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Opinion

Influenza A Viruses: Understanding Human Host Determinants

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Previous influenza A virus (IAV) pandemics have invariably been caused by the introduction of an emergent IAV strain from an animal host into a human population with no or only little pre-existing immunity to the novel strain. Although zoonotic spillover of IAVs into humans can be associated with severe disease and a high fatality rate, these strains are typically poorly adapted to humans and are unable to establish sustained transmission between humans. Given the presumably very high degree of exposure to animal populations with endemic IAV, the number of pandemics remains surprisingly low. In this review, we provide an updated perspective on the molecular mechanisms underlying the adaptation of zoonotic IAV to human hosts, and discuss the implications for future pandemics.

Highlights

The ability of influenza A virus (IAV) to alter its genotype through antigenic drift and antigenic shift leads to recurring host switch events in which a zoonotic strain becomes established in humans.

Such novel IAV strains are the cause of IAV pandemics in humans, who have no to little protective immunity to viral surface proteins of avian origin.

The major barriers to human infection and transmission by zoonotic IAV are the cellular entry receptor sialic acid (SA), the human antiviral restriction factor myxovirus resistance protein 1 (MxA), and intracellular proteins required for efficient viral replication.

While the IAV surface proteins hemagglutinin (HA) and neuraminidase (NA) appear capable of switching from avian to human hosts with few mutations, MxA antagonism requires the concurrent accumulation of multiple mutations in nucleoprotein (NP), which attenuate the virus in avian hosts, thus pointing to a requirement for an intermediate 'mixing vessel' host species, such as swine.

Prerequisites for IAV Spillover Infections

Adaptation of a zoonotic IAV to a human population presents advantages and hurdles for the virus. On the one hand, there is likely little to no pre-existing humoral or cellular immunity among the human population against a completely novel strain. This is a common feature of the four major IAV pandemics since the beginning of the 20th century: in 1918 (Spanish influenza; H1N1); in 1957 (Asian influenza, H2N2); in 1968 (Hong Kong influenza, H3N2); and in 2009 (pandemic 2009 virus, H1N1pdm09) [1]. On the other hand, as an intracellular parasite, the virus is highly dependent on numerous interactions with the host cell, many of which differ in their molecular specificity between humans and animal hosts [2]. Moreover, IAV must also contend with the antiviral mechanisms of the human innate immune system [3]. A well-known characteristic of IAVs compared with other viruses is their ability to rapidly adapt to new hosts through two distinct mechanisms: **antigenic drift** (see Glossary), that is, the slow accumulation of point mutations in individual genes; and **antigenic shift**, that is, the **reassortment** of genome segments between two (or more) strains within one co-infected cell [4].

The IAV replication cycle requires numerous interactions of this manner between the virus and the host cell, for which the cellular structure may differ between host species, thereby presenting a barrier to cross-species transmission (Figure 1). Entry into the host cell is facilitated by the viral **hemagglutinin (HA)** protein, which binds to the cellular entry receptor on the cell surface. The viral particle then enters the cell by endocytosis and, upon acidification of the endosome, the **viral ribonucleoprotein (vRNP) complex** is released into the cytosol. The vRNP enters the nucleus, where it both transcribes and replicates the viral genome. Following synthesis of new viral components, progeny vRNPs assemble into viral particles at the cellular membrane. Finally, these budding particles are released from the infected cell by the activity of the viral **neuraminidase (NA)**. Here, we describe the individual hurdles that must be overcome by emergent human-adapted strains.

HA-Mediated Host Cell Entry

Cross-species transmission of IAVs is aided by the fact that all IAVs utilize ubiquitous cell receptors. Conventional IAVs (HA subtypes H1–H16 and NA subtypes N1–N9) use sialylated glycans as their

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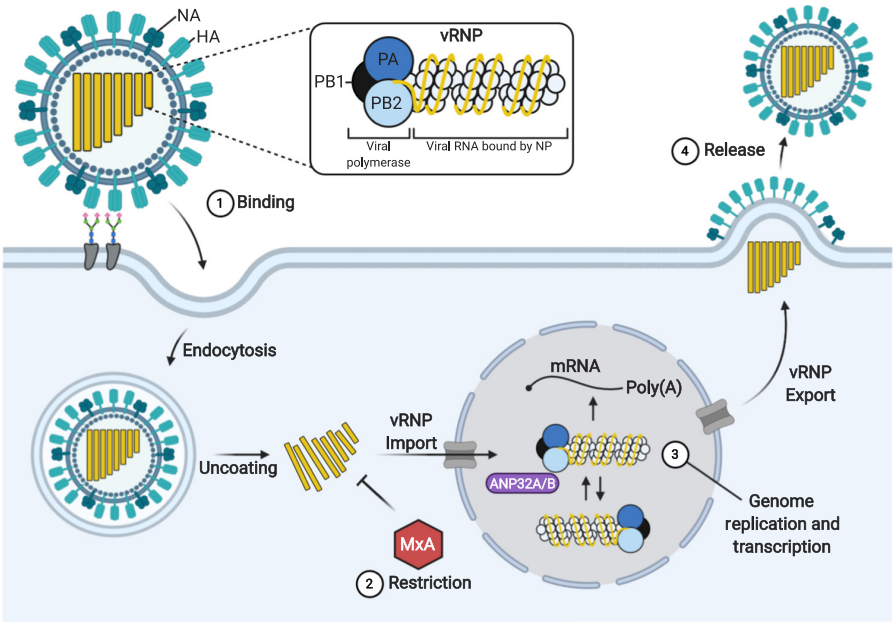

Figure 1. Overview of the Influenza A (IAV) Replication Cycle. Important steps that constitute crucial host determinants are highlighted and numbered. The IAV replication cycle is initiated upon binding of hemagglutinin (HA) to receptors on the host cell surface bearing sialic acid (SA) residues (1). Following receptor-mediated endocytosis, the segmented viral genome is released into the host cell cytoplasm in the form of viral ribonucleoprotein (vRNP) complexes and is subsequently imported into the nucleus. The human myxovirus resistance protein 1 (MxA) is thought to inhibit import of vRNP complexes, thereby exerting its antiviral activity (2). Viral replication and transcription occurs in the host cell nucleus (3). The viral polymerase relies on certain host factors, such as ANP32A and ANP32B (ANP32A/B). Newly synthesized vRNP complexes are exported to the cytoplasm and transported to the cell membrane-based sites of virus assembly, where the viral genome is incorporated into budding particles. Finally, release of progeny virions from the host cell membrane is facilitated by neuraminidase (NA) activity (4). The inset depicts a schematic representation of the vRNP complex comprising the heterotrimeric polymerase complex (PB2, PB1, and PA), oligomeric nucleoprotein (NP), and the viral RNA. Figure generated with BioRender.

cell entry receptors. However, these conventional HA proteins do differ in the type of **sialic acid (SA)** recognized based on their origin, because both the linkage between SA and the subterminal galactose and the organ distribution of SA vary among species. Whereas avian IAV HAs (H1–H16) prefer α2,3-linked SA, the HAs of human-adapted viruses (H1, H2, and H3) bind α2,6-linked SA [5–7]. Studies examining the pattern of SA distribution in human tissue have revealed that α2,6-linked SAs are predominantly found in the nasopharynx and the mucus-producing cells of the upper respiratory tract, whereas α2,3- and α2,6-linked SAs are equally abundant in the lower respiratory tract, including the trachea, lungs, and bronchus [8–10]. Analyses of SA distribution in pig tissues showed that they harbor both α2,3- and α2,6-linked SA receptors, with α2,6-linked SAs being dominant in the trachea and bronchus, and a gradual increase in α2,3-linked SAs towards the alveoli [11,12]. By contrast, the abundance and tissue distribution of α2,3- and α2,6-linked SAs is heterogeneous among avian species. For example, both α2,3- and α2,6-linked SA receptors are present in the respiratory and intestinal tracts of chickens and quails, whereas ducks lack α2,6-linked SAs in the intestine [13–15].

Structural analyses of conventional IAV HAs have demonstrated that their receptor-binding site (RBS), which is responsible for binding to sialylated glycans, uniformly comprises an edge and

Glossary

Antigenic drift: accumulation of individual point mutations resulting from the infidelity of the influenza virus polymerase.
Antigenic shift: reassortment of genome segments to form a new IAV strain due to the segmented nature of the IAV genome.
Hemagglutinin (HA): IAV surface protein that facilitates entry into host cells by binding SA. 18 subtypes have been identified to date (H1–H18).
Host switch: ability of an IAV strain circulating within one species to establish sustained infection within and transmission between individuals in a different species.
Myxovirus resistance protein 1 (Mx1/MxA): intracellular, dynamin-like GTPases that exert antiviral activity against IAV and numerous other RNA viruses.
Neuraminidase (NA): IAV surface protein that facilitates egress from the host cell by destroying the SA receptor.
Reassortment: exchange of genome segments between distinct parental IAVs upon co-infection of the same host cell.
Sialic acid (SA): cellular entry receptor for all known IAV excluding the bat IAV subtypes H17N10 and H18N11. In the context of IAV entry, SA represents the terminal glycan on numerous glycosylated proteins and may exhibit various linkages to the penultimate galactose residue.
Spillover infection: transmission of a pathogen from a reservoir species to a novel host species.
Viral ribonucleoprotein (vRNP) complex: comprises RNA-dependent RNA polymerase (PA, PB1, and PB2), oligomeric NP, and viral RNA.

a base section located on top of the HA globular head domain. The edge is composed of the structural elements 130-loop, 190-helix, and 220-loop, which together form a pocket that accommodates SA receptors. The base section comprises the four conserved residues, Y98, W153, H183, and Y195, which are responsible for anchoring the SA moiety [16,17]. However, the RBS of human and swine influenza HAs, which can accommodate α2,6-linked SAs, have been shown to be wider than the narrower conformation of avian HAs that bind to α2,3-linked SAs [18,19]. In line with this observation, earlier studies reported that avian H2 and H3 HAs acquired no more than two mutations in the 220-loop (Q226L and G228S) following zoonotic transmission, resulting in a widening of the RBS and a switch from α2,3- to α2,6-receptor specificity [20–22]. Insertion of these substitutions into the avian H5 HA by reverse genetics (partially) altered the binding specificity from avian α2,3- to human a-2,6-linked SA receptors [23,24]. Moreover, human-adapted H1 HAs prefer α2,6-linked SA owing to two amino acid substitutions in the 190-helix (E190D) and the 220-loop (G225D) [22,25]. Thus, a few mutations may be sufficient to switch receptor specificity to the human binding type.

Intriguingly, the HA proteins of the New World bat IAVs H17N10 and H18N11 are unable to bind SA residues, despite a high degree of sequential and structural homology [26–28]. Instead, their HA molecules (H17 and H18) MHC-II molecules for cell entry [29,30]. Despite the ability of these viruses to infect cells expressing human MHC-II *in vitro*, the relevance of this interaction remains unclear since these viruses have to date only been identified by sequencing in bats [31].

NA-Facilitated Release

Within the viral life cycle, NA performs a complementary function to HA and is responsible for facilitating particle release from an infected host cell by cleaving terminal SAs. NAs of avian origin specifically catalyze the cleavage of α2,3-linked SAs, while NAs of human IAV strains cleave both α2,3-and α2,6-linked SAs [32,33]. Thus, following zoonotic spillover of avian IAVs, differences in the glycan composition of the new host require not only adaptation of the HA receptor specificity, but also co-adaptation of NA [32,34]. Variations in the length of the NA stalk can similarly contribute to host tropism. The deletion of ~20 amino acids in the stalk region of some avian NA subtypes was found to interfere with the release of virions from cells, not by reducing the enzymatic activity itself, but by reducing the accessibility of the NA active site towards large SA substrates [35,36]. Interestingly, these stalk deletions are frequently selected after the transmission of IAVs from waterfowl to domestic poultry [36–38]. Although the biological significance of the selection of NA stalk deletions in poultry remains unclear, it has been suggested that the reduced ability of shorter NA variants to cleave SA [35,38] is required to counterbalance the decreased receptor affinity of the zoonotic HA [38,39]. However, in the ferret model, which is currently the animal model that best reflects influenza infections in humans, viruses bearing NA stalk deletions exhibit impaired (droplet) transmissibility, owing to their reduced ability to penetrate mucus and prevent virion clumping [40].

Polymerase Complex

Influenza viruses perform transcription and replication of their genome in the host cell nucleus, utilizing elements of the host cell transcription machinery. Thus, the heterotrimeric IAV polymerase complex (subunits PB2, PB1, and PA) requires interactions with numerous host cell factors for successful replication, and multiple mutations in the polymerase proteins have been shown to confer species tropism. Perhaps the best-known adaptive mutation is the amino acid substitution E627K in PB2: human-adapted IAVs typically encode $PB2_{627K}$, whereas avian IAVs code for $PB2_{627E}$ [41]. Notably, several studies reported that $PB2_{627K}$ alone is sufficient to allow avian IAVs to overcome mammalian host restriction and increase their virulence [41–43]. To date, three out of four pandemic IAVs (1918, 1957, and 1968) and all seasonal strains in the 20th

century have harbored $PB2_{627K}$ [44]. By contrast, the 2009 pandemic 'swine flu' virus and its presently circulating descendants encode the avian-like $PB2_{627E}$, which is compensated by two alternative substitutions at positions 590 and 591 (G590S and Q591R) in PB2 that also confer similarly efficient replication properties in humans [45,46]. Position 627 in PB2 is also involved in the species-specific regulation of the polymerase activity via the host factors acidic leucine-rich nuclear phosphoprotein 32 family member A (ANP32A) and B (ANP32B) [47,48]. The human homologs of ANP32A and ANP32B bolster the activity of human-adapted polymerases carrying $PB2_{627K,}$ but not polymerases harboring the avian-like $PB2_{627E}$ [47,48]. The activity of polymerases containing $PB2_{627E}$ is instead increased in the presence of the avian ANP32A variant, which has an avian-specific stretch of 33 additional amino acids [47,48]. Interestingly, G590S and Q591R in PB2 of H1N1pdm09 appear to compensate for the lack of 627K and enable efficient usage of ANP32A and ANP32B [45,49]. Finally, human importin-α1 and -α7 have previously been identified as positive regulators for human-adapted, $PB2_{627K}$-containing polymerases but had no enhancing effect on avian-like polymerases with $PB2_{627E}$ [50,51]. In addition to PB2, selective mutations in other components of the viral polymerase can also contribute to human species adaptation [45].

Antiviral Mx1/MxA System

The innate immune system constitutes a rapidly active first line of defense against pathogens such as influenza viruses. To restrict viral replication at an early stage and prevent further viral spread, the innate immune response induces soluble antimicrobial cytokines, most importantly type I and III interferons (IFN). Specifically, virus-infected cells synthesize and secrete type I (α/β) and III (λ) IFNs that stimulate susceptible neighboring cells to express IFN-stimulated genes (ISGs) [52,53]. Comprehensive screens have revealed that the group of ISGs comprises several hundred different factors [54,55], many of which exert direct antiviral activity. One of the most potent of these factors for suppressing IAV replication is the human **myxovirus resistance protein 1** (**MX1**, hereinafter referred to by its historical name, **MxA**) [56]. Interestingly, despite a close phylogenetic relationship, all avian MxA orthologs (designated Mx1 in non-human species) tested to date lack any detectable antiviral activity [57,58]. The degree of IAV sensitivity to MxA-mediated restriction was previously shown to be determined by NP [59]. While human-adapted influenza strains can overcome MxA suppression, influenza viruses of avian origin are generally more sensitive and are unable to replicate efficiently in the presence of MxA [60]. The ability of human-derived IAVs to overcome MxA restriction has been putatively mapped to several surface-exposed patches of distinct amino acids in NP, with characteristic signatures identified in NP of the 1918 and 2009 H1N1pdm09 strains as well as all of their seasonal descendants [61]. The fact that these amino acid signatures are almost absent in NPs of avian isolates suggests that human but not avian IAVs are under constant evolutionary pressure by MxA restriction. As demonstrated for the H1N1pdm09 virus and more recently for a Eurasian avian-like swine IAV, (partial) MxA resistance can also emerge upon circulation in intermediate hosts, such as swine, the Mx1 protein of which also exhibits antiviral activity [61,62]. Consequently, human MxA might not represent an impenetrable barrier to some swine IAV strains if their NPs require only minor adaptation to acquire full MxA resistance [61].

Mechanisms of Zoonotic Transmission

The natural ecology of IAV is complex. The natural hosts of conventional IAVs are aquatic waterfowl and shorebirds [63], in which infection often proceeds without causing any signs of disease [64–66]. However, the virus can also readily infect other avian species, most notably domestic poultry, as well as numerous mammalian species, including pigs, horses, and humans [63]. Given the proximity of humans to many of these animal species, this broad theoretical tropism raises the question of which reservoir poses the greatest threat of zoonotic spillover and generation of (pre-)pandemic IAV, and which precise mechanisms underlie adaptation to human hosts.

Due to limited data on the actual exposure rates of humans to zoonotic transmission events, it is difficult to estimate or predict the feasibility of IAV adaptation to humans. For the main zoonotic avian IAVs, including the Asian H5N1 and H7N9 subtypes, serological surveys covering more than 15 years reported a relatively low seroprevalence (<4%) among villagers and poultry workers in China and South East Asia [67–72], indicating that the previously described molecular barriers sufficiently block interspecies transmission. Direct transmission from poultry to humans does appear responsible for sporadic H5N1 and H7N9 **spillover infections**, which have caused 455 deaths among 861 cases documented from 2003 to 2020 (H5N1)[i] and 616 deaths among 1568 cases reported between 2013 and 2020 (H7N9)[ii]. Thus, despite a high case fatality rate (~52% for H5N1 and ~39% for H7N9), productive infections by avian IAV appear rare, and human-to-human transmission even more rare. Productive avian IAV infection might predominantly occur among persons with comorbidities or genetic defects in the innate and adaptive immune systems. Indeed, disease-enhancing genetic defects in the innate immune response have previously been described for patients with circulating human IAV [73,74]; however, conclusive genetic studies of patients infected with H5N1 or H7N9 are lacking. These observations suggest that the number of concomitant adaptive mutations required for direct transmission of IAV from avian to human hosts is so high as to render such an occurrence exceedingly rare.

In contrast to avian hosts, transmission of IAV from pigs to humans appears to be a more common event. Accordingly, most recent data demonstrate that the seroprevalence for antibodies to swine-derived H1N1 is significantly higher in swine workers (~10%) compared with poultry workers (<4%) [67–72,75]. Importantly, swine harbor several human-like molecular features that are lacking in poultry, including α2,6-linked SAs in their respiratory tract [11,12] and the earlier-mentioned antiviral porcine Mx1 protein. Intriguingly, the presence of both α2,3- and α2,6-linked SA receptors in the respiratory tract of pigs makes them (at least theoretically) highly susceptible to concurrent infections with both avian-derived IAV and mammalian-derived IAV, allowing co-infection with multiple strains and consequent reassortment (Figure 2A). Therefore, pigs are considered an optimal 'mixing vessel' and are thought to be pivotal for the emergence of new pandemic IAVs. Moreover, reverse human-to-pig infections and circulation of human IAVs in pig populations have both been reported, including for H1N1pdm09 [76].

The ability of a zoonotic IAV to establish sustained transmission chains between humans depends on two factors: adaptation to human host restriction factors such as the cellular entry receptor and MxA; and the procurement of a novel antigenic specificity that is not efficiently restricted by pre-existing immunity within the human population. While the unique ability of influenza viruses to alter their genome through both point mutations and genome segment reassortment provides two potent mechanisms for adaptation, the precise contributions of each remain unclear. Several point mutations conferring efficient human replication are observed with high regularity: for example, the rapid selection of the human-like $PB2_{627K}$ in patients with H5N1 or H7N9 viruses. These mutations may even occur after the initial **host switch** event. By contrast, there are no reports suggesting that other determinants, such as SA specificity of HA or MxA-mediated restriction, can be overcome by avian-derived viruses in index patients, but rather appear to require reassortment in the human or an animal host. This reassortment matches surface proteins harboring unique antigenic signatures with internal viral proteins that are already partially or even fully adapted to the human host. In the case of MxA, insertion of MxA escape mutations into the NP of avian IAV were shown to reduce the viral fitness [77], and accordingly there are no reports to date of stable reverse host switching from humans to birds. In swine, however, MxA escape mutations can be acquired by gradual adaptation to the less-potent swine Mx1 [61], bolstering the concept that pigs may not only serve as a mixing vessel, but also as a favorable intermediate host that allows the selection of NP variants with partial or

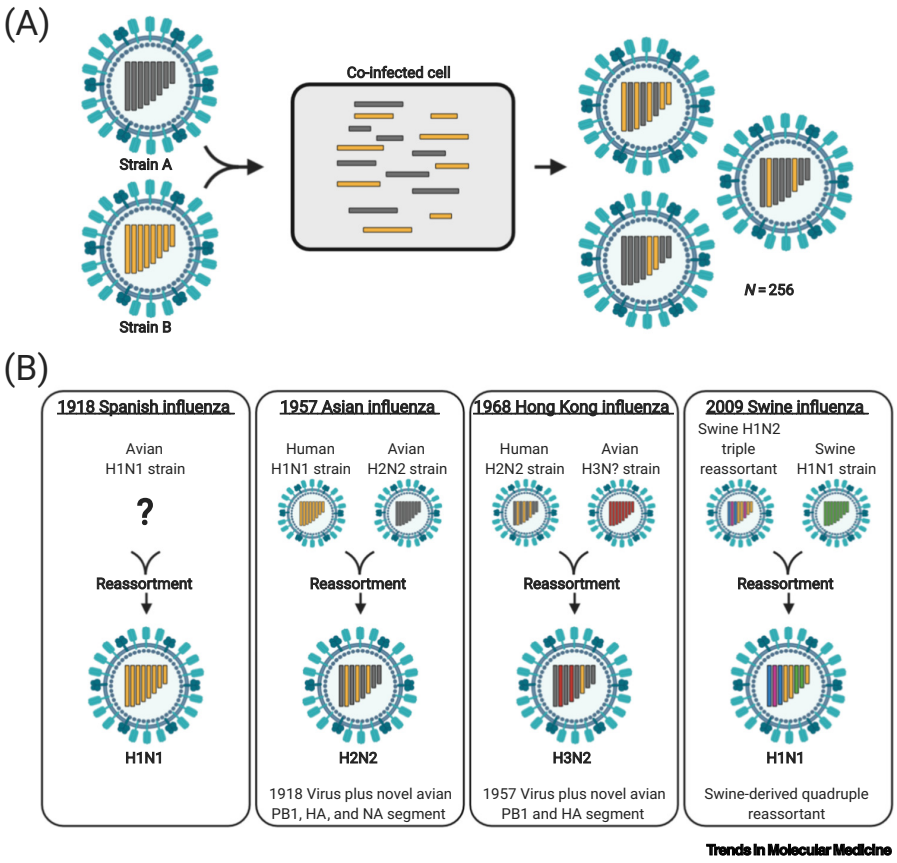

Figure 2. Reassortment Is the Key Driver in Influenza A (IAV) Evolution. (A) Co-infection of a cell with two distinct parental viruses allows the exchange of genome segments (i.e., reassortment) and the generation of progeny virions with a novel genomic composition. In theory, co-infection with two distinct strains can give rise to 256 different genotypes. (B) Reassortment was responsible for at least three of four pandemic IAVs in the past. It is currently unclear whether all eight genome segments of the 1918 pandemic virus were of avian origin or whether reassortment in humans or swine preceded the pandemic outbreak. Descendants of the 1918 pandemic virus remained in the human population as seasonal influenza epidemics and were subject to antigenic drift. Reassortment between the drifted 1918 strain and an avian H2N2 strain resulted in the Asian influenza pandemic in 1957. Similarly, reassortment between the drifted 1957 pandemic virus and an avian H3N? virus gave rise to the 1968 Hong Kong influenza pandemic. Until 2009, the H1N1 and the H3N2 strains co-circulated as seasonal influenza epidemics in the human population. In 2009, the H1N1pdm09 swine influenza virus emerged as a quadruple reassortant between a triple reassortant virus of the North American swine lineage and an H1N1 Eurasian avian-like swine virus. The H1N1pdm09 virus replaced the descendants of the 1918 virus, but not the H3N2 virus in the human population. Figure generated with BioRender.

Clinician's Corner

IAVs cause both annual epidemics and occasional pandemics. While annual epidemics are caused by IAV strains that are already established in the human population and to which some degree of population-wide protective immunity exists ('seasonal flu'), pandemics occur when an antigenically novel strain of zoonotic origin is introduced to the human population against which there is little pre-existing cross-immunity.

Although efficacious vaccines exist that protect against seasonally circulating, epidemic IAV, these vaccines do not protect against pandemic strains because the latter, by definition, are antigenically novel. Due to advances in vaccine production technology, vaccines against new IAV strains can be produced and distributed within months, as was the case for the 2009 IAV pandemic. Nonetheless, such vaccines are usually not available during the early phase of the pandemic. Numerous efforts are in progress to develop 'universal' IAV vaccines that would provide protection against not only seasonal IAV but also, in theory, novel zoonotic IAV.

Several therapeutic agents are approved in various jurisdictions for the treatment of IAV, including oseltamivir, zanamivir, peramivir, and baloxavir. The former three drugs target the viral NA, while the latter inhibits the viral polymerase. Resistance to all IAV antiviral drugs is possible through the accumulation of escape mutants, and development of resistance is periodically observed in both seasonal IAV and the H1N1pdm09 strain. The genetic basis of resistance to each drug is well understood and, therefore, the sensitivity of a circulating strain can be predicted by genetic analysis.

even full MxA resistance. Thus, these emerging mechanistic data support the hypothesis that MxA and its homologs exert evolutionary pressure on IAVs.

The genetic composition of the 1957, 1968, and 2009 pandemic strains provides strong evidence that MxA antagonism may represent an underappreciated major host restriction factor. The NP-encoding segment, which is the primary determinant of MxA resistance, was derived from human IAV by reassortment for the pandemic 1957 and 1968 influenza viruses, while the 2009 H1N1pdm09 virus acquired a pre-adapted NP genome segment from a circulating swine IAV strain. In each case, this human-like NP segment was paired with surface proteins of putative

avian origin [1] (Figure 2B). Although the immediate predecessor of the 1918 influenza pandemic remains a controversial topic, it is tempting to speculate that it reassorted with circulating human IAV or became established in swine as an intermediate host between its avian progenitor and host switch to humans [78,79], which would support the need for adaptation of NP in a non-avian host. The hypothesis that MxA resistance is a primary human restriction factor for IAV that cannot be overcome by a single-point mutations may explain why direct avian-to-human transmission does not appear capable of causing sustained transmission chains, and suggests that reassortment in humans or a third species is indispensable for the stable establishment of zoonotic IAV in humans.

Concluding Remarks

Based on the observations and data presented in this review, the likelihood that a presently circulating avian IAV (such as an Asian H5N1 or H7N9 strain) will directly cause the next pandemic appears to be low. As a result of the insights described herein, the focus of public health surveillance has recently begun to emphasize sequencing IAV among domestic swine populations to identify potentially pandemic strains [75,76]. Since 2009, globally occurring reverse zoonotic infections have re-introduced a set of pandemic IAV genome segments into swine populations, where they gave rise to novel reassortants. Initial reports confirmed the presence of triple-reassortant strains bearing numerous genetic markers indicative of the potential for human infection and transmission. Nonetheless, some caution may also be warranted: due to the increase in swine surveillance, it remains unclear whether these potentially pandemic strains are new occurrences due to the modernization of swine production and other developments [80], or whether similar viruses have been present among this population for years yet escaped detection in the absence of comprehensive surveillance. Furthermore, the 2009 H1N1pdm09 exhibited several genetic signatures that were previously thought to result in inefficient replication in humans (see Outstanding Questions). Despite an unprecedented repository of previously successful adaptive IAV mutations, there remains a real possibility that a strain may emerge that overcomes the molecular barriers described in this paper using as-yet unidentified genetic variations. Hence, it may be advisable to move away from large-scale sequencing of animal-derived isolates, and the inherent potential for false positives, towards a more rational approach combining genetic screening of animal populations with laboratory testing of selected isolates for their ability to efficiently use human host cell receptors, to escape pre-existing humoral immunity and to overcome MxA-imposed restriction.

Outstanding Questions

Should other animal populations also be screened for the presence of potentially prepandemic IAV strains?

Do the known adaptive mutations represent the optimal fitness for human-adapted viruses, or could there be novel sequences or sequence combinations that might go unnoticed in large-scale surveillance? If so, can high-throughput human-like model systems be developed to functionally test the adaptation of IAV circulating among animals?

Given the apparent presence of numerous prepandemic strains in the global swine population, are there additional human restriction factors that remain unknown? In the absence of this knowledge, is there a risk of excessive false positives that may have a detrimental effect in light of public weariness of viral pandemics?

Is a national stockpile of IAV vaccines adequate for preparation and what would be the best mechanism to select for donor strains?

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Resources

[i] www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

[ii] www.fao.org/ag/againfo/programmes/en/empres/H7N9/situation_update.html

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