

Human Influenza Virus Infections

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Abstract

Seasonal and pandemic influenza are the two faces of respiratory infections caused by influenza viruses in humans. As seasonal influenza occurs on an annual basis, the circulating virus strains are closely monitored and a yearly updated vaccination is provided, especially to identified risk populations. Nonetheless, influenza virus infection may result in pneumonia and acute respiratory failure, frequently complicated by bacterial coinfection. Pandemics are, in contrary, unexpected rare events related to the emergence of a reassorted human-pathogenic influenza A virus (IAV) strains that often causes increased morbidity and spreads extremely rapidly in the immunologically naive human population, with huge clinical and economic impact. Accordingly, particular efforts are made to advance our knowledge on the disease biology and pathology and recent studies have brought new insights into IAV adaptation mechanisms to the human host, as well as into the key players in disease pathogenesis on the host side. Current antiviral strategies are only efficient at the early stages of the disease and are challenged by the genomic instability of the virus, highlighting the need for novel antiviral therapies targeting the pulmonary host response to improve viral clearance, reduce the risk of bacterial coinfection, and prevent or attenuate acute lung injury. This review article summarizes our current knowledge on the molecular basis of influenza infection and disease progression, the key players in pathogenesis driving severe disease and progression to lung failure, as well as available and envisioned prevention and treatment strategies against influenza virus infection.

Keywords

- ▶ influenza virus
- ▶ seasonal
- ▶ pandemic
- ▶ pneumonia
- ▶ lung injury
- ▶ ARDS
- ▶ vaccination
- ▶ antiviral therapy

Influenza viruses cause annually recurrent respiratory disease in humans with significant impact on human health as well as on economy. Although influenza B viruses are almost exclusively found in humans, influenza A viruses (IAVs) circulate in the human population as an annually recurring epidemic disease, and emerge from a huge zoonotic reservoir. Characterized by their ability to rapidly acquire adaptive mutations in a process called *antigenic drift*, IAV gradually evade the human immune response.^{1,2} Additionally, the special arrangement of viral genetic information on multiple RNA segments allows for mixing genetic information of different IAV strains giving rise to novel, gene-reassorted virus strains, a process called *antigenic shift* that periodically results in emergence of virus strains with largely altered

characteristics and the ability to infect immunologically naive humans with increased pathogenicity during pandemic outbreaks. In this regard, special concern is raised toward zoonotic viruses circulating in birds as well as in swine populations that display high mortality rates when introduced into the human host. So far, these viruses, H7N9 or H5N1 “bird flu” viruses, only have a low replicative potential in humans and rarely transmit between humans,^{3,4} but are feared to adapt to highly efficient human-to-human transmission and are therefore closely monitored. Although influenza viruses usually cause moderate respiratory illness, infection of the lower respiratory tract of humans can result in pneumonia with progression to acute respiratory distress syndrome (ARDS), and death from respiratory failure.

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Influenza-mediated damage to the alveolar epithelium results from both intrinsic viral pathogenicity attributable to its tropism to alveolar epithelial cells and a robust host immune response, which, although contributing to viral clearance, can worsen the severity of lung injury. Therefore, IAVs pose a substantial threat to global human health and are closely monitored to record local influenza activity to design annual vaccines and to counteract outbreaks of novel strains which have acquired the ability to cross species barriers to infect humans. Besides existing strategies including vaccination and antiviral therapy, showing variable efficacy due to antigenic variation and the occurrence of resistant virus variants, novel approaches have been recently developed to treat influenza virus-induced lung injury by targeting an unbalanced host immune response in addition to antivirals.

In this review, we discuss features and origin of seasonal and pandemic influenza, focused on IAV, its clinical presentation, and current as well as possible future intervention strategies.

Seasonal and Pandemic Influenza

Two different genera of the virus family *Orthomyxoviridae*, influenza A and B, can cause a contagious respiratory disease in humans. They cause annual epidemics of varying severity, including mild common cold symptoms to severe lung injury with fatal outcome. IAVs, on which we focus in this review, show a very wide variety and thus are further subgrouped by the antigenic properties of their surface proteins hemagglutinin (HA) and neuraminidase (NA). Besides the well-defined strains circulating in humans (of the subgroups H1N1 and H3N2), IAVs are present in a large number of mammalian species, poultry, and particularly wild birds, with currently 18 or 11 variants known for HA or NA, respectively, where the recently discovered bat-isolates H17/18 and N10/11 seem to be quite distinct from avian and mammalian strains.^{5,6}

Influenza virus epidemics usually occur during the cold season in temperate regions (Northern hemisphere: December to April; Southern hemisphere: June to September) when low humidity and temperature ambient conditions are suggested to prolong virus shedding and transmission.⁷ In subtropical and tropical regions, influenza seasons are less clearly defined, allowing recurrent infections all over the year. Thus—and due to other reasons such as close proximity to domestic poultry—it has been proposed that the Asian continent represents a particular “reservoir” for not only endemic human but also zoonotic influenza virus strains.^{8,9} Overall, seasonal IAV affects up to 10% of the adult population and 20% of children annually¹⁰ and displays a substantial morbidity. A cohort study estimates that between 2003 and 2009 in the United States, seasonal influenza was responsible for 3.8% of all admissions for respiratory failure and evaluates the incidence of influenza-associated acute respiratory failure at 2.7 per 100,000 persons per year.¹¹ This results in considerable clinical and economic burden. For the United States, direct medical costs covering health care for influenza-induced illness as well as economic losses due to lost working days and

declined production are proposed to account for at least 26 billion USD annually.¹²

Compared with the seasonal influenza, pandemics have occurred every 20 to 30 years and are generally associated with more severe symptoms which also affect a healthy young population and potentially lead to increased mortality rates.^{13–17} Pandemic IAV strains are newly introduced into an immunologically naive human population and usually derive from avian reservoirs and have reassorted in an intermediate host such as swine, where they acquire further adaptive mutations, enabling them to infect the human respiratory tract and to effectively transmit from human to human, causing a human disease^{18,19} (→Fig. 1).

Wild birds constitute a primary reservoir of influenza viruses, but IAVs are also widely found in pigs. IAVs have further been identified in other mammalian intermediate hosts, especially aquatic mammals as elephant seals²⁰ and also in horses and recently in dogs.²¹ With respect to avian influenza, the major threats for human health currently are influenza A H5N1 and H7N9²² widely found in domestic farms and confirmed to induce severe disease in humans.^{22,23} Following a first poultry outbreak at the end of 2003 in Thailand, until now more than 840 cases of human infection with H5N1 have been reported from more than 15 countries^{24,25} with reported mortality rates of more than 50% and an overall increased morbidity in children (<15 years)²⁶; since 2013, poultry-to-human transmission of H7N9 was confirmed in more than 560 cases, leading to severe pneumonia with multiorgan failure and mortality rates of approximately 40%.^{27,28} Interestingly, H7N9 is less pathogenic in birds than H5N1 but displays signatures of human adaptation, as it presents with a better affinity for human than for avian influenza receptors.^{29,30}

As the swine respiratory tract expresses both avian and human influenza receptors,³¹ pigs can act as “mixing vessels” for the reassortment of IAV strains from different origins.³² The pandemic risk of triple reassortant strains of swine origin has been confirmed during the 2009 “swine” pandemic, where a triple (avian, swine, human) reassortant caused the first pandemic of the 21st century.¹⁹

Influenza pandemics constitute a major threat to global health, and emerging strains, in animal reservoirs as well as in the human population, are kept under tight surveillance by the authorities. The World Health Organization (WHO) has defined the progression of a pandemic into six stages, beginning by the first animal infections in phase 1 to the outbreak spreading over a large geographical zone in phase 6 (→Fig. 1), to assess any new pandemic risk (WHO Web site). The Centers for Disease Control and Prevention (CDC) has described a new influenza risk assessment framework (IRAT) to evaluate qualitatively and quantitatively the potential of new strains to give rise to pandemic events based on virus properties, host response of the human population, and the ecology and epidemiology associated with the strain.³³

Influenza pandemic episodes have been described since the end of the 19th century and virus characteristics as well as associated host responses vary from one pandemic to another. The first, even if less documented, known pandemic was the

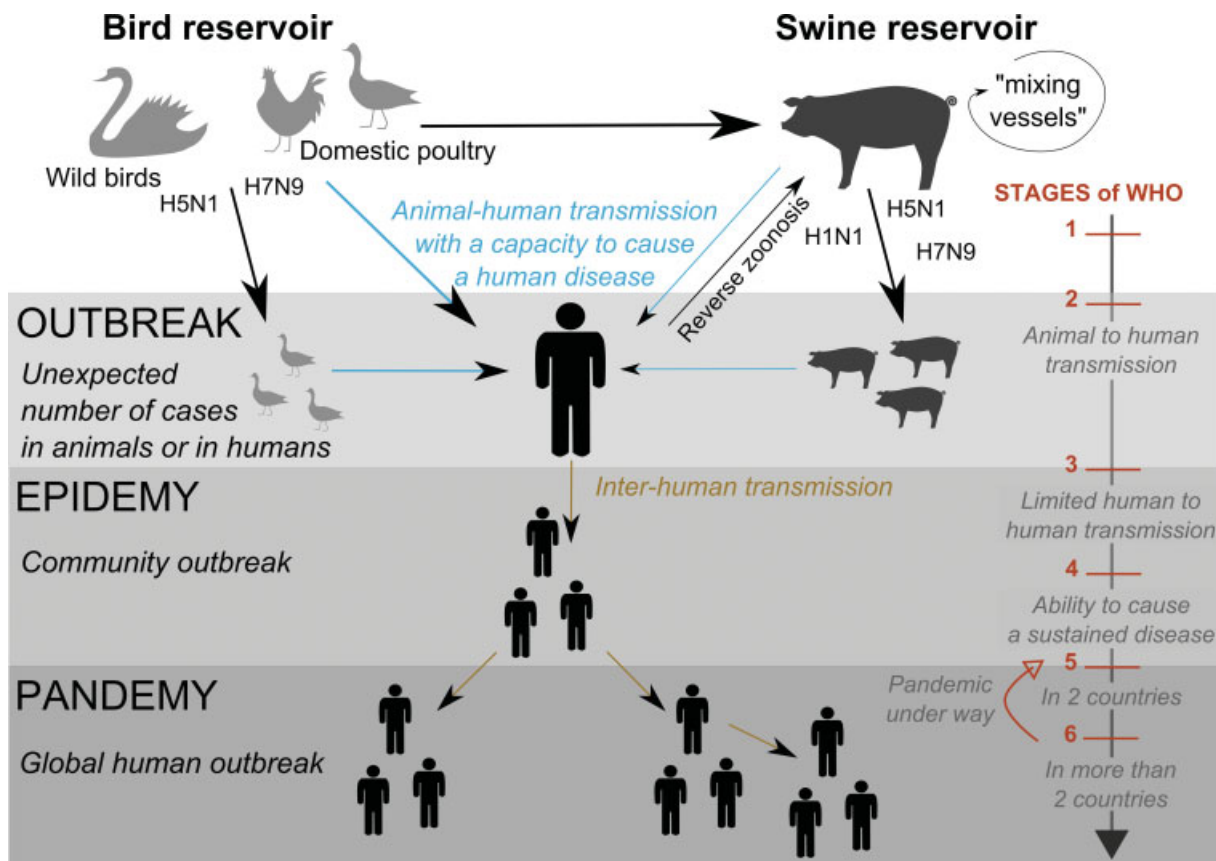


Fig. 1 A schematic representation showing major events involved in emergence of an influenza pandemic, from the two major animal reservoirs to the global outbreak in humans. Evolution steps of emerging strains are attentively followed by World Health Organization and classified into six stages.

1889 “Russian flu” (H3N8)³⁴ followed by the 1918 “Spanish flu” (H1N1), the “Asian flu” in 1957 (H2N2),^{35,36} the “Hong-Kong flu” in 1968 (H3N2),³⁷ and most recently the 2009 “swine flu” (H1N1). Interestingly, the most well-known influenza pandemic, the 1918 Spanish flu, and the most recent pandemic, the swine influenza from 2009, were caused by an H1N1 virus. Also called “the mother of all pandemics” by Taubenberger and Morens, the 1918 virus infected around one-third of the world population and was responsible for the death of at least 50 million of people within a year, appearing in three successive waves.³⁸ Although the 1918 pandemic has been extensively studied, the virus characteristics responsible for its fast spread, associated with a high mortality rate especially in the population of 20 to 40 years of age, largely remain obscure.³⁹ Yet, studies in mice have shown that the Spanish strain induces a larger immune response, especially in terms of macrophage and neutrophil recruitment, as low pathogenic viruses.⁴⁰

The 2009 “swine flu,” since then renamed as “novel influenza A (H1N1)” or “pandemic 2009 H1N1 flu,” resulted in 18,500 reported laboratory cases and a modeling study estimates that, in total, the 2009 H1N1 virus caused more than 200,000 influenza-associated deaths due to respiratory and 80,000 deaths due to cardiovascular failure. Importantly, more than 80% of these lethal cases affected a young population (<65 years).⁴¹ Contrary to the 1918 virus, the 2009 strain

seems to be able to suppress the early innate host immune response which may contribute to a high transmission rate and pathogenicity.⁴²

Viral Adaptations Needed for Host Switch and Determinants of Pathogenicity

To successfully adapt to humans as a new host—either via the direct transmission from birds to humans or a possible further adaptation process in swine—IAVs need to ensure efficient replication and interspecies transmission. Contrary to birds, where IAV replicates in the gastrointestinal epithelia, the primary target cells of IAV in humans as well as in pigs are found in the epithelium of the respiratory tract. Consequently, a successful adaptation to the human host requires mutations in the viral replication machinery.

The first step of IAV replication in the human host is the cell attachment, when the virion binds via the viral surface protein HA to sialic acids (SAs) expressed on the upper or lower airway or alveolar epithelial cell. While avian epithelia highly express α 2,3-linked SA, the α 2,6-linked SA is predominant in the human trachea. After attachment, virions enter the endosomal pathway.⁴³ Subsequently, an HA-induced fusion of viral and endosomal membrane is followed by the dissociation of the viral ribonucleoprotein complex (vRNP) consisting of viral RNA, the polymerase complex (viral

proteins PA, PB1, and PB2), and the viral nucleoprotein (NP), from the M1 protein.⁴⁴ Interestingly, avian and mammalian IAVs show different pH optima for this HA-mediated membrane fusion, and the respective mutations in the HA protein (amino acids N104D and T115I) have been associated with the capacity to sufficiently release the viral genetic information into the host cell.^{45,46} In the next step, the vRNP is trafficked from the cytosol toward the nucleus. This import is mediated by the cellular importin- α 7 in the mammalian system and importin- α 3 in the avian host,^{47–49} and the switch in usage of importin isoforms depends on both NP and PB2.⁴⁹ Within the nucleus, the negative-oriented viral (v)RNA is transcribed into messenger (m)RNA, giving rise to new viral proteins by the cellular translation machinery, as well as positive-oriented copy (c)RNA and high amounts of newly transcribed negative-sense vRNA. Herein, the avian polymerase is adapted to working temperature of 41°C as found in the avian gastrointestinal tract, whereas in human strains, mutations at position 627 in the PB2 gene are thought to contribute to the tolerance toward lower temperatures found in the human lung.⁵⁰ Virus assembly takes place at the apical cell membrane, where viral proteins and newly formed RNPs accumulate at lipid raft domains, resulting in budding, scission, and release of newly formed virions.⁵¹ During release, cleavage from cell surface expressed SA by the viral NA is essential to prevent clumping and retention of virions. NA has to switch from cleaving α 2,3- to α 2,6-bound SA when introduced into the human host.^{52,53} Furthermore, NA activity has been shown to be temperature- as well as pH-dependent, and multiple alterations promote optimal performance in the human respiratory tract.^{53,54}

Especially with regard to pandemic preparedness, an important task is to evaluate the occurrence of virus variants with increased pathogenicity. These IAV variants often show enhanced replication properties or means of immune evasion, some of which will be highlighted here (for a more detailed review, see Baigent and McCauley⁵³ as well as Tscherne and García-Sastre⁵⁵). Many mutations enhancing replication efficiency can be found in the polymerase complex (reviewed in Mänz et al⁵⁶); most prominent is the E627K substitution in the PB2 gene that is suggested to stabilize the interaction with the NP, to enhance replication rates and to induce higher pathogenicity in the 1918, 1957, and 1968 pandemic IAV.^{57,58} It is found in 32% of circulating H5N1 variants as well as H7N9 isolates.^{59–61} An important contributor to effective viral spread is the viral nonstructural NS1 protein that plays a major role in limiting the host innate immune response.^{62,63} It prevents viral RNA recognition by the innate system and thus blocks the production of antiviral type I interferons (IFNs) by influenza-infected epithelial cells.⁶⁴ Additionally, NS1 inhibits downstream signaling of protein kinase R (PKR), downregulates the IFN-receptor, and deregulates processing of cellular mRNA.⁶⁴ Viruses with reduced or lacking NS1 activity are substantially compromised in pathogenicity.^{65,66} Another major determinant of influenza virus pathogenicity is the viral HA. Highly pathogenic avian influenza (HPAI), in contrast to seasonal human IAV and low pathogenic avian influenza strains, bear a multi-

basic amino acid motif in the HA cleavage site, which promotes HA cleavage by a higher variety of proteases.^{3,67} HPAI-induced disease is therefore related to increased disease severity and higher mortality in humans.^{68,69} Additionally, influenza strains newly introduced into the population generally show no or only one to two N-linked glycosylation site (s) in the globular head domain of the HA, whereas seasonal strains acquire more sites to mask their antigenic epitopes^{70,71}; over the past 40 years, H3N2 has increased the number of sites of glycosylation in parallel with its pathogenicity decrease.⁷²

The Host Response to Influenza—Key Players in Antiviral Defense and in Driving Epithelial Injury

In patients who succumb to IAV infection, lung autopsies almost always show diffuse alveolar damage, but viral RNA is present in only a subset of patients.⁷³ These results and findings from published studies of IAV infection in animals suggest that mortality due to IAV infection may rather result from an overly exaggerated immune response than from uncontrolled viral spread. Several subsets of immune cells were found to contribute to damaging host responses, and some of the underlying molecular mechanisms have been recently defined, providing novel targets for therapeutic intervention^{64,74} (→ Fig. 2).

As mentioned earlier, the primary target cells of IAVs are the epithelial cells that mount an anti-inflammatory response upon recognition of pathogen-/danger-associated molecular patterns (PAMPs/DAMPs) by diverse pattern recognition receptors. Viral uncapped 5'-triphosphorylated RNA interacts with RIG-I (RNA helicases retinoic acid-inducible gene-1), leading to a subsequent interaction with MAVS (mitochondria-associated antiviral signaling protein), TRIM25 (tripartite motif-containing protein 25), and IPS-1 (interferon- β -promoter stimulator-1) and inducing IRF-3 and IRF-7 (interferon regulatory factor)-dependent transcription and translation of types I and III IFNs.^{75,76} Additionally, recognition of viral patterns by PKR activates NF- κ B (nuclear factor “kappa-light-chain enhancer” of activated B cells) translocation to the nucleus and transcriptional activation of proinflammatory, proapoptotic, and antiviral gene clusters.^{77–79} Other receptors sensing IAV infection include the NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome, and also endosomal toll-like receptors (TLR3 and TLR7).^{80–82} Recent evidence suggests that upon viral infection, DAMPs such as high-mobility group box 1 (HMGB1), S100A9, or purine metabolites are crucially involved in influenza disease progression.^{83,84}

Similarly to epithelial cells, lung resident macrophages represent a first line of defense and initiate an immune response following viral pattern recognition. They phagocytose viral particles as well as infected apoptotic cells and represent a major source of type I IFNs and further cytokines and chemokines.^{85,86} Thus, resident alveolar macrophages have repeatedly been shown to be crucial for limiting viral spread, morbidity, and mortality after influenza virus

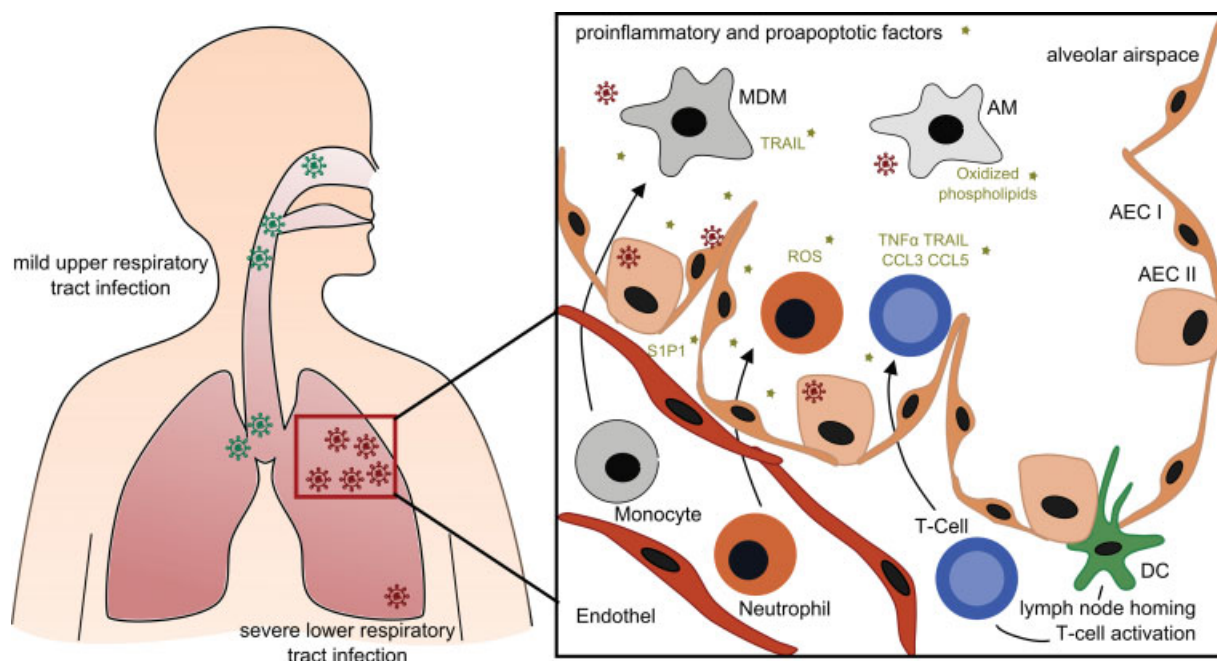


Fig. 2 Key players in influenza A virus (IAV)-induced lung injury. While upper respiratory tract infection results in mild symptomatic IAV infection, severe cases of IAV usually involve spread of the virus to the lower respiratory tract. Here, resident alveolar macrophages (AM) and dendritic cells (DC) sense IAV infection of alveolar epithelial type I (AEC I) and type II (AEC II) cells. Cytokine release and establishment of a proinflammatory milieu in the alveolar lumen lead to recruitment of additional monocyte-derived macrophages (MDM) as well as neutrophils from the blood vessels to the site of infection. DC migration to the lymph nodes further induces generation of antigen-specific T cells into the vessel. An exuberant immune response with massive release of proinflammatory and proapoptotic mediators contributes to IAV-induced lung injury.

infection.^{87,88} At the same time, they initiate innate immune responses as well as adaptive immunity by stimulation of CD8⁺ T cells that may finally result in immune-related pathologies. For example, oxidized phospholipids produced upon IAV infection in a TLR4-dependent way have been reported to potentiate macrophage cytokine production and are crucially involved in the progression of IAV-induced lung injury.⁸⁹

Neutrophils are highly recruited early after IAV infection into the alveoli and were found to play an ambivalent role in IAV infection. On one hand, depletion of neutrophils early in disease is associated with uncontrolled viral spread and progression from mild to severe influenza-induced illness.^{88,90,91} On the other hand, excessive neutrophilic recruitment, found in highly pathogenic H1N1 and H5N1 infections,⁴⁰ contributes extensively to lung injury.⁹² Already the intercellular migration process of neutrophils recruited in large numbers from the blood vessel into the alveoli requires a structural opening of epithelial junctional complexes, contributing to alveolar barrier disintegration and disease progression to ARDS.⁹³ Furthermore, neutrophilic release of various signaling molecules significantly contributes to lung injury, including further cytokines; extracellular proteases; neutrophilic extracellular traps, of which especially histone components have been proven to be injurious in IAV infection^{92,94}; and reactive oxygen species.⁹⁵ Besides neutrophilic superoxide dismutase and NADPH oxidase, myeloperoxidase also has been reported to significantly affect the alveolar epithelial barrier integrity. In line, abrogation of CXCL2- or CXCL10-driven neutrophil recruitment

during IAV infection improves outcome in preclinical models.^{96,97}

Following establishment of a proinflammatory milieu in the infected lung, circulating blood monocytes are recruited to the alveolar lumen and differentiated to monocyte-derived alveolar macrophages and dendritic cells (DCs).^{98,99} The former are, in contrast to the resident alveolar macrophages, directly associated with enhanced severity of influenza-induced injury.^{100,101} They release large amounts of proinflammatory as well as proapoptotic mediators. Among these, especially the type I IFN-induced TRAIL (TNF-related apoptosis inducing ligand) has been shown to induce and sustain collateral lung damage by induction of alveolar epithelial cell apoptosis via its receptor DR5 (death receptor 5).^{102–105} Both inhibition of the recruitment of monocyte-derived alveolar macrophages and abrogation of TRAIL signaling have been found to decrease influenza-induced lung injury, and recent evidence reveals a role of TRAIL in inhibition of edema resolution after IAV-induced lung injury in mice.^{102,103,106}

DCs reside in the airway and alveolar epithelium, extending dendrites into the alveolar lumen and thus can be directly infected or sense viral infection via antigen uptake from dying cells. Especially the CD103⁺ DC subset has been reported to be the major subset migrating to the draining lymph nodes in response to activating cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF)¹⁰⁷ and also in response to NLRP3 inflammasome cytokines, interleukin (IL)-1, and IL-18.¹⁰⁸ Subsequently activated DC can cross-present antigen to naive CD4⁺ and CD8⁺ T cells, thus priming adaptive immune response.¹⁰⁹ Interestingly, DC numbers are

strongly increased in severe H5N1 infection in nonhuman primates¹¹⁰ and DCs have been shown to secrete by themselves the NLRP3 inflammasome-dependent mediators IL-1 β , IL-1 α , and IL-18.^{111,112} The T-cell-mediated adaptive immunity to respiratory IAV infection is highly complex and influenced by multiple signaling events derived from epithelial cells, DC, and neutrophils.⁶⁴ Activated CD8⁺ T cells become cytotoxic effectors able to lyse IAV-antigen presenting infected epithelial cells, and they contribute to the induction of cell apoptosis by production of tumor necrosis factor- α (TNF α) and TRAIL.^{113–115} Although specific removal of epithelial infected cells by release of cytotoxic granules actively participates to virus clearance, the nonspecific activity of proinflammatory cytokines as TNF α , CCL3, and CCL5 released by activated T cell can additionally impact on noninfected epithelial cells and may thereby enhance lung inflammation and injury.¹¹⁶

Additionally, the lung endothelial cells are also affected by influenza virus infection and can impact on epithelial injury in murine influenza models.¹¹⁷ They not only mediate monocyte and leukocyte lung intra- and extravasation by expression of adhesion molecules and chemokines such as E-selectin, P-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, CXCL9, and CXCL10^{118,119} but also release inflammatory mediators such as IL-6 and TNF α ,^{120,121} further promoting the proinflammatory milieu during IAV infection. In contrary, endothelial cells are a major source for angiotensin-converting enzyme 2 (ACE2), which downregulates angiotensin 2, and increased serum levels of ACE2 after IAV infection correlates closely with epithelial cell protection and improved outcome after human H5N1 and H7N9 infection.^{122,123} Another important endothelial signaling event orchestrating inflammatory viral lung injury is the sphingosine 1 phosphate pathway. Blockade of the endothelial-produced sphingosine-1-phosphate 1 (S1P1) results in decreased cytokine and chemokine levels correlating with lower mortality rates in influenza-induced lung injury.^{117,124}

Clinical Presentation

Influenza viruses spread in the human population by respiratory droplet transmission.¹²⁵ Influenza infection can be an asymptomatic or mild with an uncomplicated upper respiratory tract illness; in rare cases, however, IAV induces a complicated disease with severe viral pneumonia leading to multiorgan failure or exacerbation of underlying disease conditions.¹²⁶ Seasonal IAVs especially target populations with immunodeficiencies or underlying chronic conditions, such as lung or heart disease.^{127–130}

Clinically, an acute onset of symptoms can be observed after 24 to 48 hours of incubation time, correlating with enhanced viral replication. These symptoms constitute the influenza-like illness (ILI) and include headache, cough, myalgias, malaise, chills, and fever that can persist for 2 to 8 days. Pandemic and in a less extent seasonal IAV has furthermore been described to cause gastrointestinal illness with vomiting or diarrhea, especially in children. Severe cases of influenza infection comprise spread of the virus to the

alveolar compartment, where both α 2,6- and α 2,3-SA are present—the latter highly expressed on type II pneumocytes.^{131,132} Alveolar infection causes extensive diffuse alveolar damage, histologically resembling other forms of ARDS with intra-alveolar hemorrhagic edema accumulation, fibrin deposition, massive infiltration of leukocytes, extensive bronchial and alveolar epithelial cell apoptosis, and formation of hyaline membranes.¹³³ Such patients present with progressive respiratory failure, including dyspnea, tachypnea, hypoxia, and radiological signs of diffuse bilateral pneumonia, and later on ARDS with possible fatal outcome.¹³⁴ Frequently, coinfection with colonizing bacteria aggravates the course of illness. Coinfections with *Streptococcus pneumoniae* and *Staphylococcus aureus* are the most frequent and have been observed at a high rate during pandemics leading to an increase in pneumonia-associated death.¹³⁵ The autopsy of 68 soldiers who died during the Spanish flu wave revealed evidence of bacterial pneumonia in the lung in most cases.³⁹ A cohort studies in 14 Australian ICUs demonstrated that during the 2009 influenza season, coinfections were dominant in a previously healthy younger population without comorbidities.¹³⁶ Coinfection with further respiratory viruses such as respiratory syncytial virus aggravates the severity of the disease,¹³⁷ especially in immunocompromised patients.¹³⁸

Intervention Strategies

Treatment for typical ILI is generally supportive. One major challenge in the treatment of influenza virus infection is early detection, as influenza-specific antiviral therapy is most effective in the beginning of the disease. As first symptoms are not specifically attributable to IAV but more generally to respiratory viral infection, rapid ELISA-based antigen tests eventually combined with PCR-based diagnostics tests are recommended—especially during the influenza season—to allow a fast differentiation from other causes and rapid onset of antiviral treatment. However, WHO guidelines recommend to provide initial treatment against influenza without awaiting delayed laboratory test results.¹²⁶

Currently used antivirals in clinical trials are summarized in ► **Table 1**. The primary antiviral and empiric treatment for mild as well as severe influenza A and B virus infections is administration of the NA inhibitors, recommended by the WHO to be applied as early as possible in all high-risk patients (immunocompromised, severe comorbidities, underlying chronic lung disease, age < 2 or > 65 years, morbid obesity, nursing home residents, women who are pregnant or postpartum), and in all patients with signs of severe respiratory disease. Randomized clinical trials have confirmed the efficacy of NA inhibitors to diminish symptom duration and risk of pneumonia complications¹³⁹ and length of treatment or dose may be increased in immunocompromised patients. Currently, all circulating A and B strains are susceptible to approved NA inhibitors; however, previously circulating H1N1 IAV strains have been widely resistant to oseltamivir.¹⁴⁰ A way to overcome viral resistance may be to combine antiviral drugs with different modes of action in future,¹⁴¹

Table 1 Current antiviral drugs and novel antivirals in clinical trials

Name	Trademark	Status	Mechanism of inhibition	Comments
Amantadine	Symmetrel	FDA approved (1966)	Steric inhibition of M2 ion conductance	Not recommended due to high prevalence of resistant variants
Rimantadine	Flumadine	FDA approved (1994)	Steric inhibition of M2 ion conductance	Not recommended due to high prevalence of resistant variants
Oseltamivir	Tamiflu	FDA approved (1999)	Binding to enzymatic active site of NA	Approved for patients ≥ 2 wk and older, orally
Zanamivir	Relenza	FDA approved (1999)	Binding to enzymatic active site of NA	Approved for patients ≥ 5 y and older, per inhalation
Peramivir	Rapivab, Rapiacta	FDA approved (2014)	Binding to enzymatic active site of NA	Approved for patients ≥ 18 y and older, intravenously
Laninamivir	Inavir	Approved (Japan, 2010)	Binding to enzymatic active site of NA	Approved for treatment (2010) and prevention (2013), per inhalation
AVI-7100		Phase I	Interference with expression of viral M gene segment	
DAS181-F03		Phase I	Cleavage of sialic acids from host cell surface	
Flufirvitide		Phase I	Peptide inhibitor binding thus blocking viral HA	
Favipiravir		Phase III	Nucleoside inhibitor targeting PB1	
Nitazoxanide		Phase III	Blockade of HA maturation	

Abbreviations: FDA, Food and Drug Administration; HA, hemagglutinin; NA, neuraminidase. Information used from: www.cdc.gov; www.fda.gov; www.clinicaltrials.gov (September 2015).

and to additionally target host cellular pathways known to be indispensable for viral replication at the virus–host interface.

For patients presenting with influenza-associated respiratory failure, NA treatment is highly recommended and was found to be effective in terms of outcome even when applied later than 48 hours post-symptom onset. NA inhibitors are frequently applied through the intravenous route in critical care patients.¹⁴² Complementary to antivirals, supportive treatment is necessary.¹⁴³ These measures include standard care for ARDS,^{144,145} and extracorporeal membrane oxygenation protocols were found to be beneficial,¹⁴⁶ whereas treatment by anti-inflammatory strategies such as systemic corticosteroid treatment is not recommended to date.^{147,148} As bacterial coinfections constitute a major complication which increases the severity of the disease and worsens outcome, patients with influenza-associated ARDS should be carefully monitored to start empiric antibiotic treatment as early as possible. Jain et al suggested that patients hospitalized with suspected influenza and lung infiltrates on chest radiography should receive early and aggressive treatment with both antibiotics and influenza antiviral agents.¹⁴⁹

At-Risk Populations

Influenza especially targets humans with altered immune responses and is generally associated with a U-shaped mortality curve, except for both 1918³⁸ and 2009 pandemics which display W-shape curves.¹⁵⁰ This means that high death rates are observed in children younger than 2 years and

elderly persons older than 65 years in seasonal influenza infections, whereas young adults show high mortality rates during pandemics, where the elderly may be protected to some extent due to preexisting immunity. Additional risk factors or comorbidities enhancing the likeliness to develop severe influenza-induced complications comprise age, chronic respiratory, renal, hematologic, neurologic or cardiovascular disease, diabetes, obesity, immunosuppression/deficiency, and pregnancy/postpartum period as many clinical studies on hospitalized 2009 influenza have demonstrated.^{128–130,151–153} A global pooled analysis on 70,000 hospitalized 2009 influenza patients indicate that children (<15 years) present a higher risk of being admitted to hospital, whereas older adults (>50 years) display higher mortality rates. Furthermore, morbid obesity seems to be associated with the risk of ICU hospitalization and lethal outcome.¹³⁰ Immunocompromised persons suffer from a more severe disease and are more likely to develop drug-resistance mutations.^{154,155} Numerous clinical studies especially on 2009 pandemic cases^{156,157} and WHO or CDC recommendations^{158,159} identify pregnancy as comorbidity factor for influenza and advise general vaccination of this risk population. Pregnant women are more susceptible for infection with influenza, develop a more severe disease associated with worse pregnancy outcomes, and show higher risk of ICU admission and increased mortality. Biological reasons for this predisposition are not yet defined, but changes in immune responses, particularly in antiviral responses,¹⁶⁰ associated with pregnancy are involved and the fetus-associated

complications are probably essentially due to maternal fever.¹⁶¹ As well as maternal protection, vaccination also protects newborns and infants, another risk population, for which vaccination is recommended from 6 months of age in the United States and in the European Union (EU), with the latter mostly limited to infants with increased risk of exposure.¹⁶²

Importantly, with respect to development of personalized therapies, recent studies have highlighted a link between host genetic predisposition and susceptibility to influenza viruses. Albright and colleagues have observed a hereditary contribution in lethal cases of influenza in Utah.¹⁶³ As IFN-dependent proteins and cytokines are known to be important for host defense against viruses, genome-wide association studies (GWAS) have focused on association between specific polymorphisms and susceptibility to influenza. The IFN-inducible transmembrane protein 3 (IFITM3) plays clearly an important role in host defense against H1N1¹⁶⁴ and H7N9,¹⁶⁵ but the association between its single-nucleotide polymorphism rs12252 and influenza susceptibility remains a matter of controversy.^{166,167} Recently, deficiency in IRF7 has been associated with a severe case of influenza infection.¹⁶⁸ Mutations in the *IL1A* and *IL1B* genes (coding for interleukins 1- α and - β), in the genes encoding the protease TMPRSS2 (transmembrane protease, serin 2, mediating viral HA cleavage upon infection), or galectin-1^{169–171} can furthermore impact the host susceptibility to H1N1 or H7N9.

Vaccination

Considering the aforementioned obstacles present for efficient and timely treatment of severe influenza infections, preventive vaccination is a key tool to diminish influenza morbidity as well as socioeconomic burden.¹⁷² Even if national guidelines for influenza virus vaccination vary, vaccination is generally recommended not only to high-risk patients with preexisting comorbidities or high risk of exposure but also to the whole population (6 months of age or older) to not only limit the individual risk of infection but also to improve herd immunity and the likeliness for reassortment events.¹⁷³ As influenza virus strains gradually change between seasons, the composition of the vaccine is updated yearly based on recommendations of the WHO that monitors the global influenza virus occurrence. The currently approved vaccinations are either based on egg- or cell culture-grown inactivated subunit vaccines that are administered intramuscularly, or live, attenuated influenza viruses that are used for intranasal administration in children in the United States and Europe.¹⁷⁴ The inactivated trivalent or quadrivalent vaccination preparations contain antigens of two IAV strains (currently a descendant of the pandemic H1N1 and a circulating H3N2 strain) and of one or two influenza B virus strains (Yamagata and Victoria line), respectively, and mainly trigger an immunoglobulin G (IgG) serum response.¹⁷⁵ This IgG-mediated protection can be suboptimal especially in cases of strong divergence between the antigens used for vaccination and those of the currently circulating seasonal influenza strains, when prediction is suboptimal. To minimize reduced

efficacy, efforts are taken to develop vaccines that elicit cross protection against several strains and are directed against a conserved HA stem region.^{176–178} Live, attenuated vaccine strains are usually generated on an H2N2 backbone and reassorted with the WHO-recommended strains to carry the current HA and NA genetic information and surface protein expression adapted to growth at 25°C, thus limiting spread in the human host.¹⁷⁹ They elicit a stronger immune response including IgG and IgA production as well as T-cell memory, but are considered less safe, as they bear the potential risk of reversion to a nonattenuated form and a possible reassortment platform for circulating virus strains.¹⁸⁰ Therefore, efforts are made to improve safety of live, attenuated vaccines, for example, by engineering variants unable to circumvent the antiviral IFN response by deleting or inserting a truncated NS1 protein.¹⁷⁴

Novel Therapeutic Approaches

In the light of the growing prevalence of drug-resistant IAV strains, substantial efforts are taken to establish new strategies to limit viral replication and improve outcomes of IAV infection, some of which are currently tested for human application in clinical trials (► **Table 1**). One class of drugs comprises classical antiviral agents, directly targeting viral proteins and their function. Neutralizing antibodies against both the HA and the M2 are tested that are active against a broader range of influenza strains.^{181,182} The viral HA is further targeted by decoy receptors or SA-containing inhibitors as well as carbohydrate-binding drugs, which block the HA by binding to its glycosylation sites and thus prevent cell adsorption (i.e., cyanovirin-N 56).¹⁸³ Favipiravir is a nucleoside inhibitor currently evaluated in phase III trials that blocks the activity of the viral polymerase complex and revealed very limited development of resistant IAV.^{184,185} Moreover, classical SA synthetic analogues that act similarly as oseltamivir and zanamivir have been developed resulting in admission of laninamivir and peramivir recently.^{186–188} Other strategies to directly target viral components include the use of small interfering RNA^{189,190} and small molecule inhibitors.¹⁹¹ Moreover, combination therapies using different antiviral drugs are currently studied in detail and have shown efficacy in limiting IAV replication.

Another class of novel antiviral drugs influences host cell proteins or signaling pathways necessary for viral infection or replication. DAS181 (Fludase) abrogates HA adsorption to the host cell by cleavage of SA from the host cell surface.^{192–194} HA is further targeted by agents preventing correct posttranslational processing of the transmembrane protein (Nitazoxanide), as well as by blocking cleavage of HA by the internal proteases HAT and TMPRSS2 (Aprotinin).^{192,195} Another approach is the inhibition of the transcription factor NF- κ B that has been proven to be activated upon influenza infection and to be supportive for virus replication.¹⁹⁶ Given that NF- κ B inhibition resulted in reduced viral replication, less inflammation, and improved outcome in preclinical models, an inhaled compound, L-lysine acetylsalicylate glycine (LASAG), has been tested in phase II trials.¹⁹⁷ Other strategies interrupt

virus replication through specific modulation of lipid raft domains (Viperin),¹⁹⁸ the MAP kinase pathway, pyridine metabolism, protectin D expression, and even IFN modulation,^{191,199} where the latter is either enhanced early in IAV infection or suppressed by neutralizing antibodies late in disease progression.²⁰⁰ Further strategies are aimed to modulate an unbalanced, overshooting inflammatory response during virus-induced lung injury, to promote pulmonary host defense and to drive repair of the damaged epithelium after virus clearance. GM-CSF is an important modulator of macrophage and DC-mediated antiviral immunity, and also plays a major role in the repair of the alveolar epithelium.^{201–203} As such, we suggested that local administration of GM-CSF to patients with pneumonia- and IAV-induced ARDS will improve host defense, oxygenation, and outcome,²⁰⁴ and a placebo-controlled, double-blind, randomized multicenter phase II trial on inhaled GM-CSF for treatment of ARDS induced by bacterial or viral pneumonia has been launched. Such host-modulating therapies are envisioned to be combined with antivirals in future to improve outcome of influenza-induced respiratory failure.¹⁴¹

Finally, future therapeutic options are stem cell-based approaches. Interestingly, preclinical studies have shown the beneficial effects of the application of bone marrow-derived mesenchymal stromal cells (MSCs) which possess immunomodulatory and regenerative properties²⁰⁵ for the treatment of acute lung injury,²⁰⁶ and MSCs are currently tested as therapy for human ARDS.²⁰⁷ Even if the potential of MSC has not been studied in preclinical models of influenza pneumonia so far, cell therapy—applied either systemically or locally into the injured lung—may constitute an innovative approach to treat IAV-induced ARDS patients.²⁰⁸ Moreover, endogenous epithelial stem/progenitor cells in the murine lung have been recently defined and were found to repopulate distal lung epithelium and therefore to actively participate to the repair phase after influenza-induced injury through activation of a p63/krt5 regeneration program.^{209,210} Our own data reveal that these regeneration pathways are induced and particularly modulated by influenza viruses during infection of the stem cell niche²¹¹, highlighting endogenous stem cells and their mediators as novel putative targets to improve lung regeneration after severe viral damage.

Conclusion

Taken together, IAV-induced lung injury still shows a major impact on human health, but substantial work is invested in the monitoring and the prevention of IAV-induced disease as well as in promising concepts to improve outcomes of IAV-induced ARDS. A key challenge in influenza research is to design innovative host-based therapies able to boost antiviral immunity and to improve lung regeneration. Translational approaches should be focused on better understanding of viral–host interactions as well as immunopathogenic mechanisms to define signaling pathways on a molecular basis amenable to specific targeting. Meanwhile, defining host susceptibility factors will accelerate recognition and treatment of a new category of risk patients and also promote the

discovery of novel strategies for personalized therapies. It will also be necessary to understand the mechanisms underlying the increased susceptibility to bacterial outgrowth associated with influenza infection, to reduce the prevalence of secondary infections with increased morbidities. Finally, ongoing research on stem cell-based therapies, including injury- or pathogen-specific programming of these cells prior to application to improve their particular modes of action in different forms of acute lung injury, reveals promising results which will likely be the basis of future therapies against ARDS.

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