Pandemic Influenza

Taisuke Horimoto* and Yoshihiro Kawaoka

Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Abstract: Influenza viruses cause annual epidemics and occasional pandemics that have claimed the lives of millions. The emergence of novel strains continues to challenge the public health and scientific communities. Recent outbreaks of highly pathogenic avian influenza A virus infections (including those of the H5N1 subtype) in poultry and in humans (through contact with infected birds), since 2003, have had important economic repercussions and have raised concerns that a new influenza pandemic involving H5N1 viruses is imminent. In the spring of 2009, a novel swine-origin H1N1 virus, whose antigenicity is quite different from those of human H1N1 strains, emerged in Mexico and readily transmitted and spread among humans, resulting in international outbreaks. This H1N1 virus was shown to be a triple reassortant comprising genes derived from avian, human, and swine viruses. On 11 June 2009, the World Health Organization declared that the infection caused by this new strain had reached pandemic proportion. Here, we review our current knowledge of pandemic influenza.

Keywords: Influenza, pandemic, H1N1, H5N1, reassortant, pig

1. INFLUENZA A VIRUSES

Influenza viruses, which belong to the family Orthomyxoviridae, are divided into three types, A, B, and C, based on the antigenicity of their internal proteins, such as nucleoprotein (NP) and matrix protein 1 (M1). Although all three types of viruses can be isolated from humans, seasonal influenza is caused by either type A or B viruses. Influenza A viruses are classified into 16 hemagglutinin (HA) subtypes (H1-H16) and 9 neuraminidase (NA) subtypes (N1-N9), and their antigenicity is entirely determined by the HA-NA combination, e.g., H3N2 virus. The influenza A virus genome contains eight segments of single-stranded, negative-sense RNA that each encodes one or two proteins (Fig. **1**). These viruses infect a large array of animals including mammals, such as humans, pigs, horses, etc., and avian species [1].

The HA protein on the surface of the virus is critical for binding to cellular receptors and fusion of the viral and endosomal membranes. The matrix M2 protein on the viral envelope possesses ion channel activity and plays a role in virus uncoating. Replication and transcription of viral RNAs are performed by the RNA polymerase, which consists of the PB2, PB1, and PA subunits, as well as NP in the cell nucleus. Newly synthesized vRNAs are exported from the nucleus to the cytoplasm as viral ribonucleoprotein (vRNP) complexes with the help of NS2/NEP (nuclear export protein) and M1. The eight vRNP segments are selectively assembled and packaged into virions at the plasma membrane. The virions are released from infected cells by the removal of sialic acids from the cell surface and viral glycoporteins by the NA protein. Non-structural NS1 and PB1-F2, produced in virus-infected cells, function as an interferon antagonist and apoptosis inducer, respectively.

*Address correspondence to this author at the Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan; Tel: +81-3-5449-5281; Fax: +81-3-5449-5408;
E-mail: horimoto@ims.u-tokyo.ac.jp

2. INFLUENZA PANDEMICS IN THE 20TH CENTURY

Influenza A viruses cause recurrent epidemics and pandemics. Epidemics have occurred as a result of antigenically drifted H1N1 and H3N2 viruses. Antigenically drifted influenza B viruses could similarly cause an epidemic. Pandemics are typically caused by the introduction of an antigenically shifted virus with an HA subtype that is new to human populations. In the 20th century, three major pandemics, Spanish influenza (H1N1 virus) in 1918, Asian influenza (H2N2 virus) in 1957, and Hong Kong influenza (H3N2 virus) in 1968, as well as one minor pandemic, Russian influenza (H1N1 virus) in 1977, occurred (Fig. **2**) [2]. Of these, the Spanish influenza was the most devastating, killing as many as 50 million people worldwide. Both the Asian and Hong Kong influenza pandemics killed more than a million people worldwide. The current seasonal influenza A viruses originated from the Russian (H1N1) and Hong Kong (H3N2) pandemic influenza viruses.

Although the Spanish influenza virus was not isolated during the outbreak in 1918-1919, its genome sequences has since been determined and revealed to be an avian-like H1N1 virus that contains human-like signature amino acids in several proteins [3, 4]. Asian and Hong Kong influenzas were caused by human/avian reassortants, in which a new HA gene was introduced from an avian virus [5]. Hypothetically, these human/avian reassortants could be generated in pigs serving as a "mixing vessel" [6], since both human and avian viruses can infect these animals. Thus, pigs may play a role in the generation of pandemic viruses.

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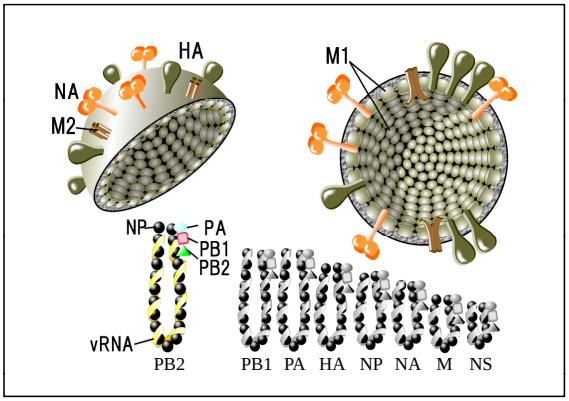

Fig. (1). Schematic diagram of an influenza A virus virion. Two glycoprotein spikes, HA and NA, and the M2 protein are embedded in the lipid bilayer derived from the host plasma membrane. The ribonucleoprotein complex (RNP) consists of a viral RNA segment associated with the nucleoprotein (NP) and three polymerase proteins (PA, PB1, and PB2). The M1 protein is associated with both the RNP and the viral envelope. A small amount of NS2 (not shown) is also included in virions, but its localization has not been determined. NS1 is the only nonstructural protein of influenza A virus and, as such, is not shown in this figure.

3. HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (H5N1)

In 1997, six individuals died following infection with highly pathogenic avian influenza viruses (H5N1) in Hong Kong, marking the first reported fatal infections of humans with pure avian influenza viruses [7]. Again, in 2003, other fatal cases with H5N1 avian viruses were reported. Since then, H5N1 viruses have evolved through frequent reassortment and have spread from Asia to Europe and Africa, becoming enzootic in poultry populations in many countries [8, 9]. Concomitantly, human infections increased resulting in severe respiratory illness with high mortality rates; 471 human infections with H5N1 virus have been confirmed, with 282 deaths (as of 28 January, 2010). Although several family clusters of H5N1 virus infection have been reported, sustained human-to-human transmission of the virus has yet to occur. Human H5N1 infections cause severe pneumonia and lymphopenia, and are characterized by high levels of cytokines and chemokines [10-13].

4. PANDEMIC SWINE-ORIGIN INFLUENZA A VIRUS (H1N1)

An outbreak of influenza-like respiratory illness was recognized in the Mexican town of La Gloria, Veracruz, in February of 2009 [14]. In early April, Mexican public health authorities reported the outbreak in Veracruz to the regional office of the World Health Organization (WHO). By mid-April, the Centers for Disease Control and Prevention (CDC) had isolated swine-origin influenza A viruses of the H1N1 subtype from multiple patients with mild respiratory symptoms in California, USA. At that time, the Public Health Agency of Canada determined that the Mexican isolates were similar to the H1N1 viruses isolated from the American patients. On 27 April, international spread and clusters of human-to-human transmission of this virus, probably due to little or no pre-existing immunity to the new strain, prompt the WHO to raise the pandemic alert from phase 3 to phase 4 on 27 April, and to phase 5 on 29 April. On 11 June, the WHO declared that the infections caused by the new strain had reached pandemic proportion (phase 6). Most human

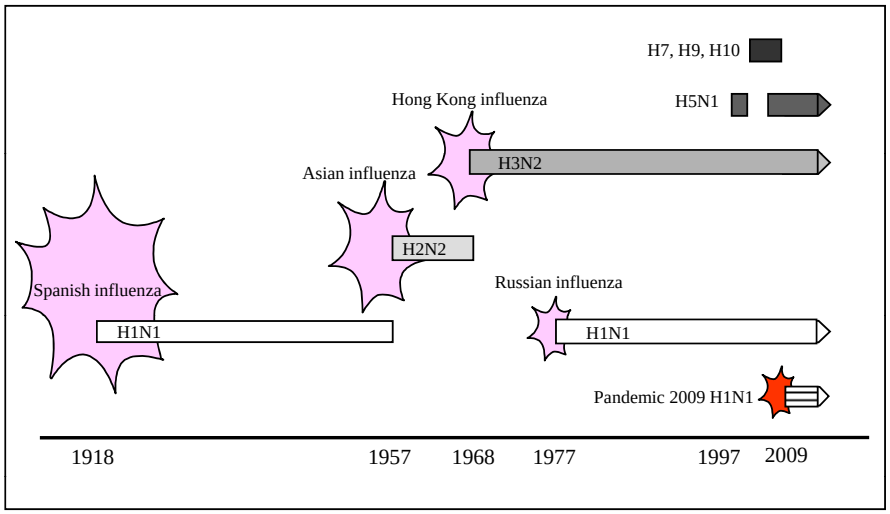

Fig. (2). Influenza pandemics. Phylogenetic studies suggest that an avian influenza virus was transmitted to humans directly or possibly via an undefined intermediate animal, leading to a devastating pandemic in 1918 (Spanish influenza; H1N1 subtype). A reassortant virus possessing avian PB1, HA, and NA genes, with all of its other genes coming from an H1N1 human virus, caused the 1957 pandemic (Asian influenza; H2N2 subtype). This H1N1 virus subsequently disappeared from circulation. In 1968, a reassortant possessing its PB1 and HA genes from an H3 avian virus, and the remainder from an H2N2 human virus, emerged as a new pandemic strain (Hong Kong influenza; H3N2 subtype), followed by the disappearance of the H2N2 virus. In 1977, an H1N1 virus that was nearly identical genetically to those circulating in humans in the 1950's appeared and spread rapidly among immunologically naive younger people (Russian influenza). The H1N1 and H3N2 viruses that have evolved from the strains responsible for Russian and Hong Kong influenza, respectively, continue to circulate in humans and to produce annual epidemics as seasonal influenzas. Since 1997, purely avian influenza viruses, including H5N1, H9N2, and H7N7 subtypes, have been directly transmitted to humans, raising concern over the possibility of a new influenza pandemic. In 2009, a swine-origin H1N1 virus with quite different antigenicity from seasonal H1N1 viruses emerged causing a new pandemic.

infections appeared to be mild [15]; however, a substantial number of individuals who did not have underlying health issues were hospitalized, attesting to the pathogenic potential of this H1N1 virus.

Data on the genetic composition of the virus indicated that it probably arose from the reassortment of recent North American swine viruses (H1N1, H1N2, or H3N2) with Eurasian avian-like swine viruses (H1N1) (Fig. **3**). As the North American swine viruses are "triple" reassortants, containing North American avian virus, classic swine H1N1 virus, and human H3N2 virus, this pandemic virus is actually a "quadruple" reassortant; deriving its PB2 and PA genes from the North American avian virus, its PB1 gene from a human H3N2 virus, its HA (H1), NP, NS genes from classic swine virus, and its NA (N1) and M genes from Eurasian avian-like swine virus [16-18]. It is unclear when and where this reassortant emerged, and why it was first identified in Mexico.

5. MOLECULAR CHARACTERISTICS OF THE PANDEMIC H1N1 2009 VIRUS

5.1. HA Protein

Influenza virus pathogenicity is determined multigenically, depending on the animal species. In poultry, however, viral pathogenicity is mainly determined by HA cleavability [19]. HA cleavage is essential for viral infectivity because fusion between the viral envelope and the cell endosomal membrane, an essential step for virus replication, only occurs with cleaved HA. HA cleavability is regulated by the amino acid sequences upstream of the cleavage site. Highly pathogenic H5 and H7 viruses possess a cluster of basic amino acids at this site. These highly pathogenic avian viruses can replicate in all organs of poultry, since their HAs are cleaved by ubiquitous proteases, such as furin, leading to severe infections and the death of the infected animals.

By contrast, other viruses, including low pathogenic avian viruses and all human and swine viruses, possess a single Arg residue at the HA cleavage site that limits their replication to only respiratory and intestinal organs due to limited HA cleavage by local proteases. The HA of the pandemic H1N1 2009 virus originated from a classic swine virus. Therefore it possesses the avirulent-type sequence at the HA cleavage site. Accordingly, the pandemic H1N1 2009 virus can only replicate in respiratory organs.

Because HA mediates the binding of the virus to host cell receptors, it is an important determinant for the host specificity of influenza viruses. Human viruses preferentially bind to sialic acid linked to galactose by an α2,6-linkage (SA α2,6Gal), whereas avian viruses preferentially bind to SA α2,3Gal [20]. This receptor-binding specificity of human and avian viruses suggests that avian virus HAs could acquire the ability to recognize human-type receptors on epithelial cells in upper respiratory organs, by mutations, and cause a pandemic in humans. A considerable number of avian-type receptors have been found in the lower region of human lungs, such as the bronchioles and alveolar type II cells, explaining the severe infection seen with highly pathogenic H5N1 viruses in some humans [21, 22].

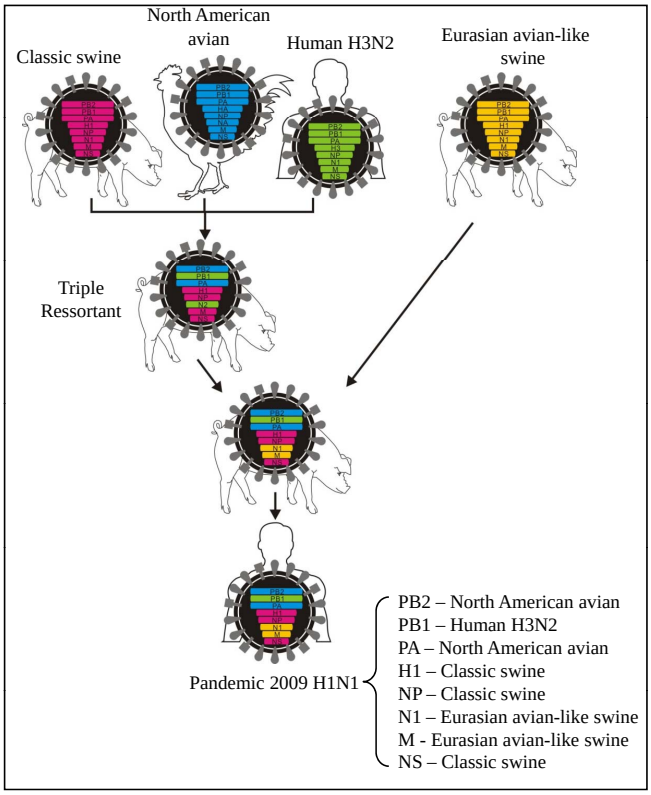

Fig. (3). Origin of the pandemic 2009 H1N1 virus. Since the late 1990s, various triple reassortant viruses between human H3N2, North American avian, and classic swine viruses have been circulating in the North American pig population. A reassortant between such a triple reassortant and a Eurasian avian-like swine virus was generated in pigs, and transmitted to humans, resulting in the pandemic 2009 H1N1 virus. Modified from Neumann, Noda, and Kawaoka [18].

Receptor-binding specificity is determined by the amino acid residues in the HA receptor-binding site [23, 24]. Earlier studies established that Gln at position 226 and Gly at 228 of H2 and H3 HAs confer binding to avian-type receptors, whereas Leu and Ser at these positions determine binding to avian-type receptors. For H5N1 viruses, the amino acids at positions 186, 192, and some others have been shown to confer human-type receptor recognition [25-27]. For H1 HAs, the amino acids at position 190 and 225 (H3 numbering) determine receptor-binding: Asp190 and Asp/Glu225 confer binding to human-type receptors, whereas Glu190 and Gly225 confer binding to avian-type receptors. The pandemic H1N1 2009 virus possesses human-type amino acids at both of these positions of HA, supporting its efficient transmissibility among humans.

5.2. PB2 Protein

The PB2 protein, a subunit of the viral RNA polymerase complex, is recognized as an important contributor to viral pathogenicity by affecting virus growth in host cells. It is well established that almost all human influenza viruses possess Lys at position 627 of the PB2 protein, whereas most avian viruses, with the exception of some H5N1 highly pathogenic avian viruses, possess Glu at this position. H5N1 viruses with Lys at PB2-627 are pathogenic in mice, whereas most of those with Glu are non-pathogenic in these animals [28, 29]. In addition, viruses possessing Lys at PB2-627, but not those possessing Glu, grow efficiently in the upper respiratory tract of mammals (33^{o}C), although both viruses grow efficiently at 37 ^{o}C [30]. Thus, a Glu-to-Lys mutation at position 627 of avian virus PB2 could be required for avian viruses to replicate efficiently in mammalian cells and be highly transmissible. A functional interaction between PB2 and NP may be regulated by the amino acid at this position of PB2 [31].

Similarly, the amino acid at position 701 of PB2 is a determinant of virulence by its interaction with a cellular nuclear import factor, importin α. An Asp to Asn mutation at this PB2 position appears to be required for the virus to replicate efficiently in mammalian cells [32]. The pandemic H1N1 2009 virus possesses the low-pathogenic type amino acids, Glu and Asp, at positions 627 and 701, respectively. Recently, a basic amino acid at position 591 in PB2 of the pandemic H1N1 2009 virus was shown to function similarly to Lys at position 627 [33]. It will be interesting to see whether this pandemic strain will further mutate to possess Lys at position 627.

Additionally, other components of the RNA polymerase complex, such as PB1 and PA, may contribute to viral pathogenicity [34]. Structural data of the complex is now available, which will help in the analysis of the molecular interactions among PB2, PB1, and PA, potentially showing that Lys at PB2-627 is part of a basic groove that is disrupted if replaced with Glu [35, 36].

5.3. Other Proteins

The NS1 protein functions as an interferon antagonist possibly by counteracting the activation of RIG-I signaling [37]. The NS1 proteins of H5N1 viruses confer resistance to interferon-mediated antiviral effects, resulting in the induction of high levels of pro-inflammatory cytokines, which may contribute to the severe systematic manifestations with high mortality observed in human infections with H5N1 viruses [38]. Several amino acids in NS1 are known to affect virulence [39, 40]. However, the pandemic H1N1 2009 viruses possess the low-pathogenic type amino acids at these positions.

The four C-terminal amino acids of NS1 form a PDZ ligand domain motif that may regulate signal pathways for some cellular events [41]. The NS1 protein of the pandemic H1N1 2009 virus lacks this domain as a result of an 11 C-terminal amino acid deletion.

The PB1 gene of most influenza viruses encodes a second protein, PB1-F2, expressed from the alterative reading frame [42]. PB1-F2 induces apoptosis by interaction with mitochondrial proteins and enhances inflammation in mice, thereby affecting viral virulence via secondary bacterial infections [43, 44]. It may also affect virulence by interacting with the PB1 protein to retain it in the nucleus for efficient virus replication [45]. The pandemic H1N1 2009 viruses encode a truncated PB1-F2 protein of only 11 amino acids, as do classic swine viruses, although the PB1 gene of the virus originates from the human H3N2 virus that contains the full-length PB1-F2 protein.

6. CONCLUDING REMARKS

Molecular analyses of the pandemic H1N1 2009 viruses indicate low-pathogenic features for humans (Table **1**) [46, 47], although worldwide transmission of the virus and a con-

Table 1. Molecular Characteristics of the Pandemic H1N1 2009 Virus

Protein	Position	Characteristic	Function
HA	Cleavage site	A single Arg (avirulent-type	Tissue tropism
	190	Asp (human-type)	Receptor binding
	225	Asp (human-type)	
PB2	627	Glu (avian-type)	Viral RNA replication
	701	Asp (avian-type)	
PB1－F2		Truncated (classic swine-type) (avirulent?)	Induction of apoptosis
NS1	92	Asp (avirulent-type)	Interferon response
	C-terminus	Deletion of PDZ domain (avirulent?)	Signal transduction

siderable numbers of lethal cases with acute pneumonia have been observed, even in the first wave of the current pandemic. Historically, the first wave of pandemic Spanish influenza was characterized by relatively low pathogenicity in humans, but the virus might have mutated into a more pathogenic form within a few months. Therefore, careful monitoring of isolates is of critical importance to detect more virulent variants. In addition, surveillance studies are essential to closely watch for the possible emergence of reassortant viruses between the pandemic H1N1 2009 virus and seasonal oseltamivir-resistant viruses, or H5N1 highly pathogenic avian viruses in H5N1 virus-endemic countries. Mass vaccination to protect against the pandemic H1N1 2009 virus will likely help control further waves of the current influenza pandemic.

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