



Published in final edited form as:

Discov Med. 2015 January ; 19(102): 33–40.

Why is co-infection with influenza virus and bacteria so difficult to control?

Linda S. Cauley and Anthony T. Vella

Department of Immunology, UCONN Health, Farmington, Connecticut, USA

Abstract

Influenza viruses are genetically labile pathogens which avoid immune detection by constantly changing their coat proteins. Most human infections are caused by mildly pathogenic viruses which rarely cause life-threatening disease in healthy people, but some individuals with a weakened immune system can experience severe complications. Widespread infections with highly pathogenic strains of influenza virus are less common, but have the potential to cause enormous death tolls among healthy adults if infection rates reach pandemic proportions. Increased virulence has been attributed to a variety of factors, including enhanced susceptibility to co-infection with common strains of bacteria. The mechanisms that facilitate dual infection are a major focus of current research, as preventative measures are needed to avert future pandemics

The death rates that are associated with influenza virus infections follow a cyclic pattern which fluctuates with seasonal changes in humidity. Occasional spikes in the numbers of fatalities mark random changes in pathogenicity, which occur when new mutations are introduced by the error-prone viral polymerase. More pronounced changes in pathogenicity mostly occur when two viruses exchange RNA-segments, thus producing a hybrid (reassorted) strain with virulent properties, or when a zoonotic virus first starts replicating in human populations (Greenbaum et al., 2012). Some avian viruses, including variants of the H5N1 and H7N9 strains that recently began causing sporadic infections in humans, are highly pathogenic to people without pre-existing immunity and have the potential to exact substantial death tolls in all age groups including healthy adults (Watanabe et al., 2012). Infections with novel avian viruses have become more frequent during recent years, fueling speculation that another devastating pandemic could soon develop.

The “Spanish flu” of 1918 was the most severe influenza pandemic on record, when 95% of the mortality was attributed to bacterial co-infection (Morens et al., 2008). Several common strains of bacteria were found in sputum samples from cadavers, including *Streptococcus pneumoniae* (Morens et al., 2008). Other pandemics occurred in 1957 and 1968 but the death tolls were less severe and coinfections with *Staphylococcus Aureus* were more prevalent, which may reflect increased use of antibiotics and the emergence of drug-resistant bacteria (ROBERTSON et al., 1958; McCullers, 2006). Preventing infections with

Correspondence should be addressed to: Linda S. Cauley Ph.D., Department of Immunology, UCONN Health., 263 Farmington Avenue, Farmington, CT 06032-1319, Telephone: 860-679-3866, Fax: 860-679-1868, lcauley@UCHC.edu.

Disclosure Statement: Authors have no known conflicts of interest.

methicillin-resistant *Staphylococcus aureus* (MRSA) has become an integral part of hospital care, underscoring the need for systematic control of anti-bacterial medication and providing an impetus in the search for alternative approaches. The mortality rates that were recorded during prior pandemics were so severe that stockpiling antibiotics and vaccines for pneumococcal infections has been touted as a major priority in preparation for future pandemics (Klugman and Madhi, 2007).

Influenza viruses became a major focus of research efforts after the ‘Spanish Flu’ caused an estimated 50 million deaths around the globe (Loo and Gale, Jr., 2007; Luk et al., 2001; Shanks, 2014). Influenza virus-related deaths were not a novel occurrence at that time, but the monumental loss of human life was unprecedented and garnered enormous public support for research efforts which ultimately led to the implementation of government-sponsored vaccination programs in many developed countries, including the US. Understanding why secondary infections with common strains of bacteria often emerge during influenza virus infection is an important frontier for current research efforts. While some progress has been made, much remains to be learned about the way that the virus interferes with immune regulation and undermines protective T cell responses. Susceptibility to bacterial co-infection increases with age and the most severe complications usually arise in people greater than 65 years old. Age-related changes in immunity also reduce the impact of vaccination due to weak B cell responses and declining antibody production (Duraisingham et al., 2013; Kogut et al., 2012).

Bacterial replication and adherence

The human body is host for a diverse constellation of bacterial species, collectively referred to as the microbiota (Hooper et al., 2012). Most of these bacteria are commensal strains which are maintained in mutually beneficial symbiosis with the host and provide many useful functions such as the provision of essential nutrients and immune protection through competition with more pathogenic strains. Sometimes small quantities of harmful bacteria are maintained within the microbiota without severe consequences. Many pathogenic microbes (or pathobionts) colonize exposed tissues such as the skin, where they will be well-positioned to invade the body should the opportunity arise. People that are genetically predisposed to subclinical infections can become ‘carriers’ of pathogenic bacteria, including MRSA. Carriers may be particularly vulnerable to co-infection with bacteria and influenza virus, since disruption of the mucosal barrier is sometimes sufficient for outgrowth of a previously harmless microbe. It is likely that viral infections also attract immune cells to new regions of the body and thus distract resident cell populations from the task of controlling bacterial growth.

Influenza viruses enter the body from the oral or nasal cavities and attach to the membranes of host epithelial cells. These tissues are also frequently sites of bacterial colonization. A pseudo-stratified epithelial layer includes specialized cells which produce mucus and surfactant. The primary function of the mucus is to trap noxious organisms before they adhere to the walls of the airways. Some particulate matter becomes trapped in the mucus and is expelled from the airways by ciliated epithelial cells. Influenza viruses with highly glycosylated coat proteins are more readily expelled from the lungs by the mucociliary

escalator, than poorly glycosylated variants which are more likely to cause deep lung infections and facilitate bacterial entry into the mucosa (McCullers, 2006). Some bacteria use mucinases to degrade the host proteins, while others take advantage of virus-induced damage in the lungs. Neuraminidase is a viral coat protein with enzymatic activity which is required for viral replication (Nayak et al., 2009). Bacterial colonization in the lungs increases when the viral neuraminidase disrupts a layer of sialylated mucins on the host cells, which act as decoy receptors for invading microbes, thus exposing cryptic sites for bacterial adherence (Peltola et al., 2005). Large quantities of sloughed host cells and increased mucus production in the airways also provide nutrients for bacterial growth (Siegel et al., 2014). Damage to the epithelial layer uncovers the basement membrane and reveals extracellular matrix proteins which provide additional sites for bacterial attachment (McCullers, 2006).

Cytokines cause immune damage and promote wound healing in the lungs

Enhanced pathogenesis during bacterial superinfection is the result of complex interactions between immune cells which elicit synergistic inflammatory responses and disrupt the integrity of the epithelial barrier. Current data, mostly derived from animal-models, point to multifactorial mechanisms of co-pathogenesis including strain-specific virulence factors of the virus. Some variations in disease severity have been linked to viral products such as the cytotoxin encoded by PB1-F2 (Alymova et al., 2014; McAuley et al., 2007), which causes host cell death and contributes to a 'cytokine storm' during infection with some pandemic and avian strains (de Jong et al., 2006; Conenello et al., 2007). Cytokines belong to large families of soluble molecules with pro- or anti-inflammatory properties, including interleukins (IL) which are primarily released by white blood cells and orchestrate the response to infection. In the lungs a cytokine storm can lead to irreparable tissue destruction as proinflammatory cytokines damage the alveoli. Defects in immune activation have also been attributed to the virally-encoded non-structural protein 1 (NS1) (Fernandez-Sesma, 2007), which interferes with signaling molecules and nuclear translocation (Jia et al., 2010).

Human deaths occur in two waves during infections with highly pathogenic strains of influenza virus. The 'cytokine storm' is responsible for many early deaths, resulting from fever, anorexia and severe lung damage leading to hypoxia. Loss of barrier function in the lower respiratory tract can facilitate acute-respiratory distress syndrome (ARDS). Neutrophils and macrophages are scavenger cells that engulf and destroy noxious organisms, including extracellular bacteria and debris from infected cells. During respiratory virus infection, severe damage to the lungs is accentuated by early and excessive infiltration of myeloid cells, including mononuclear phagocytes which kill alveolar epithelial cells by releasing a lytic molecule called TRAIL (Brincks et al., 2008). Macrophages play a key role in mediating inflammation by releasing large quantities of pro-inflammatory cytokines after they encounter necrotic cells and other debris. The proinflammatory cytokines include interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) are required for immune protection, but can become toxic when released in large amounts. Neutrophils are recruited into the lungs by IL-8 and may be important for controlling bacterial replication, however, an excessive neutrophil response is associated with a poor prognosis for ARDS susceptible patients (Tate et al., 2011). Symptoms of ARDS include diffuse

alveolar damage with accumulations of dead cells in protein-rich fluid which inhibits oxygen exchange.

The bacteria that are most commonly found in human sputum during influenza virus infection include strains that colonize the nasal passages of many healthy people (Ballinger and Standiford, 2010; Iverson et al., 2011) including *Staph. Aureus* (Metersky et al., 2012; McCullers, 2006) and group A streptococcus (GAS), which is a gram-positive bacteria that can also colonize human tonsils. Symptoms of infection with *Strep. pneumoniae* in children often include *otitis media*, while data from animal models suggest that influenza virus infection assists bacterial migration from the nasopharynx to the middle ear (Wren et al., 2014). Bacterial co-infection often becomes evident as the clinical symptoms of viral infection begin to improve and some patients experience recrudescence of fever, dyspnea and cough with milky sputum.

A variety of factors contribute to the synergy that is observed during viral and bacterial coinfection, including suppression of innate immune cells even during a robust cytokine response. Interferons are a family of proinflammatory cytokines with antiviral properties (Bonjardim et al., 2009). In animal models increased susceptibility to *Strep. pneumoniae* was linked to high concentrations of type I Interferon (IFN $\alpha\beta$) and negative-regulation of $\gamma\delta$ T cells, which are an important source of interleukin-17 (IL-17) and recruit neutrophils into the lungs (Li et al., 2012). Reduced numbers of neutrophils are often found in the lungs during the recovery from influenza virus infection, partly due to prolonged desensitization of Toll-like receptors (TLR) which are involved in recognition of bacteria (Didierlaurent et al., 2008). Some data from animal models indicate that neutrophils do not play a major role in immunity during early bacterial infection, but may become more important as the infection progresses (Sun and Metzger, 2008).

Cells of the adaptive immune system help control subclinical bacterial infections in healthy people, including specialized CD4⁺ T cells which make IL-17 (called Th17 cells) (Cohen et al., 2011). Development of Th17 cells requires exposure to transforming growth factor- β (TGF- β) and IL-6 (Yang et al., 2008; Bettelli et al., 2006), which are expressed in the lungs during influenza virus infection (Schultz-Cherry and Hinshaw, 1996; Dienz et al., 2012). These cytokines induce mildly pathogenic Th17 cells which make suppressive cytokines (i.e. IL-10 and TGF- β) (McGeachy et al., 2007), as well as interleukin-21 (IL-21) which promotes auto-proliferation. The pathogenicity of the Th17 response increases when IL-1 and IL-23 are present, leading to reduced IL-10 and IL-21 expression (Cua et al., 2003). Although IL-6 is important for immunity to influenza virus infection (Dienz et al., 2012) excessive quantities can prove detrimental by reducing the numbers of regulatory T cells which synthesize suppressive cytokines such as IL-10 and TGF β (Rincon, 2012).

As the viral titers decline, an anti-inflammatory state is induced by suppressive factors such as IL-10, which is required to restore homeostasis but may also increase susceptibility to bacterial co-infection (Sun et al., 2009). Macrophages change their cytokine response after consuming host cells that are undergoing controlled cell death (apoptosis) by producing anti-inflammatory cytokines (i.e. TGF β and IL-10). Phagocytosis must occur quickly to prevent the dying cells from undergoing secondary necrosis, when intracellular components are

released and expose danger associated molecular patterns (DAMPs) which rekindle inflammation. During the recovery from influenza virus infection, macrophage activity is suppressed through inhibitory receptors such as CD200 (Snelgrove et al., 2008) and insensitivity to bacterial products which are recognized by Toll-like receptors (Didierlaurent et al., 2008). Influenza virus infection induces high concentrations of T cell-derived interferon (i.e. IFN- γ), which has been linked to slow clearance of microbial infections by alveolar macrophages due to reduced expression of scavenger receptors that are used to engulf particulate matter including extracellular bacteria and dying cells (Sun and Metzger, 2008). Other cytokines promote wound healing, including interleukin-22 (IL-22) which reduced lung damage during co-infection with *Strep. pneumoniae* (Ivanov et al., 2013). Some injured host cells express unusual surface proteins during wound healing (Puchelle et al., 2006), thus exposing additional cryptic sites that can be used by bacteria for adherence to the walls of the airways where they can resist the action of the mucociliary escalator (McCullers, 2006).

TGF- β is released from the host cells as a latent complex which is normally activated by extracellular proteases (Jenkins, 2008), but can also be activated by the viral neuraminidase (Schultz-Cherry and Hinshaw, 1996), making some seasonal strains of influenza virus less pathogenic than newer avian strains (Carlson et al., 2010). New data suggest that viral neuraminidase may promote bacterial growth in the lungs by altering the concentrations of TGF β . Some bacteria use integrins which are regulated by TGF β for adherence (Li et al., 2015). In addition TGF β is an important regulator of the adaptive immune response, which reduces the numbers of activated CTLs in the lungs and induces regulatory CD4 T cells (Treg) that make IL-10 (Berod et al., 2012; Brincks et al., 2013). In animal models, reduced numbers of Th17 cells were linked to severe co-infection with *Strep. Pneumoniae* by a mechanism that involved IL-10 (van der Sluijs et al., 2004; McKinsty et al., 2009; Sun et al., 2009). In contrast, studies of human cells suggest that antigen-specific Tregs utilize CTLA-4 and PDL-1 (i.e. surface receptors involved in negative regulation of T cells) to suppress the Th17 response during control of commensal *Strep. pneumoniae* (Pido-Lopez et al., 2011). Similarly *Klebsiella pneumoniae* is a gram-negative bacteria that can be found in the lungs during influenza virus infection and is controlled by Th17 cells (Chen et al., 2011) suggesting that related mechanisms may be involved in susceptibility to co-infections with a variety of bacteria.

Interleukin-1 (IL-1) is a pyrogenic cytokine which is expressed in the host cells during influenza virus infection. Activation requires multiprotein complexes known as 'inflammasomes' which are an important trigger of the immune response (Ichinohe et al., 2009). Influenza virus infected mice became highly susceptible to infection with *Staph. Aureus* when Th17 responses were suppressed by a mechanism involving reduced IL-1 β expression (Robinson et al., 2013; Robinson et al., 2014). Suppressed Th17 responses also promoted colonization with *Staph. Aureus* by reducing the concentrations of an antimicrobial peptide (i.e. neutrophil gelatinase-associated lipocalin) that limits bacterial growth (Robinson et al., 2014). Other studies linked enhanced susceptibility to co infection with *Staph. Aureus* with an impaired response by natural killer (NK) cells and weak antibody-dependent cellular cytotoxicity (ADCC) (Small et al., 2010).

The impact of dual infection is not limited to enhanced bacterial growth, but also impacts immunity to the virus. Animal models have consistently shown increased viral titers during bacterial superinfections and delayed clearance. Studies suggest that *Staph. Aureus* produces proteases which cleave hemagglutinin, thus producing fusion-competent virus particles with enhanced infectivity (Tashiro et al., 1987). Symptoms of viral replication were also exacerbated during coinfection with methicillin-resistant *Staph. Aureus* (MRSA), when IL-13 suppressed the interferon response (Rynda-Apple et al., 2014). Reduced numbers of NK cells, which have lytic activity, could also delay viral control during Infection with *Staph. Aureus*. Similarly suppressed anti-viral CTL responses and cytokine expression were observed in the lungs during co-infection with *Strep. pneumoniae* (Blevins et al., 2014). Further defects in immune activation occur when dendritic cells become infected with influenza virus, leading to suboptimal maturation and increased apoptosis (Bender et al., 1998; Fernandez-Sesma et al., 2006). As ‘mature’ dendritic cells are essential for T cell priming, suboptimal activation may suppress immunity to both bacterial and viral infections thereby permitting outgrowth of pathogens that would normally be suppressed. Collectively these concepts are presented in Figure 1.

Preparations for a new pandemic – a case for stockpiling anti-viral medications and antibiotics

The drugs that are currently approved by the Food and Drug Administration (FDA) for treating influenza virus infections in humans target two viral genes. The most widely used medications are neuraminidase inhibitors (e.g. *Oseltamivir* and *Zanamivir*) which promote early recovery by reducing the amount of virus that is released into the lungs (Nayak et al., 2009). Other drugs, such as such as *Amantadine* and *Rimantadine*, block M2 proton channels which are required for virus to enter the cytoplasm of the host cells (Moorthy et al., 2014). The available drugs are becoming less efficacious as resistant viruses emerge (van der Vries et al., 2011; Thorlund et al., 2011) and the search for new pharmaceuticals must be a top priority in preparations for managing future influenza pandemics. New strategies that are under consideration include inhibitors of the viral polymerase (Furuta et al., 2013), as well as enzymes which impede infection by cleaving sialic acid residues from the surface of the host epithelial cells (Marjuki et al., 2014). Data from animal models suggest that treatment with Doxycycline (an inhibitor of matrix metalloproteinases (MMPs) can reduce lung injury, although viral replication and weight loss were not changed (Ng et al., 2012). Similarly, ARDS patients experienced reduced lung injury during treatment with a neutrophil elastase inhibitor (Iwata et al., 2010). As drug-resistant bacteria become more prevalent the new treatments must target ubiquitous strains such as MRSA. Some progress has been made as recent studies suggest that synthetic antibiotics (Pasberg-Gauhl, 2014), or plant extracts such as flavonoids (Wang et al., 2014) can augment bacterial clearance when used in combination with conventional remedies. Similar responses were observed when Chevalone E (an extract from marine animals) was used in combination with antibiotics (Prompanya et al., 2014).

Concluding remarks

For many years vaccines have been produced from inactivated components of ubiquitous viruses which are used to induce high concentrations of serum antibodies (Baez et al., 1980; Cox et al., 2004). These vaccines are not optimal because they do not include pandemic strains and offer little protection against viruses with novel coat proteins (Luke and Subbarao, 2006; Vardavas et al., 2010). Increasingly frequent of infections with new strains of avian influenza virus (including H5N1 and H7N9) have been reported during recent years (Watanabe et al., 2012; Belser et al., 2011) highlighting the possibility that another pandemic could occur in the not too distant future. Ideally preparations for such an event will include the development of new classes of vaccines for both viral and pneumococcal infections, which target drug-resistant strains (Tripp and Tompkins, 2014; Hoft et al., 2011; Klugman and Madhi, 2007; Chung and Huh, 2015).

The advent of new medications and inventions that improve hygiene are likely to reduce the severity of a future influenza pandemic. Though such improvements are cause for optimism, other aspects of modern life are cause for concern as widespread access to high speed transportation and high density animal husbandry can facilitate the transmission of highly contagious pathogens. Drug-resistant viruses and bacteria are becoming increasingly commonplace with widespread use (and abuse) of antibiotics or anti-viral medications, including neuraminidase inhibitors (Thorlund et al., 2011). Complacency could have dire consequences, since relatively low death rates (1–2%) could be sufficient to cause massive numbers of casualties on a global scale. Animal models faithfully recapitulate many salient features of bacteria co-infections, thus revealing important insights into the mechanisms that promote synergy between different organisms, but much more remains to be learned before effective treatment and prevention strategies will be ready to provide immunity on a global scale. Promising research initiatives include high-throughput RNA-sequencing and computer modeling to analyze cytokine signatures in patients with community-acquired infections.

Acknowledgments

Funding for this article was provided by National Institutes of Health grant PO1 AI056172.

Reference List

- Alymova IV, Samarasinghe A, Vogel P, Green AM, Weinlich R, McCullers JA. A novel cytotoxic sequence contributes to influenza A viral protein PB1-F2 pathogenicity and predisposition to secondary bacterial infection. *J Virol.* 2014; 88(1):503–515. [PubMed: 24173220]
- Baez M, Palese P, Kilbourne ED. Gene composition of high-yielding influenza vaccine strains obtained by recombination. *J Infect Dis.* 1980; 141(3):362–365. [PubMed: 7365284]
- Ballinger MN, Standiford TJ. Postinfluenza bacterial pneumonia: host defenses gone awry. *J Interferon Cytokine Res.* 2010; 30(9):643–652. [PubMed: 20726789]
- Belser JA, Zeng H, Katz JM, Tumpey TM. Infection with highly pathogenic H7 influenza viruses results in an attenuated proinflammatory cytokine and chemokine response early after infection. *J Infect Dis.* 2011; 203(1):40–48. [PubMed: 21148495]
- Bender A, Albert M, Reddy A, Feldman M, Sauter B, Kaplan G, Hellman W, Bhardwaj N. The distinctive features of influenza virus infection of dendritic cells. *Immunobiology.* 1998; 198(5): 552–567. [PubMed: 9561373]

- Berod L, Puttur F, Huehn J, Sparwasser T. Tregs in infection and vaccinology: heroes or traitors- Microb Biotechnol. 2012; 5(2):260–269. [PubMed: 21951341]
- Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006; 441(7090):235–238. [PubMed: 16648838]
- Blevins LK, Wren JT, Holbrook BC, Hayward SL, Swords WE, Parks GD, Alexander-Miller MA. Coinfection with *Streptococcus pneumoniae* Negatively Modulates the Size and Composition of the Ongoing Influenza-Specific CD8+ T Cell Response. J Immunol. 2014
- Bonjardim CA, Ferreira PC, Kroon EG. Interferons: signaling, antiviral and viral evasion. Immunol Lett. 2009; 122(1):1–11. [PubMed: 19059436]
- Brincks EL, Katewa A, Kucaba TA, Griffith TS, Legge KL. CD8 T cells utilize TRAIL to control influenza virus infection. J Immunol. 2008; 181(7):4918–4925. [PubMed: 18802095]
- Brincks EL, Roberts AD, Cookenham T, Sell S, Kohlmeier JE, Blackman MA, Woodland DL. Antigen-specific memory regulatory CD4+Foxp3+ T cells control memory responses to influenza virus infection. J Immunol. 2013; 190(7):3438–3446. [PubMed: 23467933]
- Carlson CM, Turpin EA, Moser LA, O'Brien KB, Cline TD, Jones JC, Tumpey TM, Katz JM, Kelley LA, Gaudie J, Schultz-Cherry S. Transforming growth factor-beta: activation by neuraminidase and role in highly pathogenic H5N1 influenza pathogenesis. PLoS Pathog. 2010; 6(10)
- Chen K, McAleer JP, Lin Y, Paterson DL, Zheng M, Alcorn JF, Weaver CT, Kolls JK. Th17 cells mediate clade-specific, serotype-independent mucosal immunity. Immunity. 2011; 35(6):997–1009. [PubMed: 22195749]
- Chung DR, Huh K. Novel pandemic influenza A (H1N1) and community-associated methicillin-resistant *Staphylococcus aureus* pneumonia. Expert Rev Anti Infect Ther. 2015; 13(2):197–207. [PubMed: 25578884]
- Cohen JM, Khandavilli S, Camberlein E, Hyams C, Baxendale HE, Brown JS. Protective contributions against invasive *Streptococcus pneumoniae* pneumonia of antibody and Th17-cell responses to nasopharyngeal colonisation. PLoS One. 2011; 6(10):e25558. [PubMed: 22003400]
- Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathog. 2007; 3(10):1414–1421. [PubMed: 17922571]
- Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. Scand J Immunol. 2004; 59(1):1–15. [PubMed: 14723616]
- Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature. 2003; 421(6924):744–748. [PubMed: 12610626]
- de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Van Vinh CN, Khanh TH, Dong VC, Qui PT, Van Cam B, Ha dQ, Guan Y, Peiris JS, Chinh NT, Hien TT, Farrar J. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med. 2006; 12(10):1203–1207. [PubMed: 16964257]
- Didierlaurent A, Goulding J, Patel S, Snelgrove R, Low L, Bebie M, Lawrence T, van Rijt LS, Lambrecht BN, Sirard JC, Hussell T. Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. J Exp Med. 2008; 205(2):323–329. [PubMed: 18227219]
- Dienz O, Rud JG, Eaton SM, Lanthier PA, Burg E, Drew A, Bunn J, Suratt BT, Haynes L, Rincon M. Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. Mucosal Immunol. 2012
- Duraisingham SS, Roupheal N, Cavanagh MM, Nakaya HI, Goronzy JJ, Pulendran B. Systems biology of vaccination in the elderly. Curr Top Microbiol Immunol. 2013; 363:117–142. [PubMed: 22903566]
- Fernandez-Sesma A. The influenza virus NS1 protein: inhibitor of innate and adaptive immunity. Infect Disord Drug Targets. 2007; 7(4):336–343. [PubMed: 18220965]

- Fernandez-Sesma A, Marukian S, Ebersole BJ, Kaminski D, Park MS, Yuen T, Sealton SC, Garcia-Sastre A, Moran TM. Influenza virus evades innate and adaptive immunity via the NS1 protein. *J Virol*. 2006; 80(13):6295–6304. [PubMed: 16775317]
- Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. *Antiviral Res*. 2013; 100(2):446–454. [PubMed: 24084488]
- Greenbaum BD, Li OT, Poon LL, Levine AJ, Rabadan R. Viral reassortment as an information exchange between viral segments. *Proc Natl Acad Sci U S A*. 2012; 109(9):3341–3346. [PubMed: 22331898]
- Hoft DF, Babusis E, Worku S, Spencer CT, Lottenbach K, Truscott SM, Abate G, Sakala IG, Edwards KM, Creech CB, Gerber MA, Bernstein DI, Newman F, Graham I, Anderson EL, Belshe RB. Live and inactivated influenza vaccines induce similar humoral responses, but only live vaccines induce diverse T-cell responses in young children. *J Infect Dis*. 2011; 204(6):845–853. [PubMed: 21846636]
- Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012; 336(6086):1268–1273. [PubMed: 22674334]
- Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J Exp Med*. 2009; 206(1):79–87. [PubMed: 19139171]
- Ivanov S, Renneson J, Fontaine J, Barthelemy A, Paget C, Fernandez EM, Blanc F, De TC, Van ML, Dumoutier L, Huerre MR, Eberl G, Si-Tahar M, Gosset P, Renaud JC, Sirard JC, Faveeuw C, Trottein F. Interleukin-22 reduces lung inflammation during influenza A virus infection and protects against secondary bacterial infection. *J Virol*. 2013; 87(12):6911–6924. [PubMed: 23596287]
- Iverson AR, Boyd KL, McAuley JL, Plano LR, Hart ME, McCullers JA. Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *J Infect Dis*. 2011; 203(6):880–888. [PubMed: 21278211]
- Iwata K, Doi A, Ohji G, Oka H, Oba Y, Takimoto K, Igarashi W, Gremillion DH, Shimada T. Effect of neutrophil elastase inhibitor (sivelestat sodium) in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): a systematic review and meta-analysis. *Intern Med*. 2010; 49(22):2423–2432. [PubMed: 21088343]
- Jenkins G. The role of proteases in transforming growth factor-beta activation. *Int J Biochem Cell Biol*. 2008; 40(6–7):1068–1078. [PubMed: 18243766]
- Jia D, Rahbar R, Chan RW, Lee SM, Chan MC, Wang BX, Baker DP, Sun B, Peiris JS, Nicholls JM, Fish EN. Influenza Virus Non-Structural Protein 1 (NS1) Disrupts Interferon Signaling. *PLoS One*. 2010; 5(11):e13927. [PubMed: 21085662]
- Klugman KP, Madhi SA. Pneumococcal vaccines and flu preparedness. *Science*. 2007; 316(5821):49–50. [PubMed: 17412937]
- Kogut I, Scholz JL, Cancro MP, Cambier JC. B cell maintenance and function in aging. *Semin Immunol*. 2012; 24(5):342–349. [PubMed: 22560930]
- Li N, Ren A, Wang X, Fan X, Zhao Y, Gao GF, Cleary P, Wang B. Influenza viral neuraminidase primes bacterial coinfection through TGF-beta-mediated expression of host cell receptors. *Proc Natl Acad Sci U S A*. 2015; 112(1):238–243. [PubMed: 25535343]
- Li W, Moltedo B, Moran TM. Type I interferon induction during influenza virus infection increases susceptibility to secondary *Streptococcus pneumoniae* infection by negative regulation of gammadelta T cells. *J Virol*. 2012; 86(22):12304–12312. [PubMed: 22951826]
- Loo YM, Gale M Jr. Influenza: fatal immunity and the 1918 virus. *Nature*. 2007; 445(7125):267–268. [PubMed: 17230179]
- Luk J, Gross P, Thompson WW. Observations on mortality during the 1918 influenza pandemic. *Clin Infect Dis*. 2001; 33(8):1375–1378. [PubMed: 11565078]
- Luke CJ, Subbarao K. Vaccines for pandemic influenza. *Emerg Infect Dis*. 2006; 12(1):66–72. [PubMed: 16494720]
- Marjuki H, Mishin VP, Chesnokov AP, De La Cruz JA, Fry AM, Villanueva J, Gubareva LV. An investigational antiviral drug, DAS181, effectively inhibits replication of zoonotic influenza A virus subtype H7N9 and protects mice from lethality. *J Infect Dis*. 2014; 210(3):435–440. [PubMed: 24569063]

- McAuley JL, Hornung F, Boyd KL, Smith AM, McKeon R, Bennink J, Yewdell JW, McCullers JA. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. *Cell Host Microbe*. 2007; 2(4):240–249. [PubMed: 18005742]
- McCullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev*. 2006; 19(3):571–582. [PubMed: 16847087]
- McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol*. 2007; 8(12):1390–1397. [PubMed: 17994024]
- McKinstry KK, Strutt TM, Buck A, Curtis JD, Dibble JP, Huston G, Tighe M, Hamada H, Sell S, Dutton RW, Swain SL. IL-10 deficiency unleashes an influenza-specific Th17 response and enhances survival against high-dose challenge. *J Immunol*. 2009; 182(12):7353–7363. [PubMed: 19494257]
- Metersky ML, Masterton RG, Lode H, File TM Jr, Babinchak T. Epidemiology, microbiology, and treatment considerations for bacterial pneumonia complicating influenza. *Int J Infect Dis*. 2012
- Moorthy NS, Poongavanam V, Pratheepa V. Viral M2 Ion Channel Protein: A Promising Target for Anti-influenza Drug Discovery. *Mini Rev Med Chem*. 2014; 14(10):819–830. [PubMed: 25342196]
- Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis*. 2008; 198(7):962–970. [PubMed: 18710327]
- Nayak DP, Balogun RA, Yamada H, Zhou ZH, Barman S. Influenza virus morphogenesis and budding. *Virus Res*. 2009; 143(2):147–161. [PubMed: 19481124]
- Ng HH, Narasaraju T, Phoon MC, Sim MK, Seet JE, Chow VT. Doxycycline treatment attenuates acute lung injury in mice infected with virulent influenza H3N2 virus: involvement of matrix metalloproteinases. *Exp Mol Pathol*. 2012; 92(3):287–295. [PubMed: 22421441]
- Pasberg-Gaahl C. A need for new generation antibiotics against MRSA resistant bacteria. *Drug Discov Today Technol*. 2014; 11:109–116. [PubMed: 24847660]
- Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. *J Infect Dis*. 2005; 192(2):249–257. [PubMed: 15962219]
- Pido-Lopez J, Kwok WW, Mitchell TJ, Heyderman RS, Williams NA. Acquisition of pneumococci specific effector and regulatory Cd4+ T cells localising within human upper respiratory-tract mucosal lymphoid tissue. *PLoS Pathog*. 2011; 7(12):e1002396. [PubMed: 22144893]
- Prompanya C, Dethoup T, Bessa LJ, Pinto MM, Gales L, Costa PM, Silva AM, Kijjoa A. New Isocoumarin Derivatives and Meroterpenoids from the Marine Sponge-Associated Fungus *Aspergillus similanensis* sp. nov. *KUFA 0013*. *Mar Drugs*. 2014; 12(10):5160–5173. [PubMed: 25317534]
- Puchelle E, Zahm JM, Tournier JM, Coraux C. Airway epithelial repair, regeneration, and remodeling after injury in chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2006; 3(8):726–733. [PubMed: 17065381]
- Rincon M. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. *Trends Immunol*. 2012
- Robertson L, Caley JP, Moore J. Importance of *Staphylococcus aureus* in pneumonia in the 1957 epidemic of influenza A. *Lancet*. 1958; 2(7040):233–236. [PubMed: 13564806]
- Robinson KM, Choi SM, McHugh KJ, Mandalapu S, Enelow RI, Kolls JK, Alcorn JF. Influenza A exacerbates *Staphylococcus aureus* pneumonia by attenuating IL-1beta production in mice. *J Immunol*. 2013; 191(10):5153–5159. [PubMed: 24089191]
- Robinson KM, McHugh KJ, Mandalapu S, Clay ME, Lee B, Scheller EV, Enelow RI, Chan YR, Kolls JK, Alcorn JF. Influenza A virus exacerbates *Staphylococcus aureus* pneumonia in mice by attenuating antimicrobial peptide production. *J Infect Dis*. 2014; 209(6):865–875. [PubMed: 24072844]
- Rynda-Apple A, Harmsen A, Erickson AS, Larson K, Morton RV, Richert LE, Harmsen AG. Regulation of IFN-gamma by IL-13 dictates susceptibility to secondary postinfluenza MRSA pneumonia. *Eur J Immunol*. 2014

- Schultz-Cherry S, Hinshaw VS. Influenza virus neuraminidase activates latent transforming growth factor beta. *J Virol*. 1996; 70(12):8624–8629. [PubMed: 8970987]
- Shanks GD. How World War I changed global attitudes to war and infectious diseases. *Lancet*. 2014; 384(9955):1699–1707. [PubMed: 25441200]
- Siegel SJ, Roche AM, Weiser JN. Influenza promotes pneumococcal growth during coinfection by providing host sialylated substrates as a nutrient source. *Cell Host Microbe*. 2014; 16(1):55–67. [PubMed: 25011108]
- Small CL, Shaler CR, McCormick S, Jeyanathan M, Damjanovic D, Brown EG, Arck P, Jordana M, Kaushic C, Ashkar AA, Xing Z. Influenza infection leads to increased susceptibility to subsequent bacterial superinfection by impairing NK cell responses in the lung. *J Immunol*. 2010; 184(4): 2048–2056. [PubMed: 20083661]
- Snelgrove RJ, Goulding J, Didierlaurent AM, Lyonga D, Vekaria S, Edwards L, Gwyer E, Sedgwick JD, Barclay AN, Hussels T. A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. *Nat Immunol*. 2008; 9(9):1074–1083. [PubMed: 18660812]
- Sun J, Madan R, Karp CL, Braciale TJ. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat Med*. 2009; 15(3):277–284. [PubMed: 19234462]
- Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. *Nat Med*. 2008; 14(5):558–564. [PubMed: 18438414]
- Tashiro M, Ciborowski P, Klenk HD, Pulverer G, Rott R. Role of Staphylococcus protease in the development of influenza pneumonia. *Nature*. 1987; 325(6104):536–537. [PubMed: 3543690]
- Tate MD, Ioannidis LJ, Croker B, Brown LE, Brooks AG, Reading PC. The role of neutrophils during mild and severe influenza virus infections of mice. *PLoS One*. 2011; 6(3):e17618. [PubMed: 21423798]
- Thorlund K, Awad T, Boivin G, Thabane L. Systematic review of influenza resistance to the neuraminidase inhibitors. *BMC Infect Dis*. 2011; 11:134. [PubMed: 21592407]
- Tripp RA, Tompkins SM. Virus-Vectored Influenza Virus Vaccines. *Viruses*. 2014; 6(8):3055–3079. [PubMed: 25105278]
- van der Sluijs KF, van Elden LJ, Nijhuis M, Schuurman R, Pater JM, Florquin S, Goldman M, Jansen HM, Lutter R, van der Poll T. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. *J Immunol*. 2004; 172(12):7603–7609. [PubMed: 15187140]
- van der Vries E, Schutten M, Boucher CA. The potential for multidrug-resistant influenza. *Curr Opin Infect Dis*. 2011; 24(6):599–604. [PubMed: 22001947]
- Vardavas R, Breban R, Blower S. A universal long-term flu vaccine may not prevent severe epidemics. *BMC Res Notes*. 2010; 3(1):92. [PubMed: 20367882]
- Wang SY, Sun ZL, Liu T, Gibbons S, Zhang WJ, Qing M. Flavonoids from *Sophora moorcroftiana* and their synergistic antibacterial effects on MRSA. *Phytother Res*. 2014; 28(7):1071–1076. [PubMed: 24338874]
- Watanabe Y, Ibrahim MS, Suzuki Y, Ikuta K. The changing nature of avian influenza A virus (H5N1). *Trends Microbiol*. 2012; 20(1):11–20. [PubMed: 22153752]
- Wren JT, Blevins LK, Pang B, King LB, Perez AC, Murrah KA, Reimche JL, Alexander-Miller MA, Swords WE. Influenza a virus alters pneumococcal nasal colonization and middle ear infection independently of phase variation. *Infect Immun*. 2014; 82(11):4802–4812. [PubMed: 25156728]
- Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS, Tian Q, Jetten AM, Dong C. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity*. 2008; 28(1): 29–39. [PubMed: 18164222]

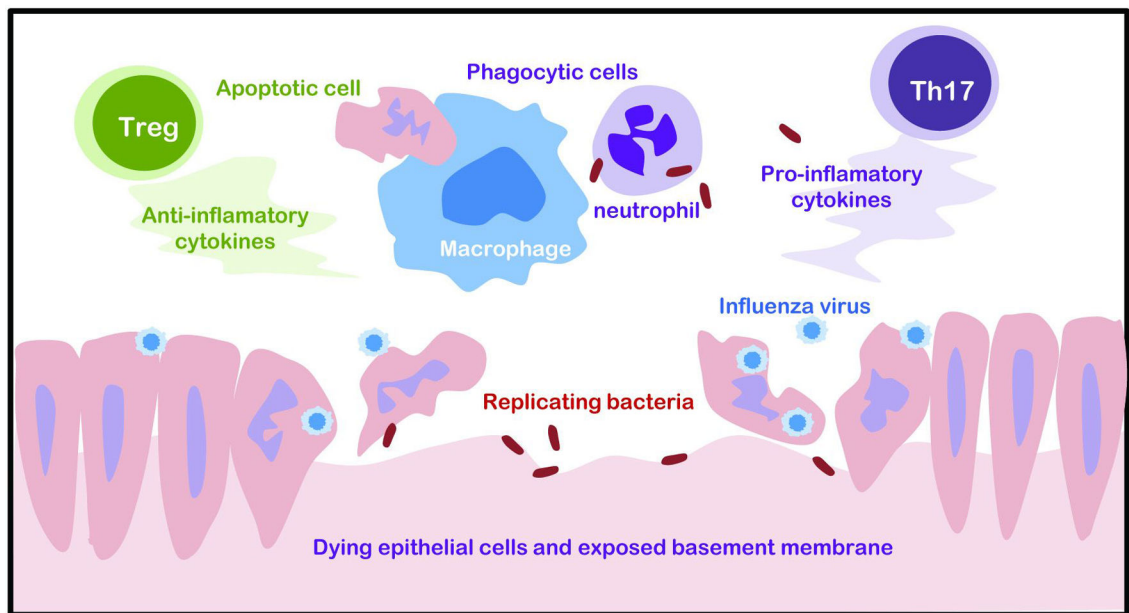
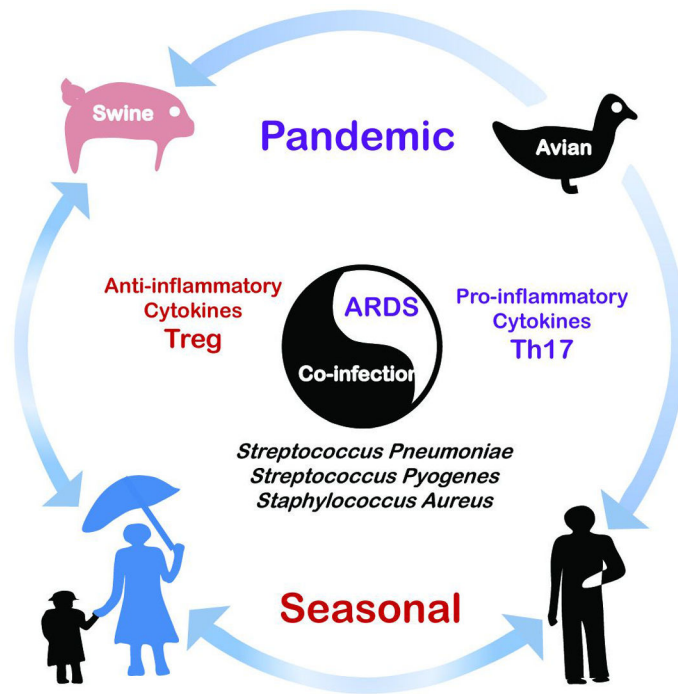


Figure 1. Influenza virus infection damages the lungs and promotes bacterial growth by changing the cytokine response. Severe damage to the alveoli usually occurs during infection with highly pathogenic strains of influenza virus, including reassorted strains that are adapted for replication in birds or swine. Bacterial growth often begins when the virus disrupts the epithelial barrier and induces cytokines which reduce the numbers of Th17 cells in the lungs. Immune protection is further compromised by inefficient phagocytosis by macrophages and suppressive cytokines which are released by regulatory T cells. Many current antibiotics and

anti-viral medications are not sufficient to protect against drug-resistant strains of influenza virus or bacteria such as MRSA.