via an MyD88-independent pathway, implicating a role for TRIF and type I IFNs in regulating proinflammatory cytokines and chemokines during bacterial and influenza infections (Biswas and others 2007).

Other Possible Mechanisms for Increased Susceptibility to Bacterial Pneumonia After Influenza Infection

Impaired Th17 responses

While the current literature has described a number of mechanisms to account for the increased susceptibility to bacterial pneumonia after influenza infection, other factors almost certainly contribute. Th17 T cells have recently been appreciated for their role in linking the innate and the adaptive immune responses (Gaffen 2008). The derivation of Th17 cells is complex and requires expression of orphan nuclear receptor RORγT as well as the presence of IL-6, TGF-β, IL-1, IL-21, and IL-23 (Littman and Rudensky 2010). In addition to producing IL-17 (both IL-17A and IL-17F), Th17 cells also produce IL-22 and IL-21, which are important for neutrophil activation, tissue remodeling and repair, and production of antimicrobial proteins (Littman and Rudensky 2010). IL-17 binds to multimeric receptor complexes and activate NF-κB and MAPK pathways to induce an array of proinflammatory cytokines and chemokines (Gaffen 2008). In additional to CD4 cells, CD8$^+$ T cells, activated memory $\gamma\delta$ T cells, and invariant NK T cells are significant cellular sources of IL-17 (Peck and Mellins 2010). Previous work has shown that Th17 T cell subsets play an important role in the generation of autoimmunity. However, these cells also contribute to antimicrobial immunity in bacterial and viral infections. For instance, mice infected with *Klebsiella pneumoniae*, *Bordetella pertussis*, and *S. pneumoniae* all mount a robust Th17 response, and disruption of IL-17 signaling increases susceptibility to these pathogens (Peck and Mellins 2010). During influenza infection, IL-17, produced by $\gamma\delta$ T cells and signaling through the IL-17RA, is important for neutrophil migration and activation but not the recruitment of CD8$^+$ T cells (Crowe and others 2009). However, recent studies have suggested a role for type I IFNs to suppress IL-17 expression and Th17 differentiation (Tilg and others 2009). Moreover, a decrease in the number of recruited DCs to the lung has been described during later stages of influenza infection (Aldridge and others 2009). As DCs are the primary source of IL-23, it is tempting to speculate that reduced IL-23 production may adversely influence the ability to mount an effective IL-17 response. Additional studies are required to define the role of IL-17 in postinfluenza bacterial pneumonia.

Type III IFNs

A novel class of cytokines in the IFN family are the type III IFNs (also referred to as IFN-lambda/IL-28/IL-29). Like type I IFNs, type III IFNs are induced in certain viral infections, inhibit viral replication, and induce typical IFN-inducible antiviral genes (Ank and others 2008). Type III IFNs mediate the antiviral effects of TLR3 or TLR9 activation, and the target of lambda IFNs appears to be epithelial cells (Ank and others 2008; Wang and others 2009). Both IL-28 (IFN-λ2) and especially IL-29 (IFN-λ1) are expressed by human AECs in response to influenza, and promote strong antiviral responses (Wang and others 2009). Treatment of AECs with IL-29 in culture reduced release of infectious viral proteins and chemokine release. Whether the inhibitory effect of IL-29 on chemokine production in this model was due to decreased viral load or direct suppressive effects on chemokine gene express has not been determined.

IL-1-receptor-associated kinase-M

IL-1-receptor-associated kinase-M (IRAK-M), also named IRAK-3, is a member of the IRAK family. In contrast to other IRAK family members, IRAK-M lacks kinase activity and functions as a negative regulator of TLR signaling by blocking the disassociation of IRAK-1 from the toll-IL-1 signaling domain. This protein has been shown to mediate tolerance induced by LPS and other PAMPs (Kobayashi and others 2002; Nakayama and others 2004). We have found that IRAK-M is upregulated in AM during experimental sepsis, and mediates both the suppression of macrophage cytokine responses and impaired bacterial clearance after secondary *P. aeruginosa* challenge (Deng and others 2006). Our lab has more recently reported that IRAK-M is upregulated in lung macrophages and AECs during influenza infection, and this protein is vital to dampening the magnitude of proinflammatory cytokine expression and lung injury during primary infection (Seki and others 2010). IRAK-M is considerably upregulated in the lungs of influenza-infected animals after secondary bacterial challenge. Whether or not IRAK-M contributes meaningfully to influenza-induced suppression of antibacterial host defense has not yet been determined.

Conclusions

Secondary bacterial pneumonia is an all-too-common and feared complication of influenza. There are multiple mechanisms accounting for enhanced susceptibility to postinfluenza

bacterial pneumonia, including host- and virus-induced alterations of respiratory epithelium that facilitate bacterial adherence and invasion, impaired recruitment and/or activation of innate cells, and expression of cytokines that promote an immunosuppressive microenvironment favoring bacterial growth rather than eradication. As our population ages, there will be a burgeoning number of patients at risk for the development of influenza-related disease. Moreover, there is dramatic shift in the types of bacterial pathogens causing respiratory infection in both the community and nosocomial setting, which will almost certainly impact the epidemiology of postinfluenza pneumonia. Consequently, more research is needed to define mechanisms of immune suppression during the evolution of influenza infection, which may allow for the development of novel approaches to interrupt these deleterious pathways in patients at risk.

Author Disclosure Statement

No competing financial interests exist.

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