EXPERT REVIEWS

Influenza vaccines to control influenza-associated bacterial infection: where do we stand?

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Ioanna Christopoulou[1,2], Kenny Roose[1,2], Lorena Itatí Ibañez[1–3] and Xavier Saelens*[1,2]

[1]*VIB Inflammation Research Center, Technologiepark 927, Ghent, Belgium*
[2]*Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, Ghent, Belgium*
[3]*ICT-Milstein – CONICET, Ciudad Autónoma de Buenos Aires, Argentina*
**Author for correspondence: xavier.saelens@irc.vib-ugent.be*

Influenza A virus is a pathogen that is feared for its capacity to cause pandemics. In this review, we illustrate the clinical evidence which support the theory that bacterial co-infection is a considerable risk factor for exacerbated disease during pandemic and seasonal influenza, including infection with influenza B viruses. We provide an overview of the multiple and diverse mechanisms that help explain how influenza creates an opportunity for replication of secondary bacterial infections. Influenza vaccines and pneumococcal vaccines are widely used and often in overlapping target groups. We summarize the evidence for a protective effect of influenza immunization against bacterial infections, and *vice versa* of pneumococcal vaccines against influenza-associated pneumonia and lethality. It is important that future implementation of broadly protective influenza vaccines also takes into account protection against secondary bacterial infection.

Keywords: influenza virus • pandemic • *S. pneumoniae* • secondary bacterial infection

Many pathogens enter the human body at mucosal surfaces. This means that pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* have to compete with residing commensal bacteria for space and nutrients. Respiratory and enteric viruses (e.g., influenza A and B virus, rotavirus) that predominantly replicate in the epithelial cells of the mucosa disturb microbial homeostasis at these sites, for example by inducing tissue damage. Such damage may offer a free way for pathogenic bacteria that are normally tolerated or controlled, to invade the sub mucosal layer. Influenza viruses also seem to ally with certain bacterial species by suppressing the immune response of the host to these bacteria [1]. In the case of human influenza, these interplays are clinically important: patients that are infected with influenza virus and bacteria in the lung compartment often become severely ill.

The clinical impact of influenza bacteria co-infections is very well-illustrated by a study from the Centers of Disease Control and Prevention (Atlanta, USA), reporting that influenza-associated deaths in children was increasingly associated with *S. aureus* infection between 2004 and 2007 [2]. Likewise, the 2009 H1N1 pandemic was associated with increased hospitalization and death of children. Also during that event, bacterial co-infection, again with *S. aureus*, significantly increased the risk for developing severe illness, with disseminated intravascular coagulation as a major symptom [3]. Defining the etiology of pneumonia in patients is very difficult because this requires sampling in the lower respiratory tract followed by pathotyping to determine which bacterium, virus or even fungal species is responsible for the disease. Not surprisingly, most studies in humans in which influenza-bacterial co-infection of the lungs were clearly documented are based on post-mortem analysis.

Different bacterial species are known to establish co-infections with influenza viruses, and such co-infections may occur in the upper respiratory tract, the lungs or the inner ear. In the latter case, this may result in *otitis media*, a condition that in rare cases can lead to hearing loss [4]. *S. pneumoniae* is common among incidences of influenza-bacteria co-infection in

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humans [1]. This gram-positive bacterium frequently colonizes the upper respiratory tract in humans, most of the time without causing harm. *S. pneumoniae* can cause pneumonia or *otitis media* by itself. There are more than 90 serotypes of *S. pneumoniae*, many of which are covered by the 23-valent polysaccharide vaccine (PPV23) [5,6]. *S. aureus* is also a very common cause of secondary pneumonia in influenza patients. During the 1957–1958 pandemic, *S. aureus* was probably more frequently associated with secondary bacterial pneumonia than *S. pneumoniae* [7]. Approximately one-third of the human population carries *S. aureus*, usually without any symptoms. *S. aureus* can colonize the skin and also prevails in the anterior nares [8]. This bacterium can cause a range of diseases in its own right, from mild skin infections to severe pneumonia and even sepsis. *Streptococcus pyogenes* and *Hemophilus influenzae* are also responsible for secondary bacterial pneumonia although apparently less frequently than *S. aureus* and *S. pneumoniae* [9].

In this review, we look at the clinical evidence that supports the general belief that bacterial co-infection is a frequent and disease-exacerbating event during pandemic and episodes of seasonal influenza. We address the current knowledge, based on experimental data, of the likely mechanisms that are responsible for the increased susceptibility of influenza patients for bacterial pneumonia. We summarize the evidence in support of a beneficial outcome of influenza vaccines to prevent or mitigate secondary bacterial infection and, *vice versa*, of pneumococcal vaccines on influenza disease severity. Finally, we try to forecast the research priorities for the next 5 years that could help to improve our knowledge and to develop intervention strategies to prevent and control bacteria-influenza virus co-infections.

Incidence of human influenza-bacterial co-infection

Influenza pandemics are particularly associated with increased bacterial pneumonia. Such pandemics happen when an influenza A virus that carries an hemagglutinin (HA) subtype that is antigenically very different from previously circulating human influenza A viruses infects people and is highly contagious. The 1918–1919 pandemic stands out because of its high mortality rate, and because we know very little on the origin of the 1918 influenza virus [10,11]. Nevertheless, analysis of archived pathology reports and careful examination of historical lung tissue samples from 1918 influenza fatalities revealed that co-infection with bacteria that are normally residing in the upper respiratory tract was evident in the majority of fatal cases, with only 4.2% of tested samples being negative for bacterial infection [12]. *H. influenzae*, *S. pneumoniae* and *S. aureus* were most commonly associated with fatal cases of the 1918 H1N1 virus. Therefore, it is fair to conclude that secondary bacterial infection, without antibiotics available, was a frequent and often deadly complication for victims of the 1918 pandemic virus. The Asian flu pandemic that spread in 1957–1958 was caused by an H2N2 virus and killed an estimated 2 million people worldwide. Secondary bacterial pneumonia seems to have occurred less frequently in fatal influenza cases than during the 1918 pandemic, although it still accounted for between 40% and 74% of fatalities and was mainly associated with *S. aureus* [13–15]. During the 1968 pandemic, caused by an H3N2 virus, bacterial co-infections were again reported much more often than during the preceding years of seasonal influenza. In this case, *S. pneumoniae* was most frequently isolated from patients that had succumbed to influenza [16]. Most likely, the use of antibiotics helped to control bacterial co-infections during the pandemics of 1957 and 1968.

The 2009 pandemic was caused by a fairly low pathogenic, swine-derived H1N1 virus. In all, it is estimated that approximately 200,000 people died due to infection by this virus. The occurrence of bacterial co-infection was variable for pandemic H1N1 infection, with studies reporting between 10 and 50% of such cases [17–19]. Taken together, secondary bacterial infection, in particular with *S. pneumoniae* and *S. aureus*, is historically well documented during influenza pandemics.

Following pandemic outbreaks, human influenza viruses recur in seasonal epidemics in moderate climate zones. The incidence of bacterial co-infection during seasonal influenza outbreaks is considerable, variable and probably lower than during pandemic events. Three studies have reported co-infection with bacteria in 9–31% of cases during seasonal influenza [20–22]. According to a report that analyzed scientific databases published between 1950 and 2006, *S. pneumoniae* and *Staphylococcus* spp. are the most common pathogens found during influenza. The same report also described that the occurrence of bacterial co-infection is higher during pandemic (40.8%) as compared to seasonal periods (16.6%) [23].

Disease severity associated with influenza-bacterial co-infection

Bacterial superinfection after influenza virus infection can have very severe consequences. In one study, three members of a family in Maryland (USA) with confirmed influenza died. In two of these cases, co-infection with methicillin-resistant *S. aureus* (MRSA) was documented. Three other members of the same family with confirmed H3N2 influenza were identified, two of which required hospitalization, although no *S. aureus* co-infection was detected and both patients recovered [24]. More circumstantial evidence that patients that are infected with influenza A virus and pneumococci are at risk for developing severe disease is based on a study that was performed in H1N1 2009 patients from Argentina: the presence of *S. pneumoniae* in nasopharyngeal swabs correlated strongly with disease severity [25].

Symptoms due to infection with influenza B virus (IBV) are usually comparable to those caused by seasonal H1N1 viruses and less severe than those caused by H3N2 viruses. However, IBVs circulate as two antigenically distinct lineages (so-called Victoria and Yamagata) that occasionally co-circulate and that prove to be very hard to cover by trivalent influenza vaccines [26–28]. In addition, IBVs are the major cause of human influenza every 4–5 years. There are very few reports of co-infection of bacteria and IBV, probably because this virus is

usually less pathogenic than H3N2 viruses. Three such cases have been described in previously healthy persons that were admitted to a hospital in Basel (Switzerland). During the influenza season of 2007/2008, IBV infection was found associated with *S. pyogenes* in two of these cases and with *S. pneumoniae* in one case [29]. In another study, reporting on the seasonal influenza outbreak of 2010/11, four cases of co-infection of IBV with invasive Group A streptococci were described [30]. Finally, in Hong Kong, during the influenza epidemic of 2011/2012, four previously healthy patients without chronic illness were found to be co-infected by IBV and *S. pneumoniae* or *S. pyogenes*, leading to severe pneumonia [31].

Excess disease and hospitalization due to influenza is most often seen in very young children and the elderly. However, bacterial co-infections during influenza episodes can aggravate symptoms of pneumonia in individuals of different ages [32,33]. Children are an important risk group for influenza-bacterial co-infection because this age group seems particularly at risk for severe disease outcome and also because they are considered important vectors to spread influenza and bacteria within the community. It has shown that there is a strong positive association between the presence of *S. aureus* and influenza viruses in nasopharyngeal swabs of children under 2 years of age [34]. Randolph *et al*. identified risk factors for lethal outcome of critically ill patients that were (likely) infected by 2009 H1N1 pandemic virus and admitted to pediatric intensive care units in the USA. Co-infection with MRSA came out as an important mortality risk factor from this study, and the authors concluded that 'new therapies for treating severe influenza, and new treatment strategies for MRSA pneumonia complicating influenza are urgently needed for children' [35]. A case–control study in children younger than 3 years of age carried out in Peru during 2009/2011 showed that the risk of pneumococcal acquisition increased following influenza virus infection when compared to episodes without infection [36]. Taken together, influenza is frequently associated with a co-infection of *S. pneumoniae* or *S. aureus*. This begs the question if there is a mechanistic explanation for this microbial co-existence. It is important to notice that most of the studies described here take into consideration data obtained from patients with severe disease. Few studies analyze the situation in case of milder disease or consider groups of patients with different degrees of disease severity in a large population [22]. The establishment of surveillance systems or studies including disease etiology for all patients would be more informative to understand the interactions between pathogens in different conditions.

How does influenza facilitate bacterial superinfection?

Clinical and historical data clearly show influenza infection has the ability to prime the infected host for secondary bacterial infection. But how is this accomplished? A number of research groups have employed different models to investigate which factors from the pathogens and the host play a part in this phenomenon. To investigate bacterial superinfection after influenza, researchers have employed defined strains of influenza virus and bacteria, including *S. pneumoniae*, *S. aureus* and *H. influenzae*, in different animal models such as laboratory mice, ferrets, chinchillas and monkeys. Not surprisingly, mechanistic foundations of secondary bacterial infection have been deduced mainly from the mouse model, given the abundance of research reagents and genetic tools that are available for this mammal. An overview of the cellular and molecular players that facilitate bacterial replication following influenza A virus infection is presented in Figure 1.

Influenza virus replication and the induced cell death and inflammatory response can severely damage the integrity of the epithelial lining of the respiratory system. The mere disruption of this primary barrier of defense can strongly enhance the attachment of bacterial pathogens to their receptors, which has been demonstrated in mice, ferrets and chinchillas for influenza [37–39]. In particular, pandemic strains of influenza can induce an excessive amount of lung damage, elevating secondary bacterial infections. Histopathological analysis of patients who succumbed to secondary bacterial infection during previous pandemics clearly illustrate this damage [15,40,41]. With the 2009 pandemic, the group of Jeffrey Taubenberger took an in-depth look at the underlying mechanisms that could explain the occurrence of bacterial superinfection after influenza virus infection [42]. In a mouse model for dual infection with influenza virus and *S. pneumoniae*, they showed that a 2009 pandemic influenza strain induced more severe epithelial damage when compared to a seasonal H1N1 strain. Moreover, regeneration of the epithelial layer and repair was significantly lower after pandemic influenza virus infection. This predisposed infected mice to a higher level of colonization with pneumococci, leading to increased morbidity and mortality. A detailed study by Jamieson *et al.* [43] further showed that excess mortality, in a co-infection model using *Legionella pneumophila* as secondary pathogen, is not due to the pathogenic nature of either viral or bacterial infection. Moreover, excessive inflammatory responses did not account for this phenotype either. Instead, very poor induction of tissue repair responses during the co-infection was identified as the cause for elevated mortality rates, since administration of amphiregulin, an epidermal growth factor family member involved in tissue repair after influenza infection, partially protected co-infected mice even though this intervention did not influence viral or bacterial replication in the lung [44].

Next to the direct effect influenza virus infection has on the integrity of the respiratory epithelium, two different viral factors have been shown to enhance bacterial colonization. The first of these is neuraminidase (NA). During the influenza life cycle, NA serves as a receptor-destroying enzyme, removing sialic acids on glycoproteins and glycolipids, releasing budding viruses from the host cell and avoiding agglutination of budded virions. In addition, by removing sialic acids on cellular proteins, NA can unmask cellular receptors for pneumococci. This has been shown in *ex vivo* models based on chinchilla respiratory epithelium [45–47]. The group of Jonathan McCullers

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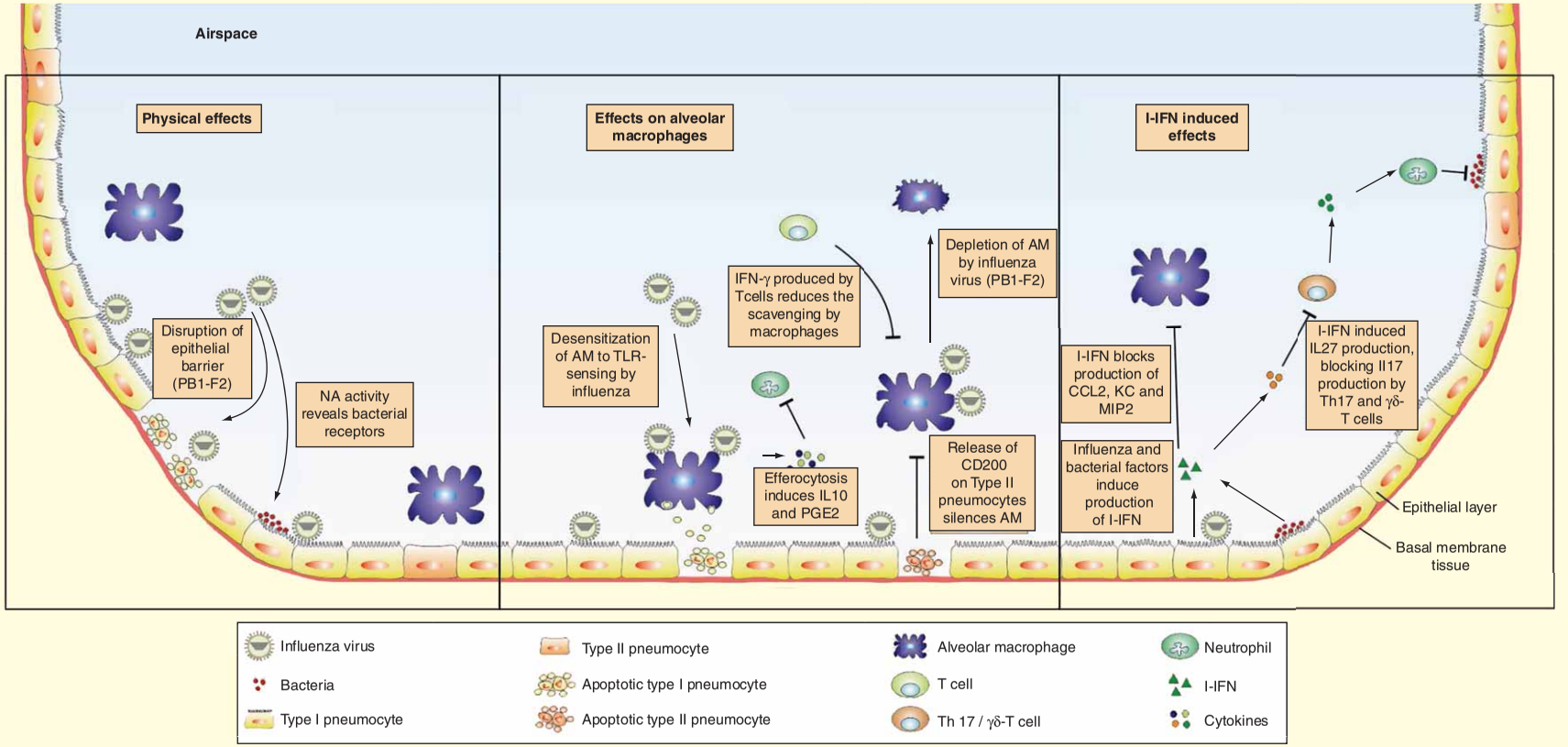

Figure 1. Mechanistic overview of the predisposition for bacterial superinfection after influenza virus infection. (left panel) Physically, influenza virus infection disrupts the epithelial layer lining the respiratory tract, partially through the action of PB1-F2, providing a niche for bacterial pathogens. In addition, viral NA activity can unmask bacterial receptors used for colonization. **(middle panel)** AMs are part of the frontline innate defense to viral and bacterial infections. AM effecter functions become silenced in different ways. Influenza virus infection will cause downregulation of bacterial sensors such as TLR2 and depletion of AM by the cytotoxic effects of PB1-F2. Apoptotic pneumocytes further quench the AM and downstream responses: efferocytosis by AM will induce the production of immune silencing cytokines such as IL-10 and PGE2, while CD200 on the surface of apoptotic bodies of type II pneumocytes will reduce the AM responses after binding with CD200R. T cell produced IFN-g will also diminish the scavenging properties of AM. **(right panel)** Upon viral and bacterial infection, infected cells will produce I-IFN. This will lead to a decrease in CCL2 production by macrophages, responsible for the recruitment of monocytes, and KC and MIP2, which are neutrophil chemoattractants. I-IFN will also induce the secretion of IL-27 by a number of cell types. IL-27 blocks the production of IL-17 by Th17 and γδ-T cells. IL-17 functions in recruiting neutrophils, main players in the clearance of bacterial pathogens.
AM: Alveolar macrophages; TLR: Toll-like receptor; I-IFN: Type I interferon.

explored this in more detail in a mouse model for co-infection [39]. Using the NA-inhibitor oseltamivir, they showed that NA activity enhances adherence of *S. pneumoniae* to respiratory epithelium by stripping of sialic acids. In a follow-up study, they investigated the influence of the NA activity in priming the respiratory tract for bacterial superinfection [48]. With increasing NA activity, colonization by *S. pneumoniae* was enhanced, leading to higher morbidity and mortality in dual infected mice. Avadhanula *et al.* showed that influenza virus, human respiratory syncytial virus (a Para-myxovirus lacking an NA function) and human para-influenza virus type 3 (a para-myxovirus with an NA function) were able to augment bacterial adhesion to primary and immortalized respiratory epithelial cells [49]. While the paramyxoviruses enhanced the expression of specific bacterial receptors on infected cells, influenza virus did not. Hence, it was proposed that influenza unmasks bacterial receptors through desialylation by the viral NA. The importance of NA and direct destruction of the respiratory epithelium by influenza virus replication are also clear from vaccination studies attempting to subdue secondary bacterial infection. Huber *et al.* showed that anti-NA immunity increased both antiviral immunity and survival following a secondary *S. pneumoniae* challenge [50]. A recent study by Siegel *et al.* provides strong evidence that the availability of sialic acid in the nasopharynx is the main driving factor that promotes pneumococcal growth during co-infection with influenza virus. This host derived sugar is catabolized by *S. pneumoniae*, leading to higher bacterial loads in the respiratory tract and extended colonization. Airway mucins are heavily glycosylated and the mammalian airway epithelium is heavily sialylated. Furthermore, influenza virus infection leads to increased mucus secretion. The sialic acid fraction of these polysaccharides is liberated by influenza and bacterial NA activity, providing *S. pneumoniae* with an abundant carbon source [51].

A second viral factor guiding bacterial superinfection is PB1-F2, encoded by influenza A but not B viruses. This non-essential 87-residue protein, encoded by the PB1 gene in a +1 open reading frame, has multiple functions influencing viral pathogenicity (for a review, see [52]). One way by which PB1-F2 contributes to pathogenicity is by inducing cell death of infected cells. This outcome has been shown to be influenza strain-specific and guided by the 27-residue long mitochondrial targeting C-terminal end of the protein [53]. A follow-up study identified key residues involved in the cytotoxic effects induced by PB1-F2, providing a fingerprint for pathogenic influenza strains, such as the Spanish flu of 1918 [54]. In addition, the same study showed the strong cytotoxic and pro-inflammatory effects induced by PB1-F2 (or derived C-terminal peptides), which prime the infected host for secondary infection by *S. pneumoniae*.

Indirectly, the effects of influenza infection on the host immune system also play a significant role. In the lung, alveolar macrophages (AMs) are part of the local innate immune system and form a very important first line of defense against pathogens. With the original discovery of the PB1-F2 protein, it was found that it induces specific depletion of AMs [55]. Since AMs operate as a communicative bridge between the lung environment and the adaptive immune system, depleting this cell population further endows the potential of bacterial superinfection. The specific killing of AMs by influenza infection and the action of PB1-F2 has been linked to predisposition of the infected host to secondary infection with *S. pneumoniae* [56]. In addition, partial restoration of AM levels through local treatment with GM-CSF protects co- infected mice from disease attributed to the bacterial pathogen [57].

Influenza virus infection will evoke a strong pro-inflammatory response early in infection. To counter this, an anti-inflammatory response will normally follow to dampen the detrimental side effects of persistent inflammation such as tissue damage. Master regulators of this balancing act are cytokines, chemokines and other cellular factors, inducing or inhibiting each other's production and secretion. Dysregulation of this system can severely influence the outcome of the ongoing infection [58]. Within the co-infection model of influenza and opportunistic bacteria, different immune regulators have been identified to play a role. At the level of AMs, immune silencing after viral infection abrogates the antibacterial function of this innate immune cell population. While influenza virus infection itself desensitizes AM to sensing bacterial products through Toll-like receptor ligation [59], the antiviral cytokine interferon (IFN)-γ will downregulate the expression of bacterial scavenger receptors [60]. Apoptotic epithelial and immune cells release CD200. Binding of this ligand to CD200R, a myeloid cell-specific surface glycoprotein, will result in an inhibitory signal and suppression of the innate signaling function of AM [61]. Finally, phagocytosis of apoptotic cells by AM, a process called efferocytosis, will induce the expression and secretion of IL10 and PGE2 [62,63]. These suppressive immune response modifiers diminish bacterial uptake and killing and slow down the recruitment and infiltration of immune cells such as neutrophils.

A hallmark of viral (and bacterial) infection is the induction of type I IFN (I-IFN) in a wide variety of cell types [64]. Although beneficial, the pleiotropic effects of I-IFN have also been implicated in guiding secondary bacterial infection in the influenza virus-infected host. Following its induction by influenza virus genomic RNA and pneumococcal factors such as pneumolysin and peptidoglycan, I-IFN has been shown to block the production of CCL2, responsible for macrophage recruitment [65], and the neutrophil chemoattractants KC and MIP2 [59,66]. The main suppressive effect of I-IFN on bacterial immunity, however, is the inhibition of both Th17 and γδ-Tcells [67]. These cell types are part of the innate immune system and are strong producers of IL17, responsible for the recruitment of scavenging neutrophils [68]. This has been enforced by showing that I-IFN lowers the production of IL23, a regulator of Th17 responses, and IL17 and IL22, produced by Th17 cells [69], leading to poor control of secondary bacterial infection. This phenotype could be rescued by overexpression of IL23 delivered by an adenoviral vector. Complementing

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these data, IL22, which functions in protection of epithelial barriers, can protect mice in a *S. pneumoniae* influenza A virus co-infection model [70]. A very detailed study by Cao *et al.* further confirms all these findings: the induction of I-IFN blocks γδ-Tcell development, leading to inhibition of IL17 production, reduced recruitment of neutrophils and poor containment of the bacterial pathogen [71]. Finally; the main mediator that is induced by I-IFN in a variety of cell types was shown to be IL27, which has also been shown to inhibit Th17 responses [72]. This way, although regarded as beneficial, the innate antiviral immune response can render the infected host susceptible to secondary opportunistic pathogens. The clinical importance of a strong Th17 response toward an opportunistic bacterial pathogen is underscored by work by the group of Richard Malley. By fractionating a killed whole-cell pneumococcal vaccine, they identified proteins from the bacterium which are able to induce strong and protective Th17 responses in a mouse model [73]. Moreover, the research has led to a Phase I clinical trial with the vaccine (termed GEN-004) which has yielded promising results, referred to in [74].

In summary, host factors that are induced and released by influenza virus infection together with influenza virus-encoded proteins disrupt airway homeostasis, leading to a condition that favors bacterial spreading (Figure 1). How relevant are the aforementioned mechanistic findings for clinical cases? That is a difficult question to answer, because there are many confounding factors in individual patients that can help explain why an influenza-bacterial co-infection was diagnosed and contributed to disease severity. For example, patients may not have been carriers of *S. pneumoniae* or *S. aureus* before and just by chance acquired the bacterium simultaneously with the flu. This assumption is supported by analysis of data from surveillance studies in Canada that counted the incidence of infections with *S. pneumoniae*, *S. pyogenes*, *H. influenzae* and *Neisseria meningitidis* and overlaying these with influenza activity [75]. After correcting for shared seasonality, only a clear association remained between the incidence of influenza B and invasive *S. pyogenes*: late peaks of influenza B counts were followed by peaks in *S. pyogenes* cases. A European study also found a strong association between influenza B and invasive group A streptococci despite a much higher activity of H1N1 2009 [30].

Impact of influenza vaccines on secondary bacterial infection and of pneumococcal vaccines on human influenza

There are currently two types of *S. pneumoniae* vaccines approved: the polysaccharide vaccine (PPV) and the conjugate vaccines (PCV). The PPV23 contains more serotypes than any other available vaccine, which in some countries covers more than 85% of serotypes [76]. It is recommended for use in adults over 65 years of age and for persons 2 years of age and older at high risk for disease. In the USA, it is also recommended for people aged 19–64, who are either smokers or asthmatics. The first pneumococcal conjugate vaccine to be licensed was the heptavalent PCV7, which in most countries has been replaced by the PCV13 or the PCV10, which has a non-typeable *H. influenzae* protein D conjugated. The current recommendations for the PCV vaccines are for all children under 5 years of age and adults with certain medical conditions, for example, immunosuppression [77]. Different studies are underway to evaluate the protective effect of the PCVs to adults over ≥65 years [78,79]. Although there are observations that the incidence of pneumococcal infections coincides with the influenza season [25,80], as well as with the occurrence of other respiratory viral infections, most studies on pneumococcal vaccines focus only on their effectiveness to the pathogen it targets in primary infections. With proved synergistic effect of influenza virus infection and bacterial co-infections, a number of research groups started to investigate the effect of pneumococcal vaccination on the outcome of secondary infections. A retrospective cohort study in elderly people over 75 in Taiwan found a 23% reduction in hospitalization in individuals who were vaccinated with both influenza and pneumococcal vaccine compared to influenza-alone vaccinated group, during influenza season [81]. In Japan, two different studies looked for an additive effect of the dual vaccination in patients with chronic respiratory disease. Furumoto *et al.* demonstrated a decrease in infectious acute exacerbation, but not pneumonia, when patients were vaccinated against both pathogens compared to influenza alone [82]. In a cohort study, Sumitani *et al.* showed a decrease in hospitalization between the two groups [83]. However, both these studies were relatively small with approximately 100 people in each group. A large cohort study in the USA in elderly persons with chronic lung disease (controlling for covariates and confounders) showed that influenza vaccination alone reduced hospitalization for pneumonia by 52% and pneumococcal vaccination by 27%, but when both vaccines were administered to subjects the reduction was 63% [84]. Studies by Christenson's group have found that simultaneous administration of PPV23 and influenza vaccine can better prevent excess hospitalizations in the elderly compared to either vaccine [85–87]. The study reported in 2004 involved 124,702 subjects, 72,107 of which received both vaccines, 29,346 only influenza vaccine and 23,249 only the pneumococcal vaccine [85]. A case–control study in Spain also showed that PPV23 vaccine is effective in preventing influenza associated hospitalization with approximately 41%, with the reduction being as high as 81% when both vaccines (pneumococcal and influenza) were used simultaneously [88]. Moreover, a prospective cohort study in the elderly, also in Spain, found a reduction of 26% in all-cause pneumonia hospitalizations compared to unvaccinated subjects [89]. A similar study in France indicated there was no effect of the pneumococcal vaccine on influenza incidence, but there was a decrease in mortality after influenza infection, thus strongly suggesting there is an additive effect of dual vaccination. Moreover, this effect was higher when the influenza vaccine had a better match with the strains circulating that season [90]. It is important to add that the true benefit of influenza vaccine effectiveness in the elderly is being debated in the literature. The vast majority of the clinical studies that concluded

that there was a reduction in influenza, influenza-like illness, hospital admissions, complications and/or deaths in this age group by influenza vaccination had limitations, and very few studies have been designed as randomized controlled trials [91]. Many studies on the efficacy of influenza vaccination in the elderly did not take into account confounding effects such as the frailty status of the subjects [92–94]. Frailty selection bias and monitoring non-specific endpoints such as all-cause mortality (instead of influenza-related mortality) exaggerate vaccine benefits [94]. However, when the available data that looked at influenza vaccine effectiveness against disease were arranged and studied according to a 'biological and conceptual framework', the efficacy against infection under conditions of virus circulation were found to be as high as 60% [95].

Table 1. Overview of vaccine studies measuring protection against secondary infections.

Study type	Age range (years)	Effectiveness (95% CI)	Location	Ref.
Pneumococcal vaccine studies measuring protection against influenza infections				
Case–control	≥18	41% (8–62)	Spain	[88]
Cohort	≥65	9% (-8–23)	France	[90]
Randomized control secondary analysis	<2	45% (14–64)	South Africa	[100]
Ecologic	<5	Not included†	Canada	[98]
Ecologic	<2	Not included†	Australia	[99]
Influenza vaccine studies measuring protection against secondary bacterial infections				
Placebo-controlled	<5	50.9% (not included)	Turkey	[101]
Placebo-controlled (pooled data)	<7	10.9% (1.3–19.7)	Multinational	[105]
IIV-controlled (pooled data)	<6	9.1% (-0.7–17.9)	Multinational	[105]
Case–control	17–35	0.34 (0.29–0.40)‡	USA	[106]

†Do not provide laboratory confirmation for effectiveness of the vaccine.
‡Adjusted odds ratio.
IIV: Inactivated influenza vaccine.

Using computational models, McGarry *et al.* showed the benefits of the introduction of PCV13 over PCV7, in case of a pandemic similar to the one of 2009 [96]. In addition, a study by Simonsen *et al.* [97] showed that the potential lethal synergy of influenza and *S. pneumoniae* can be abrogated in all age groups by vaccinating infants under 24 months of age. They observed a clear association between PCV7 coverage and reduction in influenza-attributable pneumonia hospitalizations. The latter cases were not laboratory confirmed for the presence of influenza virus. Two other studies, one conducted in Canada and one in Australia, also concluded that all-cause pneumonia was reduced following implementation of the pneumococcal vaccine [98,99].

The most convincing data on the impact of pneumococcal vaccine on viral pneumonia is based on a study that was reported by Madhi *et al.* [100]. A double-blind, randomized, placebo-controlled trial conducted in South Africa in over 37,000 infants revealed that the implementation of a 9-valent pneumococcal conjugate vaccine reduced pneumonias associated with any of seven respiratory viruses in children in hospital. A reduction of even 45% in radiological or clinically confirmed influenza-associated pneumonia was noticed in both HIV-infected and -uninfected children [100].

There are also two different vaccines approved for influenza: the live-attenuated influenza vaccine (LAIV) and the inactivated influenza vaccine (IIV), which convey protection in a different way. Are these influenza vaccines able to protect against secondary bacterial infections? A study in children 6–60 months old in daycare (a major risk factor for *otitis media*) showed that IIV is highly effective in reducing all acute *otitis media* (AOM) episodes during the flu season, with a 50.9% decrease in incidence [101]. Other studies with influenza vaccines, both LAIV and IIV, have reported similar but more conserved reductions, with percentages varying from 32 to 43.7% [102–104]. Heikkinen *et al.* pooled data from six placebo-control and two randomized studies and were able to deduce that LAIV vaccination alone was capable in reducing all-cause AOM to a level comparable with the PCV7 vaccine [105]. Given the fact that influenza-associated AOM is frequently caused by *S. pneumoniae*, the aforementioned study provides indirect evidence of the protection of influenza vaccine against secondary bacterial infection. Gathering data from the USA army, where new recruits were vaccinated against influenza, Lee *et al.* reported a strong protective effect against *Streptococcus* incidence (Table 1) [106]. It is important to realize that there are a lot of limitations concerning how each study confirms the causative agent for bacterial pneumonia. Most commonly used is the blood culture which lacks sensitivity, as it can be frequently false-negative, for example, depending on the prior use of antibiotics, or even give false-positive results. More invasive techniques like lung puncture and bronchoalveolar lavage are rarely done. New ELISA assays have higher specificity and urine-Binax assay, which is used to detect pneumococcal C polysaccharide, looks promising, but it can be more trusted in adults than in children due to nasopharyngeal carriage [107–109].

A number of studies have also been conducted in mouse models. Mina *et al.* combined LAIV vaccination with colonization by different strains of *S. pneumoniae* or *S. aureus* and

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looked at the bacterial load in the respiratory tract [110]. Remarkably, they showed that after vaccination with LAIV, there was an increase in bacterial density and duration of colonization in the upper respiratory tract, and this for up to 4 weeks after vaccination. This increase in bacterial colonization and its duration was almost equal if the vaccine was given before or after the colonization and was comparable to that induced by wildtype influenza virus infection. Notably however, at the lower respiratory tract, which is of greater significance for bacterial superinfection due to more severe complications like pneumonia, there was a clear difference between the administration of LAIV and fully replication competent influenza virus. Unlike wildtype influenza virus, the LAIV vaccination did not have an effect on bacterial colonization in the lungs of the mice even when the experiment was repeated with highly virulent strains of pneumococcus. The same group had previously reported that prophylactic LAIV vaccination is sufficient to reduce the density and duration of the pneumococcal load in the nasopharynx if it is administered 10 weeks before colonization with pneumococcus serotype 19F, thus reducing the chance of transmission, an effect that they failed to see with PCV7 [111]. As already stated, Huber *et al.* [50], driven by previous evidence that limiting NA activity reduces the severity of secondary pneumococcal infection and by the fact that some NA is present in current influenza vaccines but almost never measured for its immunogenicity showed that either an NA or an HA vaccine match with the challenge influenza virus limits the complications of secondary bacterial infections in mice [39]. Finally, Sun *et al.* reported that the seasonal LAIV vaccine FluMist was able to protect against a heterologous 2009 H1N1 pandemic influenza strain and against secondary bacterial infection [112]. FluMist-treated mice showed a strong decrease in morbidity and mortality after primary viral or secondary bacterial infection and in both viral and bacterial pulmonary loads compared to unvaccinated mice. The key mediator was found to be mucosal IFN-γ, a key player in the susceptibility to secondary bacterial infections. By limiting the primary viral infection, IFN-γ levels were strongly suppressed during the recovery phase, restoring protection to secondary bacterial infection.

Expert commentary

Viral and bacterial co-infections are a true burden worldwide and cause both clinical and economic problems. Although a lot of progress has been made in the field of vaccinology, there are still obstacles to overcome. After the introduction of PCV7, concerns of serotype replacement were raised as selective pressure on the bacteria promoted the circulation of non-PCV7 covered serotypes. With the use of PCV10 and PCV13 vaccines, serotype replacement in the field may be reduced, but monitoring for such events remains important especially in countries that have not yet implemented the broadly protective pneumococcal vaccines [76]. In addition, we are still far from developing a vaccine for *S. aureus*, a major threat, as multi-drug-resistant strains of this bacterium are now widely spread.

Concerning influenza, vaccines are targeting the induction of antibodies against HA, requiring almost yearly adaptation of the vaccine. Given the proposed role for NA in facilitating bacterial superinfection and its protective potential as a vaccine antigen against influenza, more efforts are needed to standardize and potentially top up existing influenza vaccines with NA antigen.

During pandemics, high percentage of hospitalizations and fatalities are caused by secondary bacterial infection. Although the number of people succumbing to these secondary infections is declining, the prevalence of co-infections is still high. Rapid monitoring of bacterial co-infection, as soon as possible after influenza has been confirmed in patients, should become a routine standard in primary care centers.

It is clear that better insights into the mechanisms used by these viral and bacterial pathogens to facilitate secondary infections are needed. Major efforts have been made in unraveling the cascades leading to bacterial superinfection, identifying key viral and bacterial factors and immune players. The identification of the central role that AM and I-IFN have in the host response mark the advent of new lines of investigation toward prevention and treatment of opportunistic infections after influenza.

It has become evident that vaccination against the bacterial or viral pathogens helps to reduce these secondary infections. However, more studies are needed to elucidate the true benefits. Potentially, simultaneous immunization with both vaccines is the most plausible measure to attempt to reduce co-infection in high-risk groups.

New and potentially broadly protecting influenza vaccines are being developed that could be rapidly implemented in case of a pandemic outbreak. Since pandemic influenza is often associated with bacterial pneumonia, next to their safety and efficacy their indirect effect on bacterial superinfection should also be determined.

Five-year view

Bacterial infection in the lungs of influenza patients occurs frequently and often leads to more severe disease and even lethality. We can control such bacterial infections reasonably well by timely administration of antibiotics. However, multidrug-resistant *S. pneumoniae* and *S. aureus* strains are circulating in the human population.

Given that it is now clear that co-infection with influenza and certain bacterial strains is frequent, and that techniques to detect influenza virus infection are improving, measures could be taken to improve its treatment. For example, systematical screening for influenza virus will help to avoid delay in the initiation of antiviral treatment and to reduce nosocomial transmission, especially in patients admitted in intensive care units.

Major advances have been made in unraveling the mechanism underlying the potency of influenza virus infection to allow bacterial co-infection. With clear pathogenic factors, cellular players and immune components being identified,

efforts can be put in designing specific regiments to prevent and, especially, treat secondary bacterial pneumonia.

After the introduction of pneumococcal vaccines, there is evidence that serotypes that are not covered in these vaccines can replace the targeted serotypes. 'Universal' influenza vaccines based on conserved viral antigens and relying on humoral (e.g., HA-stem, M2e) or T-cell immunity (directed against NP and M1) have reached early stage clinical trials. It will be important to show that such vaccines not only protect against influenza but also still have a beneficial impact on secondary bacterial pathogens, regardless of their serotype.

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Key issues

- Humans continuously live in close contact with a plethora of bacterial and viral species, requiring a stable balance between tolerance and immunity.
- Historical and scientific data underscore the ability of influenza viruses to grant certain bacterial species, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, the power to colonize the respiratory tract, causing severe pneumonia.
- The incidence of bacterial co-infection is drastically increased during influenza pandemics, providing a link with influenza virulence and lack of pre-existing immunity to the circulating influenza strains.
- Complicated pneumonia due to co-infection during seasonal influenza is predominant in adults older than 65 and in children less than 5 years of age; nevertheless, young adults are also at risk during pandemic influenza.
- Co-infection of bacteria and influenza is not restricted to influenza A only, also influenza B has the potential to cause significant excess morbidity and mortality due to bacterial superinfection.
- Influenza virus infection can directly create a niche for bacterial pathogens, by disrupting the epithelial barrier of the respiratory system.
- Immune silencing effects induced by influenza virus infection affects alveolar macrophage function, and induction of type I interferon further endows bacteria the ability to colonize the respiratory tract.
- By limiting primary viral infection and downstream immune responses, influenza vaccines have the ability to decrease secondary bacterial infections.
- There is some evidence that pneumococcal vaccination can reduce influenza-associated hospitalization by directly targeting the bacterial pathogens.

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