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The Epidemiology, Virology, and Pathogenicity of Human Infections with Avian Influenza Viruses

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Influenza is a global challenge, and future pandemics of influenza are inevitable. One of the lessons learned from past pandemics is that all pandemic influenza viruses characterized to date possess viral genes originating from avian influenza viruses (AIVs). During the past decades, a wide range of AIVs have overcome the species barrier and infected humans with different clinical manifestations ranging from mild illness to severe disease and even death. Understanding the mechanisms of infection in the context of clinical outcomes, the mechanism of interspecies transmission, and the molecular determinants that confer interspecies transmission is important for pandemic preparedness. Here, we summarize the epidemiology, virology, and pathogenicity of human infections with AIVs to further our understanding of interspecies transmission.

Influenza A viruses are classified into different subtypes based on the major antigenic surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). To date, 16 HA and 9 NA subtypes have been identified in aquatic birds. Aquatic birds are considered the natural reservoir in which avian influenza viruses (AIVs) typically cause asymptomatic infections with high viral replication rates. Some subtypes of AIVs can cause severe disease in poultry, especially in chickens. Based on their virulence in chickens, AIVs are therefore divided into high-pathogenicity AIVs (HPAIVs) and low-pathogenicity AIV (LPAIVs) (see fao.org/avianflu/en/index.html). All HPAIVs are of the H5 or H7 subtypes. LPAIVs of the H5 and H7 subtypes

can evolve in poultry to become HPAIVs, causing severe disease and high mortality. In 1878, Perroncito was the first to report a serious outbreak of chicken disease in Italy, which was known as fowl plague, and was confirmed as an AIV of the H7N7 subtype in 1955 (Alexander and Brown 2009). Since then, a substantial number of poultry outbreaks caused by HPAI H5 and H7 subtype viruses have been reported worldwide, posing a great challenge for the poultry industry.

AIVs have occasionally caused human infections with clinical manifestations ranging from mild illness to severe disease and even death. Although no sustained human-to-human transmission has occurred, human infections

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with AIVs remain a cause for great concern for at least two reasons: First, viruses of the H5 and H7 subtypes may cause severe human disease with high case fatality rates (CFRs) (40%–60%), and second, recent studies indicate that HPAI H5 viruses require only a few amino acid mutations to become airborne transmissible among ferrets, highlighting the potential pandemic risk caused by AIVs (Herfst et al. 2012; Imai et al. 2012).

EPIDEMIOLOGY OF HUMAN INFECTIONS WITH AIVs

Because of the species barrier, AIVs rarely infect humans. In recent decades, both HPAIVs and LPAIVs have caused sporadic human infections, which are typically transmitted directly from poultry to humans. HPAIVs (H5N1, H5N6, H7N7, H7N3, and H7N9) and LPAIVs (H7N2, H7N3, H9N2, H7N9, H6N1, H10N7,

and H10N8) have all caused human infections (Fig. 1). However, none of these avian viruses has transmitted efficiently among humans.

Human Infections with HPAI H5 Viruses

In 1997, the first laboratory-confirmed human infection with an HPAI H5N1 virus was documented in a 3-yr-old boy with acute pneumonia and respiratory distress syndrome in the Hong Kong Special Administrative Region (SAR) of China (Fig. 1). This fatal case showed that a purely AIV can cause respiratory disease and death in humans (Claas et al. 1998; Subbarao et al. 1998; Bender et al. 1999). Seventeen patients were subsequently diagnosed, including six fatal cases in Hong Kong. In 2003, human infections with HPAI H5N1 viruses reemerged in Asia (Peiris et al. 2004). Since then, as of September 2019, 861 cases of laboratory-

Figure 1. The history of human infections with avian influenza viruses (AIVs). Arrows in red and blue indicate human infections with high-pathogenicity AIVs (HPAIVs) and low-pathogenicity AIVs (LPAIVs), respectively. The length of the arrows represents the time frame during which human cases were reported. (USA) United States of America, (UK) United Kingdom, (ITA) Italy, (NLD) the Netherlands, (CAN) Canada, (EGY) Egypt, (AUS) Australia, (MEX) Mexico, (CHN) China.

confirmed human infections with highly pathogenic H5N1 AIVs have been reported in 17 countries, including 455 deaths (see who.int/influenza/human_animal_interface/2019_09_27_tableH5N1.pdf). Egypt has reported 359 human cases, and China, Indonesia, Vietnam, and Cambodia each have reported more than 50 human cases. Most of the HPAI H5N1 human cases (~75%) have occurred in young adults and children (<30 yr of age) (Qin et al. 2015).

In April 2014, the first human infection with an HPAI H5N6 virus (which possessed an HA of the same lineage as HPAI H5N1 viruses) was reported in the Sichuan Province of China (Pan et al. 2016). As of September 2019, 24 HPAI H5N6 human cases have been reported in China, 16 of which were fatal. No HPAI H5N6 human cases have been reported outside China, although poultry outbreaks caused by HPAI H5N6 viruses have been reported in other countries such as Vietnam.

Human Infections with H7 Subtype AIVs

The first influenza A virus isolate was the fowl plague virus (FPV) isolated from chickens in 1902 (Alexander and Brown 2009). In 1955, it was recognized as an influenza A virus through an antigenicity study of the viral nucleoprotein (NP). This virus is now recognized as an H7N7 subtype influenza virus (A/chicken/Brescia/1902 [H7N7]) (Alexander and Brown 2009).

In 2003, an HPAI H7N7 influenza virus outbreak resulted in the death of tens of millions of poultry in the Netherlands. During this outbreak, 89 poultry workers were infected; most of them presented with conjunctivitis, except one veterinarian who died of severe pneumonia (van Kolschooten 2003; Fouchier et al. 2004). Two H7N3 human cases were reported in Canada in 2004 (Tweed et al. 2004), caused by HPAI H7N3 and LPAI H7N3 viruses, respectively (Figs. 1 and 2) (Hirst et al. 2004).

In the spring of 2013, the first human infection with an LPAI H7N9 virus was reported in the Yangtze River Delta region of China (Gao et al. 2013b). The virus was not pathogenic to poultry, but most of the laboratory-confirmed

human infections resulted in severe illness, and even death (Gao et al. 2013b; Zhang et al. 2013b). Before the vaccination of poultry was implemented in mainland China, five waves of H7N9 outbreaks occurred (Zhu et al. 2018). During the fifth wave in 2016–2017, the LPAI H7N9 virus evolved into an HPAI H7N9 virus, which caused high mortality among chickens, and also infected humans (Deng et al. 2017; Ke et al. 2017; Yang and Liu 2017; Zhang et al. 2017a). As of September 2019, a total of 1568 laboratory-confirmed H7N9 human cases have been reported, with a CFR of ~39% (Wang et al. 2017) (see chinaivdc.cn/cnic/en/). All human H7N9 cases originated in China; two human H7N9 cases reported in Malaysia and Canada were traced back to travelers from China.

In January 2018, one human infection with an H7N4 subtype AIV was identified in the Jiangsu Province of China (Tong et al. 2018). This was the first human infection with an H7N4 virus. Since then, no additional H7N4 human cases have been reported (Figs. 1 and 2).

Human Infections with H9N2 Subtype AIVs

The first reported human infections with H9N2 AIVs occurred in Guangdong province in southern China in 1998, including two cases in Shaoguan city and three cases in Shantou city (Guo et al. 1999). The following year, two human infections with H9N2 AIVs were reported in Hong Kong (Peiris et al. 1999). As of September 2019, a total of 35 human infections with H9N2 AIVs have been reported in China, including one fatal case in an individual with underlying disease. Human infections with H9N2 AIVs have also been reported in other countries, including Pakistan, Bangladesh, and Egypt (Figs. 1 and 2). The reported number of human infections with H9N2 AIVs may be an underestimate because of the mild symptoms caused by H9N2 AIVs in humans. Serological studies indicate that additional human infections with H9N2 AIVs have occurred (Lu et al. 2008; Woo and Park 2008; Pawar et al. 2012; Shinde et al. 2012; Zhou et al. 2014; Li et al. 2017).

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Figure 2. Areas with confirmed human cases of avian influenza virus (AIV) infection since 2000. The affected countries are colored in red. Labels indicate cases other than H5N1; imported cases are shown in blue. The map was created with mapchart.net.

Human Infections with AIVs of Other Subtypes

In May 2013, the only human infection with an H6N1 virus was reported in Taiwan province, China (Wei et al. 2013). From November 2013 to February 2014, three human infections with H10N8 viruses were reported in Jiangxi province, China, two of which were fatal (Chen et al. 2014); since then, no other human infections with H10N8 viruses have been reported. Mild human infections with H10N7 AIVs have been reported previously in Egypt and Australia (Figs. 1 and 2) (Organization PAH 2004; Arzey et al. 2012). Serological evidence suggests that AIVs of the H4, H6, and H11 subtypes may also have caused human infections (Shortridge 1992; Peiris et al. 2007).

Comparative Epidemiology of Human Infections with H7N9 and H5N1 AIVs

Humans are generally not susceptible to AIV infections, mainly because of the differences in the receptor-binding specificity between human and AIVs. As a consequence, AIVs do not easily cross the species barrier to infect humans. However, as described earlier, AIVs occasionally infect humans. A major source of human infections with AIVs are live poultry markets, where people may be exposed to infected birds, to AIVs in the environment, or both (Wan et al. 2011; Han et al. 2013; Shi et al. 2013b). Limited human-to-human transmission of AIVs may have occurred (Olsen et al. 2005), including vertical transmission (Shu et al. 2006).

Most of the (fatal) human infections with AIVs have been caused by viruses of the H7N9 and H5N1 subtypes. Human H7N9 infections have mainly affected the elderly of >60-yr of age, whereas human H5N1 infections primarily have occurred in young adults. The sex ratio of human infections with H5N1 virus is balanced, but H7N9 AIVs have infected more males than females (with a ratio of 2:1). The CFR of human infections with H7N9 influenza viruses is ~39%, whereas that of human infections with H5N1 influenza viruses is ~60% (Cowling et al. 2013b; To et al. 2013; Qin et al. 2015).

VIROLOGICAL CHARACTERISTICS OF HUMAN INFECTIONS WITH AIVs

Typically, all eight viral RNA segments of AIVs that infect humans are derived from AIVs, and there has been no natural reassortment with influenza viruses prevalent in human or pig populations.

Receptor-Binding Profiles of AIVs That Cause Human Infections

Transmission of AIVs to humans is rare, in part because of host range restrictions that limit influenza virus transmissions between avian species and mammals. The host species restriction and cell and tissue tropism of influenza viruses are determined by several factors, including differences in receptor distribution between avian and mammalian cells and differences in the receptor-binding specificities of human and AIVs. The HA proteins of AIVs typically have a binding preference for sialic acid (SA) residues with an α -2,3-Gal terminating sequence found primarily on the surfaces of epithelial cells in the intestinal tract of ducks. In the human respiratory tract, α -2,6 SA receptors are predominantly expressed on ciliated cells in the upper respiratory tract (URT), whereas α -2,3 SA receptors are mainly present on nonciliated cells of the lower respiratory tract (LRT) and on type II pneumocytes (Fig. 3) (Connor et al. 1994; Shinya et al. 2006; Chandrasekaran et al. 2008). Accordingly, although AIVs can infect humans without a change in receptor-binding specificity, mutations that confer binding to α -2,6 SA receptors are likely critical to confer sustained human-to-human transmission. Replication of HPAI H5N1 viruses (which bind to α -2,3 SA receptors) in humans is mainly confined to the LRT, which may have limited the number of virus particles expelled through breathing and/or coughing.

Like all subtypes of AIVs, AIVs of the H7 subtype can be subdivided into two genetic lineages according to the geographic origin of the viruses: the North American lineage and the Eurasian lineage, with viruses from both lineages having infected humans (Fig. 1). Most Eur-

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Figure 3. Airway tract tissue tropism illustration and the receptor-binding profiles of seasonal influenza viruses, avian H5N1 viruses, and influenza A(H7N9) viruses.

asian H7 subtype viruses resemble HPAI H5N1 viruses in their preferential binding to α -2,3 SA receptors. However, the H7N9 viruses that emerged in 2013 can bind to both SA α -2,3-Gal and SA α -2,6-Gal receptors (Fig. 3) (Shi et al. 2013a; Tharakaraman et al. 2013; Watanabe et al. 2013; Xiong et al. 2013; Zhou et al. 2013). This dual receptor-binding profile may lead to more frequent human infections with H7N9 viruses, as compared with human infections with AIVs of other subtypes.

Key Mammalian-Adapting Molecular Markers of AIVs That Cause Human Infections

One of the most common amino acid substitutions associated with converting LPAI strains from avian- to mammalian-receptor specificity is the HA Q226L substitution (H3 numbering). For example, LPAI H9N2 isolates from birds can acquire a Q226L substitution that increases the viruses' receptor-binding affinity for mammalian cells and may facilitate the infection of mammalian hosts (Wan et al. 2008). With regard to human isolates of H7N9 viruses, A/Shanghai/1/2013 (encoding HA Q226) and A/Anhui/1/2013

(encoding HA L226) bind preferentially to avian α -2,3-Gal receptor analogs or to both α -2,3-Gal and α -2,6-Gal receptors, respectively (Yamada et al. 2006; Nidom et al. 2010; Yang et al. 2010; Srinivasan et al. 2013). The Q226L mutation has not been commonly observed in the HA of HPAI H5N1 field isolates. The HA G186V substitution (H3 numbering) is associated with the dual receptor-binding specificity of both HPAI and LPAI influenza H7N9 viruses (Shi et al. 2013a; Zhou et al. 2013; Zhu et al. 2017).

Although receptor-binding specificity is an important factor in the interspecies transmission of influenza viruses, additional factors are involved in the mammalian adaptation of AIVs. In 2012, Herfst and colleagues (Herfst et al. 2012) showed that introduction of the HA Q226L/G228S and PB2 E627K mutations into an H5N1 virus did not confer virus transmission via respiratory droplets among ferrets. However, respiratory transmission among ferrets occurred after the virus had been passaged 10 times in ferrets (Herfst et al. 2012), resulting in three additional mutations (PB1 H99Y, HA H110Y, and HA T160A) that might be essential for the viral respiratory droplet transmissibility in ferrets (Linster et al. 2014). In the study by Imai

et al. (2012), the artificially introduced substitutions HA N224K/Q226L (which confer binding to α -2,6-Gal receptors) and the additional HA N158D/T318I mutations (which emerged during two passages in ferrets) were required to generate an airborne-transmissible H5 virus (with the remaining seven segments from the human A/California/04/2009 H1N1 virus) in ferrets. Mutations at either position 158 or 160 resulted in loss of the N-glycosylation site at 158–160 of HA, which has been shown to affect the receptor-binding profiles and the virulence of HPAI H5 viruses (Herfst et al. 2012; Imai et al. 2012). Moreover, H1N1 virus genes encoding the acidic polymerase (PA) and nonstructural (NS1) proteins rendered the H5N1 virus transmissible by respiratory droplets between guinea pigs (Zhang et al. 2013c). Other H1N1 genes, including those that encode the NP, NA, and the matrix (M1) protein, as well as mutations in H5 HA that improve affinity for human-like airway receptors, could enhance the mammal-to-mammal transmission (Zhang et al. 2013c). These reports have established that mutant and/or reassortant H5N1 AIVs can transmit among mammals. Analyses of field viruses indicate that some circulating viruses already contain some of the mutations identified in these H5 transmission studies (Russell et al. 2012). Enhanced surveillance should be performed to closely monitor these mutations in zoonotic influenza viruses.

Besides HA-receptor binding, key factors for viral fitness in mammals include substitutions in the viral polymerase proteins, including PB2-E627K (Table 1) (Hatta et al. 2001). Avian influenza virus isolates exclusively contain glutamic acid at position 627 in PB2, whereas it is frequently mutated to lysine in human-derived isolates (Fonville et al. 2013; Manz et al. 2013). The PB2-E627K mutation is required for the airborne transmissibility of HPAI H5N1 viruses in ferrets and high viral replication in mice (Hatta et al. 2001; Herfst et al. 2012; Imai et al. 2012). However, avian H5N1 and H7N9 viruses that retained the avian-like glutamic acid at position 627 of PB2 have frequently caused fatal human infections (Zhu et al. 2015), indicating that PB2-E627K is not always strictly necessary for avian viruses to infect mammals.

EMERGENCE, EVOLUTION, AND DISSEMINATION OF AIVs THAT CAUSE HUMAN INFECTIONS

LPAI H7N9 Viruses

The influenza A(H7N9) viruses have caused five epidemic waves in humans in China. Since their emergence, studies have been conducted to learn about the viruses' origin and evolution. Most likely, the HA of these viruses originated from a duck influenza virus; the NA was related to N9 viruses detected in migratory birds, and the six internal genes were from avian influenza H9N2 viruses (Gao et al. 2013b; Liu et al. 2013).

Since the first detection of the novel H7N9 viruses in 2013, the six internal genes have frequently reassorted with those of H9N2 viruses cocirculating in poultry (Wu et al. 2013; Zhang et al. 2013a; Cui et al. 2014; Lam et al. 2015; Wang et al. 2016). From wave III onward, the evolution of the NP gene changed in that it was most frequently derived from cocirculating H7N9 viruses rather than from H9N2 viruses (Zhu et al. 2018). Dynamic reassortment events generated multiple genotypes. Most of these genotypes were transient, although some gradually became dominant. One of the genotypes, ZJ11-like viruses, emerged in the late phase of wave III, slightly increased in frequency late in wave IV, and substantially increased in frequency from the beginning of wave V. The ZJ11 genotype viruses accounted for most of the human infections in wave V (Zhu et al. 2018). Overall, the H7N9 viruses have evolved rapidly since their emergence.

A “genetic tuning” mechanism was proposed to explain the possible process of interspecies transmission of avian H7N9 viruses (Fig. 4) (Wang et al. 2014a; Zhu and Shu 2015). Through this process, H7N9 viruses may have first acquired mutations that facilitate replication in poultry before acquiring mutations that facilitate replication in mammals.

HPAI H7N9 Viruses

Currently, the LPAI H7N9 viruses have evolved into two regionally distinct lineages—the Yangtze Delta lineage and the Pearl Delta lineage—

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Table 1. Molecular markers associated with increased transmissibility in animal models of AIVs

Function	Gene	Position and AA	Reference(s)
Enhanced binding to human-type receptor	H5	Loss of glycosylation site at 158–160	Herfst et al. 2012; Imai et al. 2012
		186K	Chutinimitkul et al. 2010; Yamada et al. 2006
		190D	Chen et al. 2012; Stevens et al. 2006
		196R/H	Chen et al. 2012; Watanabe et al. 2011; Yamada et al. 2006
		222E/Q	Guo et al. 2017; Manz et al. 2010
		224K	Imai et al. 2012
		226L	Harvey et al. 2004; Herfst et al. 2012; Imai et al. 2012; Stevens et al. 2006; Chutinimitkul et al. 2010
		227N/R	Chen et al. 2012; Chutinimitkul et al. 2010; Gambaryan et al. 2006; Guo et al. 2017; Shinya et al. 2010
		228S	Chutinimitkul et al. 2010; Harvey et al. 2004; Herfst et al. 2012; Stevens et al. 2006; Wang et al. 2010
	H6	225D	de Vries et al. 2017b
		226L	Qu et al. 2017
	H7	Gain of glycosylation site at 133	Srinivasan et al. 2013
		186V	Xiong et al. 2013; Yang et al. 2010; Shi et al. 2013a; Tharakaraman et al. 2013
		193T	de Vries et al. 2017a
		224K	de Vries et al. 2017a
		226L/I	Shi et al. 2013a; Srinivasan et al. 2013; Tharakaraman et al. 2013; Xiong et al. 2013
		228S	Srinivasan et al. 2013; Tharakaraman et al. 2013
Increased polymerase activity in mammalian host	H9	226L	Wan et al. 2008
	PB2	Q591K	Chan et al. 2016; Mok et al. 2014
		E627K	Herfst et al. 2014; Mok et al. 2014; Zhu et al. 2015
		D701N	Mok et al. 2014; Zhu et al. 2015

Figure 4. Proposed "genetic tuning" mechanism for influenza A(H7N9) viruses. (Created from data in Wang et al. 2014a and Zhu and Shu 2015.)

based on the sequences of their HA and NA genes (Wang et al. 2016). Although HPAI H7N9 viruses were first isolated in the Pearl Delta region, they have exclusively belonged into the Yangtze Delta lineage (Ke et al. 2017; Yang et al. 2017). HPAI H7N9 viruses arose from the LPAI H7N9 progenitors. The acquisition of multiple basic amino acids at the HA cleavage site (a hallmark of HPAI, discussed in more detail later) occurred in late May 2016 in the Pearl Delta region. Different motifs of basic amino acids at the HA cleavage site have been detected in HPAI H7N9 viruses in poultry (Shi et al. 2017). In contrast, HPAI H7N9 viruses isolated from humans bear only two types of amino acid insertions (Yang et al. 2017).

Dynamic reassortment of the internal genes of LPAI H7N9 and H9N2 viruses has been broadly reported (Wu et al. 2013; Zhang et al. 2013a; Cui et al. 2014; Lam et al. 2015; Wang et al. 2016). Similar to LPAI H7N9 viruses, HPAI H7N9 viruses also reassorted with LPAI H7N9 or H9N2 viruses in poultry (Yang et al. 2017). Several genotypes emerged that differed in their internal genes. One of these genotypes (Genotype II [Yang et al. 2017]) may have a fitness advantage over the others, because this genotype was the only one to spread from Guangdong province to other regions.

HPAI H5N6 Viruses

HPAI H5N6 viruses are currently circulating in poultry throughout China and South East Asia and occasionally infect humans in China. The HA gene of H5N6 viruses originated from the clade 2.3.4 HPAI H5N1 viruses that emerged in chickens in China in 2005. In 2007, the HPAI H5 viruses evolved into clade 2.3.4.4. From 2009 to 2012, the clade 2.3.4.4. H5 viruses reassorted with waterfowl viruses of different NA subtypes, including N2, N6, and N8, generating HPAI H5N2/N6/N8 viruses (Yang et al. 2016). Phylogenetic and the “time to most recent common ancestor” (tMRCA) analyses suggested that two independent reassortment pathways generated two subclades of H5N6 viruses between 2011 and 2013. In the first pathway, H5N2 viruses with a clade 2.3.4.4 HA gene may have reassorted

with an H6N6 virus between mid-2011 and mid-2012 to acquire the N6 NA gene; then, further reassortment might have occurred involving the acquisition of the six internal genes of poultry clade 2.3.2.1c H5N1 viruses, generating so-called “Reassortant A” viruses. In the second pathway, reassortment likely occurred among an H5N8 virus with a clade 2.3.4.4 HA gene, an H6N6 virus with an NA gene containing a deletion in the stalk region, and poultry clade 2.3.2.1c H5N1 viruses, which provided the internal genes; the resulting H5N6 virus was designated “Reassortant B.” Since 2015, consecutive reassortment events of “Reassortant B” H5N6 viruses with the six internal genes from chicken H9N2 viruses have resulted in so-called “Reassortant C” H5N6 viruses (Yang et al. 2016).

All three reassortant viruses have caused human infections. The virus isolated from the first reported H5N6 human case in Sichuan Province in 2014 was a “Reassortant A” virus. Viruses from human cases in Guangdong Province in late 2014 had the gene cassette of “Reassortant B,” and human isolates from Yunnan and Guangdong provinces since 2015 have belonged to “Reassortant C.” Since then, HPAI H5N6 viruses have continued to evolve. In 2016, two H5N6 viruses were isolated from two patients in China. The internal gene cassettes of these two strains differ from those reported previously, representing two novel genotypes of H5N6 viruses (Zhang et al. 2017b). Another novel influenza H5N6 virus with a unique internal gene cassette was isolated from a patient in Jiangsu province in China in 2018 (unpubl. data). Most likely, novel genotypes of H5N6 viruses will continue to emerge.

CLINICAL FEATURES OF HUMAN INFECTIONS WITH AIVs

Clinical Manifestations

AIVs generally do not cause clinical symptoms in their natural reservoir, but poultry (such as chickens) infected with HPAIVs usually present with severe syndromes and high mortality. The clinical presentation of human infections with AIVs varies from mild respiratory symptoms

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and conjunctivitis to acute pneumonia and even death. Most humans infected with highly pathogenic H5 AIVs develop a severe illness with a high case fatality rate (Yuen et al. 1998; Uyeki 2009; Shinya et al. 2011). Before 2013, conjunctivitis was the main manifestation of human infections with viruses of the H7 subtype, with the exception of a fatal human infection with an HPAI H7 virus (Koopmans et al. 2004). Respiratory conditions, including pneumonia and respiratory distress syndrome, are the main manifestations of human infections with the novel H7N9 AIVs (Gao et al. 2013a; Lu et al. 2013; Yu et al. 2013a,b). Although the novel H10N8 viruses are of the low-pathogenicity type, they can cause serious illness or death (Chen et al. 2014). Symptoms of human infection with H9N2 viruses are generally mild, mainly manifested as respiratory symptoms such as fever.

Viral Factors That Contribute to Severe Clinical Symptoms in Infected People

Pathogenesis is the result of complex interactions between the virus and host, with many factors from both the virus and the host involved in this interplay. Besides the HA-mediated receptor-binding specificity discussed above, key to viral pathogenicity is the presence of a multi-basic HA cleavage site (MBCSs). HPAIV generally encode a MBCS at the HA cleavage site (Bosch et al. 1981; Webster and Rott 1987), whereas HAs from mammalian viruses or LPAIV usually encode only a single basic residue at their cleavage site (Garten and Klenk 1999; Klenk and Garten 1994). The presence of the polybasic residues allows the HA to be cleaved by enzymes that are expressed in a wide range of tissues, whereas the cleavage site that contains a single basic residue can only be cleaved by enzymes in the respiratory tract or intestinal tract. Because HA cleavage is required for viral infectivity, the polybasic cleavage site broadens the tissue range of the virus and is strongly associated with systemic spread and increased infectivity and virulence in birds and mammals (Hatta et al. 2001; Tanaka et al. 2003; Suguitan et al. 2012). HPAI H5N1 viruses with an MBCS cause severe infections in hu-

mans. Some H7N9 viruses also have acquired an MBCS to become highly pathogenic in chickens. HPAI H7N9 viruses with an MBCS showed increased disease severity in the ferret model compared with an LPAI H7N9 virus (Imai et al. 2017). However, LPAI H7N9 viruses that lack an MBCS still cause severe disease in most human cases (Gao et al. 2013a,b). Thus, the MBCS is a key virulence factor for AIVs in avian species, but not a key determinant for the severity of AIV infections in humans.

Substitutions in the ribonucleoprotein (RNP) complex (which comprises the polymerase subunits PB2, PB1, and PA and NP) are frequently linked to increased replication and virulence of viruses. PB2 is a major host range determinant of influenza viruses. One of the most critical substitutions is PB2-E627K, which, as already mentioned, contributes to human infections with AIVs. The PB2 E627K mutation is strongly linked to increased virulence in mice of HPAIV H5N1 (Hatta et al. 2001; Maines et al. 2005; Chen et al. 2007), HPAIV H7N7 (Munster et al. 2007), LPAIV H7N9 (Mok et al. 2014; Zhu et al. 2015), and the 1918 H1N1 virus (Qi et al. 2012).

Most influenza H7N9 viruses isolated from chickens cause no clinical symptoms in mice and are nonlethal in ferrets (Zhang et al. 2013b; Shi et al. 2017). LPAI or HPAI H7N9 viruses isolated from patients cause mild to severe disease or are lethal in these mammal models, respectively (Richard et al. 2013; Watanabe et al. 2013; Zhu et al. 2013; Imai et al. 2017). Of note, an HPAI H7N9 virus was shown to be more pathogenic in mice and ferrets than an LPAI H7N9 virus (Imai et al. 2017). In addition, H7N9 viruses isolated from humans are generally more transmissible in ferrets than those isolated from avian species (Zhang et al. 2013b; Zhu et al. 2013; Imai et al. 2017; Shi et al. 2017). This may be due to mammalian-adapting mutations, including PB2 E627K. Studies have shown that after replication in mice and ferrets, LPAI or HPAI H7N9 viruses isolated from avian species acquired mammalian-adapting mutations such as PB2 E627K or PB2 D701N (Zhu et al. 2015; Shi et al. 2017). However, no such mutations occurred when the virus replicated in chickens. When HPAI H7N9 viruses of avian origin ac-

quired mammalian-adapting mutations, they became lethal in mice and ferrets and highly transmissible among ferrets (Shi et al. 2017).

Some mutations in PB1 (such as PB1 473V and 598P) and NP (such as NP 357K) have also been found to increase the pathogenicity of AIVs in mammals (Xu et al. 2012; Zhu et al. 2019). Other genes, including those encoding NS1 and the nuclear export protein (NEP), also encode molecular markers associated with pathogenicity. NS1 is critical for natural infection because it inhibits the host innate immune response (Donelan et al. 2003; Hale et al. 2008) through species-specific interactions with multiple host proteins (Rajsbaum et al. 2012). Mutations in NS1 have been frequently observed during mouse adaptation of AIVs (Dankar et al. 2011; Zhang et al. 2011). When crossing the species barrier, HPAIV H5N1 could acquire the adaptive substitution M16I in NEP to escape restricted viral genome replication in human cells (Mänz et al. 2012; Reuther et al. 2014).

Host Factors That Contribute to Severe Clinical Symptoms in Infected People

Several studies have explored the host factors that contribute to the severe clinical symptoms caused by AIV infections, including cytokine storms, preexisting immunity, age biases in exposure to infected poultry (Cowling et al. 2013a; Rivers et al. 2013), and host genes. However, none of these studies can fully explain the patterns of severe disease and mortality. A recent study revealed that childhood HA imprinting could provide profound, lifelong protection against severe infection and death caused by H5N1 and H7N9 viruses (Gostic et al. 2016). This study highlighted the contribution of antigenic imprinting to the severity of clinical symptoms. Nevertheless, we still have a long way to go before we fully understand the complex interplay between the pathogen and the host.

The Cytokine Storm

One hallmark of human HPAI H5 infections is a rapid and robust cytokine response, often referred to as a “cytokine storm” or hypercytoki-

nemia. This buildup of cytokines causes an inflammatory environment at the site of infection, leading to immune cell infiltration (de Jong et al. 2006; Zhou et al. 2013; Wang et al. 2014b; Guo et al. 2015). Following infection with HPAI H5N1 viruses in animal models, including chickens, ferrets, and rhesus macaques, increased expression of specific pro-inflammatory cytokines, particularly interleukin (IL)-6 and IL-8, was observed, suggesting that IL-6 and IL-8 may be key regulators leading to worsened pathology of viruses. Upon human infections with H5N1 viruses, a similarly robust cytokine response occurs with the up-regulation of IL-6, IL-10, and tumor necrosis factor (TNF)- α . The levels of IP-10, MIG, MCP-1, IL-6, IL-8, and IFN- α in sera from H7N9 virus-infected patients were significantly higher than those of healthy people (Zhou et al. 2013). Therefore, the “cytokine storm” caused by disorder of the natural immune response may be an important contributor to severe infections. Infections of chickens with the recently emerged HPAI H5N6 viruses (which have caused several human fatalities), results in a very distinct immune response relative to other H5N6 strains by inducing much higher levels of IL-6, IL-8, and other pro-inflammatory mediators such as TNF- α (Gao et al. 2017).

Tissue Tropism

Unlike seasonal influenza virus infections, which are often confined to the respiratory system, H5N1 AIV infection can cause viremia (Likos et al. 2007). H5N1 viruses can be detected and isolated in the plasma of the patients, and the virus can spread to all organs and tissues including the brain (Chutinimitkul et al. 2006; Likos et al. 2007; Gao et al. 2010). Additionally, viruses can also be detected in human fecal specimens (Gao et al. 2010). Limited studies have shown that systemic spread of the virus may exacerbate disease progression, with megatrophs potentially playing an important role (Gao et al. 2010; Schrauwen et al. 2012). Similar to HPAI H5N1 viruses, LPAI H7N9 viruses can infect type II alveolar epithelial cells and replicate in them effectively, which may further damage lung function (van Riel et al. 2013; Zhou

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Table 2. Host genes associated with severe outcomes of H7N9 infections in humans

Host gene	SNP	Animal studies	Reference(s)
<i>IFITM 3</i>	rs12252/rs34481144	+	Wang et al. 2014b; Lee et al. 2017; Pan et al. 2017
<i>TMRPSS2</i>	rs2070788/rs383510	+	Cheng et al. 2015
<i>LGALS1</i>	rs4820294/rs2899292	+	Chen et al. 2015
	haplotype GG		
<i>CD55</i>	rs2564978	–	Lee et al. 2017

(SNP) Single-nucleotide polymorphism, (+) validated in mouse models, (–) not validated in mouse models.

et al. 2013). Compared with LPAI H7N9 viruses, which mainly replicate in respiratory tissues, HPAI H7N9 viruses can spread systemically in infected ferrets (Zhu et al. 2015; Imai et al. 2017; Shi et al. 2017). These latter viruses could infect and replicate efficiently in brain tissue, thus causing the death of the ferrets.

Host Genes

Interferon-induced transmembrane protein 3 (IFITM3) genetic variants influence the severity of clinical symptoms. The effects of genetic variation in the IFITM3 gene on influenza disease were first identified during the 2009 H1N1 pandemic (Everitt et al. 2012; Zhang et al. 2013d). This genetic variation was also associated with severe disease in some H7N9 patients (Wang et al. 2014b; Lee et al. 2017; Pan et al. 2017). Among H7N9 patients, the IFITM3 C/C genotype was associated with more severe clinical symptoms than the C/T and T/T genotypes (Wang et al., 2014b). Other host factors, including TMRPSS2, LGALS1, and CD55, were also reported to be associated with poor outcomes of H7N9 infections (Table 2) (Chen et al. 2015; Cheng et al. 2015; Lee et al. 2017).

CONCLUDING REMARKS

Influenza is an acute respiratory disease caused by influenza viruses. It can cause serious public health concerns such as epidemics, zoonotic infections, and pandemics. Influenza pandemics are unpredictable but recurring events that can have profound consequences on human health and economic well-being worldwide. Risk assessment tools and pandemic preparedness plans are essential to help mitigate the impact

of a pandemic. Recently, the United States Center for Disease Control and the World Health Organization have developed different risk assessment tools that can be used to assess the pandemic risk of influenza viruses with pandemic potential. Since 1997, AIVs of the H5 subtype have been considered zoonotic viruses with pandemic potential; fortunately, no such event has occurred to date. However, we cannot exclude the possibility of AIVs causing pandemics in future. These viruses may acquire the ability to transmit efficiently among humans, through mutations, reassortments, or both. Under the “One Health” approach, the global surveillance system for zoonotic influenza viruses should be further expanded in animals and humans, and epidemiologic investigations as well as virologic and pathogenesis research should be reinforced to provide essential scientific evidence for the risk assessment and early warning of a potential pandemic. Universal influenza vaccines and novel antiviral drugs may provide better protection against future pandemics.

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