



## REVIEW ARTICLE

# Influenza Virus: a Master of Metamorphosis

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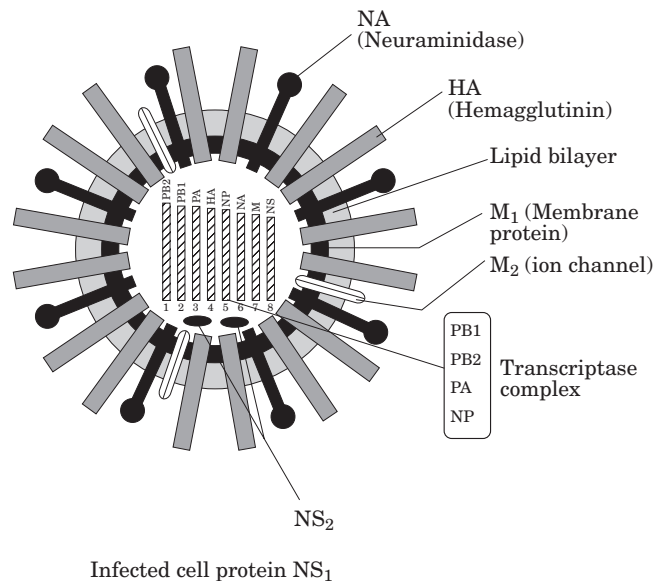
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### Introduction

In 1997, 18 Hong Kong residents were infected with an avian influenza A (H5N1) virus; six of them died.<sup>1–3</sup> This incidence of 'bird flu' has reminded virologists, public health authorities, and vaccine producers of the continuous threat of emerging influenza viruses, in particular those which can cause an influenza pandemic. The present paper discusses some aspects of such viruses, and the pandemics they may cause.

### The Structure of Influenza Viruses

Influenza viruses are enveloped viruses that contain single-stranded RNA of negative polarity (Fig. 1).<sup>4</sup> There are three types of viruses – A, B, and C – which can be differentiated on the basis of the antigenicity of their internal antigens. Type C is clinically unimportant, and will not be discussed in the present article. Anchored in the virus membrane, 400–500 spikes project out of the influenza A or B virus particle.<sup>5</sup> Each of these projections consists of a glycoprotein, either haemagglutinin (HA or H) or neuraminidase (NA or N). The HA and NA spikes occur in a ratio of about 8 : 1; the HA glycoprotein constitutes about 40% of the total mass of the virus particle.<sup>5</sup> In order to render the virus particle infectious, the HA must be cleaved at a specific site into two subunits, HA1 and HA2, by a host-derived trypsin-like enzyme. During the adsorption phase of the virus replication cycle, HA attaches to sialic acid residues present on the membrane of the host cell. This attachment initiates the penetration phase of the cycle. By virtue of similar adsorption, HA can attach to avian and mammalian erythrocytes and thus provide the virus particles with the ability to clump red blood cells – called haemagglutination – a process which can be specifically inhibited by antibodies. This inhibition is the basis of the most widely used test for identifying



**Figure 1.** Structure of the influenza A particle. Three types of membrane protein – haemagglutinin (HA), neuraminidase (NA), and small amounts of the M<sub>2</sub> ion channel protein – are inserted through the lipid bilayer of the virus membrane. Within the envelope are eight segments of single stranded genome RNA (ranging from 2341 to 890 nucleotides), individually complexed with proteins. Influenza B virus does not contain the M<sub>2</sub> protein. Instead, a glycoprotein, called NB, is spanning the envelope, probably also functioning as an ion channel. Figure derived from Lamb and Krug *et al.*<sup>4</sup>

influenza viruses and quantifying anti-influenza antibodies, namely the haemagglutination inhibition test. NA is an enzyme that cleaves sialic acid (N-acetyl neuraminic acid) from the oligosaccharide chains anchored in the cell membrane, thus enabling the newly formed influenza virus particles to detach from the host cell membrane. Anti-influenza immunity is mainly targeted at the HA1 subunit of HA and, to a lesser extent, at the NA.

### Antigenic Variability of Influenza Viruses

The main goal of parasitic microbes is not to multiply in their host as much as possible, but to ensure that they can still do so after years, decades, or even centuries. In most cases, the build-up of specific immunity to a microbe is the

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greatest obstacle to its long-term survival. The relation between influenza A virus and man is a good example to illustrate how a 'clever' virus uses a number of different strategies to achieve the antigenic heterogeneity necessary for long-term persistence in its host. Similar to the multitude of types of enteroviruses and adenoviruses, influenza A virus has developed at least 15 so-called 'subtypes' of HA and nine subtypes of NA. All of these subtypes have been shown to occur in wild aquatic birds simultaneously, although their proportions vary in time.<sup>6</sup> These subtypes can be distinguished by major differences in the antigenicity of their HA and the NA, and are only slightly subject to cross-immunity. In rare instances, an avian influenza virus subtype has succeeded in colonizing man. After being established in the human population for a couple of years, the influenza virus subtype is confronted with the specific immunity mounted during earlier infections with the same subtype. Influenza viruses react by displaying a unique kind of variability. On the basis of random mutations in the HA or the NA, a variant is selected that evades the herd immunity. This process is facilitated by the quasi-species composition of influenza virus strains. Evidence has been obtained which shows that several influenza virus subpopulations can coexist in a single person.<sup>7,8</sup> After one or more influenza seasons, when man also acquires immunity to the new variant, a new variant is again selected from the previous one by the same mechanism. Although immunity to older variants wanes after a number of years, these variants never return in the human population, perhaps because they become extinct. This process of small, almost annually occurring, accumulating antigenic changes is called antigenic drift. As long as a subtype persists in the human population, this drift process seems to continue. The

underlying mutations of subtype A (H3N2) seem to be largely confined to a set of 18 codons in the HA gene, a finding that may be helpful in the prediction of the prevailing variant of the next season.<sup>9</sup>

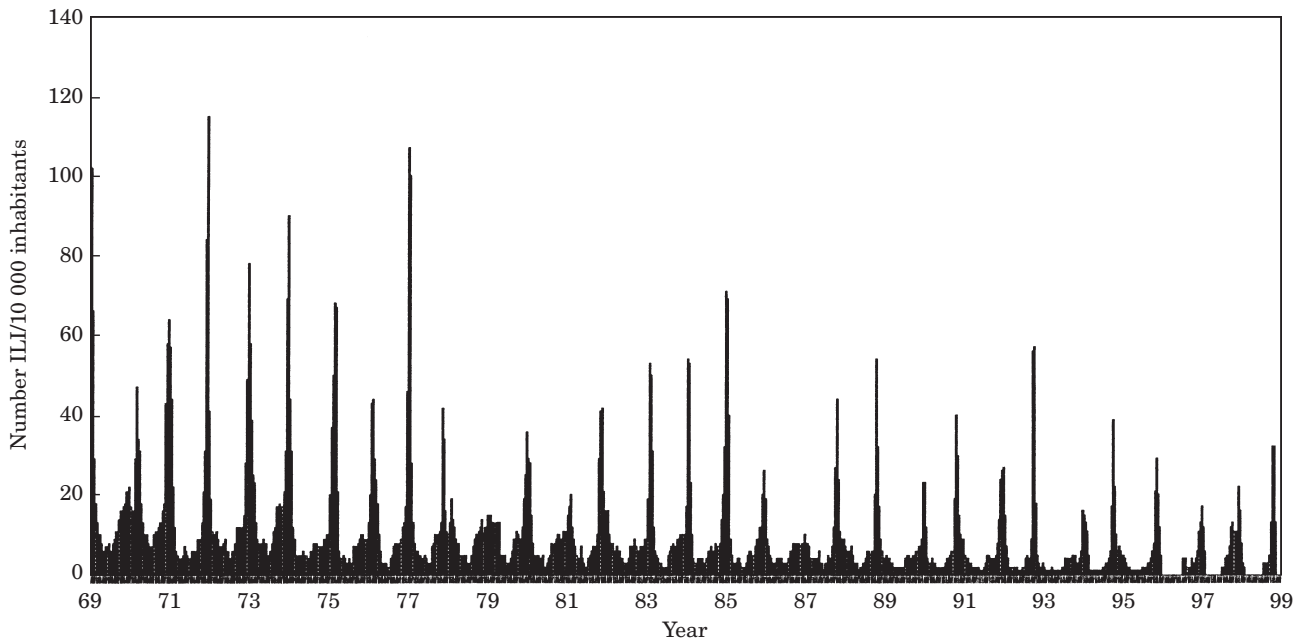
The antigenic drift of influenza A (H3N2) virus since its appearance in man in 1968 is shown in Table I.<sup>10</sup> This drift was detected by the haemagglutination inhibition test, using post-infection ferret antisera. The designation of influenza virus strains consists of type/animal/place of isolation/sequential number/year of isolation (subtype); for example, A/Goose/Guandong/1/96 (H5N1). No animal species is indicated in the designation of human isolates. No subtypes are known to exist for influenza B and C viruses. Table I shows that, in each case, antiserum prepared with a certain virus strain reacts only with the homologous virus at high titres and with strains isolated earlier or later at progressively decreasing titres. Influenza B and A (H1N1) viruses behave similarly, although the frequency of antigenic changes is considerably lower in these (sub)types. Antigenic drift contributes to the ability of influenza viruses to cause annual epidemics (Fig. 2). With the exception of South-east Asia, where influenza occurs throughout the year, influenza activity is restricted to the winter seasons in the northern and southern hemispheres. This phenomenon may be partly due to the differences between the seasons with respect to indoor relative humidities, which entail marked differences in the survival of influenza virus in aerosols.<sup>11</sup> In the United Kingdom and The Netherlands, influenza epidemics last for 10 weeks on average. One practical consequence of antigenic drift is the need to update the influenza vaccine composition almost every year. For this purpose, the WHO operates a global influenza surveillance network of virus laboratories to detect new variants.<sup>12</sup>

Table I. Haemagglutination-inhibition (HI) reactions with influenza A (H3N2) viruses.\*

Season	WHO strain	HI titres of post-infection ferret antisera against virus from								
		1968	1972	1976	1980	1984	1988	1992	1996	1998
1968/69	A/Hong Kong/1/68	<u>5120</u>	640	–	–	–	80	–	–	–
1972/73	A/England/42/72	–	<u>1280</u>	–	–	–	–	–	–	–
1976/77	A/Victoria/3/75	–	160	<u>2560</u>	320	160	–	–	–	–
1980/81	A/Bangkok/1/79	–	–	160	<u>2560</u>	2560	40	–	–	–
1984/85	A/Philippines/2/82	–	–	–	640	<u>1280</u>	320	–	–	–
1988/89	A/Sichuan/2/87	–	–	–	–	640	<u>2560</u>	40	–	–
1992/93	A/Beijing/32/92	–	–	–	–	–	40	<u>2560</u>	320	–
1996/97	A/Wuhan/359/95	–	–	–	–	–	–	320	<u>5120</u>	160
1998/99	A/Sydney/5/97	–	–	–	–	–	40	40	160	<u>2560</u>

\* Strains are indicated by type/place of isolation/sequential number/year of isolation. HI titres are given as the reciprocals of the maximum serum dilutions which completely inhibit the haemagglutination.

Homologous titres are underlined and in bold print. '–' is < 10. Table derived from de Jong, Rimmelzwaan, and Osterhaus.<sup>10</sup>



**Figure 2.** Number of influenza-like illnesses (ILI) per 10 000 inhabitants per week among patients presenting in general practices in The Netherlands for the seasons 1969/70 up to and including 1998/99. Source: NIVEL (Netherlands Institute for Primary Health Care).

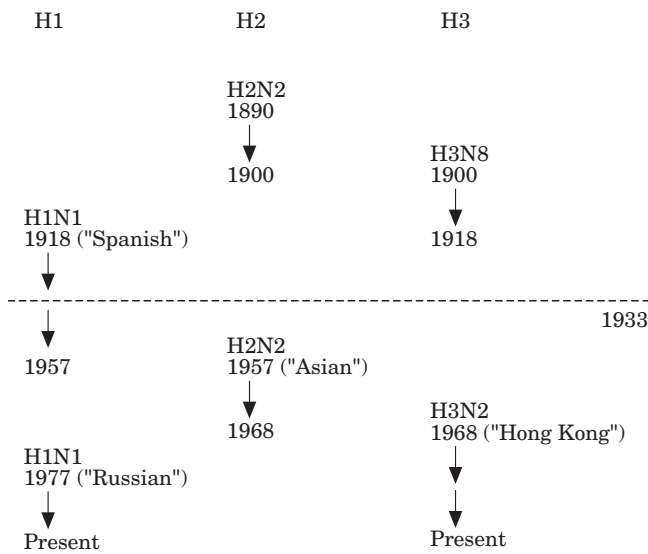
### Influenza Pandemics are Caused by Emerging Subtypes of Influenza A Virus

As mentioned above, influenza A viruses have an extensive reservoir in water-fowl, in which they usually cause asymptomatic infections.<sup>6</sup> They replicate in the intestines of the birds and are mainly transmitted by the faecal–oral route. Ponds used by migratory birds as a resting site are particularly involved in the transmission. Occasionally, these viruses infect domestic birds like chicken and turkeys via droppings, sometimes causing large epidemics with mortalities up to 100%. Ample evidence suggests that all influenza A viruses found in mammals originated at some time from those found in wild aquatic birds. These viruses are also sporadically transmitted to man. If the HA is of subtype 4–15, man is not immune to the virus. Because they are not adapted to circulation in the human host, the viruses almost never accomplish more than a few serial transmissions. In rare cases, however, a virus strain can succeed in causing extensive worldwide epidemics and in establishing itself in the human population for years or decades. By definition, such an introduction of a new avian subtype in man is called an antigenic shift and is associated with a pandemic. (Note the special use of the words ‘antigenic shift’ and ‘pandemic’. With other pathogens they only mean a large change in antigenicity and an epidemic occurring over a large area, respectively.)

Influenza pandemics have occurred three times in the present century: in 1918, when subtype H1N1 was introduced, starting in the U.S.A. (‘Spanish Influenza’); in 1957, when subtype H2N2 emerged in South-east Asia (‘Asian Influenza’); and in 1968, when subtype H3N2 established itself in the human population, starting in the same area (‘Hong Kong Influenza’) (Fig. 3).<sup>13</sup> For obscure reasons, the prevailing subtype in all these cases disappeared abruptly and completely. Subtype H1N1 was reintroduced in man in 1977. The circulation of the established subtype H3N2 was not affected at that time, and both subtypes have persisted concurrently since. The 1977 event is sometimes called a ‘half antigenic shift’. The data shown in Figure 3 for the period before 1933 (when the first influenza virus was isolated from humans) were obtained from the examination of sera from older people taken before the emergence of H2N2 in 1957 and H3N2 in 1968.<sup>14</sup>

### Reassortment

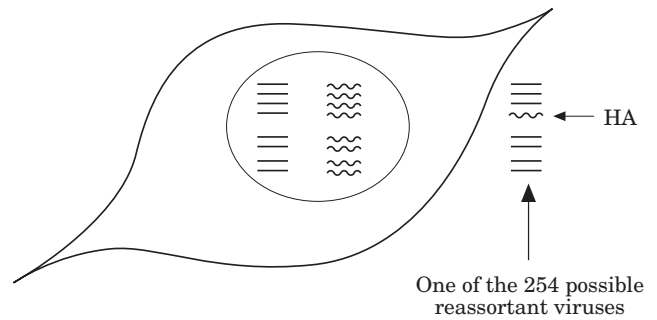
When analysing the genomes of H2N2 and H3N2 viruses, another unique mechanism for the variability of influenza viruses was discovered. Nucleotide sequencing of the genome of the 1957 H2N2 virus revealed that the RNA segments coding for HA, NA, and one of the internal proteins were of avian origin, whereas the other five genes were derived from the human H1N1



**Figure 3.** Prevalence of influenza A virus subtypes in history. H is haemagglutinin, N is neuraminidase. Every period started with a pandemic due to an antigenic shift. Popular names of the pandemics and the subtypes are given in parentheses. Data referring to subtypes circulating before 1933 (i.e. above the dashed line) were obtained by examining sera from older people – taken before the emergence of H2N2 in 1957 and H3N2 in 1968 – for the presence of antibodies to the subtypes concerned ('serum archeology').<sup>14</sup> Figure derived from de Jong, Rimmelzwaan, and Osterhaus.<sup>10</sup>

ancestor virus.<sup>6</sup> In 1968, only the genes for HA (H3), and again one of the internal proteins, were introduced from the avian reservoir. The other six genes, including the one coding for the NA, originated from the human H2N2 ancestor virus.

The mechanism for the creation of a new combination of the eight RNA segments of two different influenza viruses is called 'genetic reassortment'. The basis of reassortment is the segmentation of the RNA genome of influenza A and B viruses into eight fragments, each coding for one or two of the 10 virus proteins. In cells co-infected with two influenza A viruses with different genetic constitutions, there is an exchange of homologous segments which may result in the emergence of stable reassortants (Fig. 4). Some of the  $2^8 = 256$  mathematically possible combinations of genes might not allow the formation of an infectious virus capable of replicating in the host organism, or, from an epidemiological point of view, of being efficiently transmissible in the animal or human host population. HA and NA proteins are coded for by two different RNA fragments. With the restrictions mentioned above, any subtype of HA can be combined with any subtype of NA by reassortment. These HA–NA combinations are also called subtypes. Because there are 15 HA and nine NA subtypes, there are theoretically



**Figure 4.** Schematic representation of reassortment in influenza viruses. A host cell is infected by two different influenza viruses. The horizontal lines in the nucleus of the cell are the eight RNA fragments (genes) of the viruses. These fragments can be mixed randomly during the formation of new virus particles. One of the 254 theoretically possible reassortants is shown at the right of the cell: seven fragments are from the human parent and one – the fragment coding for HA – is from the avian parent virus. This reassortant virus could be the cause of the next antigenic shift of influenza virus and the associated pandemic. Figure derived from de Jong, Rimmelzwaan, and Osterhaus.<sup>10</sup> (–) Human; (~) Avian.

$15 \times 9 = 135$  combinations of HA and NA. Although no subtypes of influenza B virus are known, epidemiologically relevant reassortment has also been observed with this type.<sup>15</sup>

Reassortment is a strategy for antigenic variation which is known to play a major epidemiological role only in influenza viruses. It greatly facilitates the colonization of mammals, including man, by avian HA subtypes by providing the avian HA with mammalian internal proteins which, during a period of at least several decades, are adapted to support virus replication in the mammalian host. Curiously, in the pandemic viruses of both 1957 and 1968, the same polymerase protein PB1 of the new pandemic virus was an exception to this rule and was derived from the avian parent virus.<sup>16</sup> It is not known in which mammal the reassortments for the 1957 and 1968 human pandemic strains took place. Avian influenza virus strains do not normally spread in the human population. They more frequently establish long-term infection in swine.<sup>17–19</sup> Pigs are also sensitive to infection and colonization by human influenza viruses,<sup>20–22</sup> so it is not surprising that reassortants between human and swine viruses have been isolated from pigs.<sup>23</sup> It has been demonstrated that man is also susceptible to infection with swine influenza viruses.<sup>24,25</sup> Until recently, therefore, the consensus was that the pig is the most probable 'genetic mixing vessel', giving birth to pandemic strains.<sup>6,26,27</sup> In 1997, however, the Hong Kong event made it clear that man himself can also function as a mixing vessel.<sup>1,2,28</sup>

## Origin and Impact of Pandemics

For unknown reasons, the latter two pandemic strains of influenza virus emerged in South-east Asia. A few explanatory factors may be the size and density of the population, the continuous (although variable) occurrence of influenza throughout the year, and the lifestyle of the farmers, which involves frequent contacts between man, pigs, and aquatic birds, allowing the pig to play its role as genetic mixing vessel. The fact that most of the inter-pandemic variants also appear to originate in this region is more difficult to explain. Swine are not involved in the process of antigenic drift, although the size and density of the human population and the continuous occurrence of influenza may play a role. Also, the latter two pandemic strains of influenza virus emerged in South-east Asia, giving this area with respect to the process of antigenic changes a lead over other parts of the world, where the new subtypes arrived at least half a year later.

Morbidity and mortality from pandemics can be high (Table II). Pandemics usually occur in two or three waves with increasing virulence.<sup>13</sup> An estimated  $40\text{--}50 \times 10^6$  people died worldwide during the 1918–1920 pandemic of Spanish Influenza caused by subtype H1N1. In the second and third influenza wave, the death rate increased to approximately 1.5% of all clinical cases of influenza, i.e. at least 10 times higher than in all other pandemics.<sup>13</sup> Reasons for this high fatality rate are obscure, and the unique age distribution of the fatal cases still defies explanation. In the 1918 pandemic, the normal U-shape of the fatality rate curve – high values at the extremes and low values in between – was transformed into a W-shape, with an equally high peak for 20- to 40-years-olds (Fig. 5).<sup>29,30</sup> Over the following decades, the age curve gradually returned to the normal U-shape.<sup>31</sup> The W-shape may have been associated with an inherent characteristic of the concerning strains of subtype H1N1. In contrast, the H1N1 virus strains circulating after the re-emergence of the subtype in 1977 are of low virulence. In view of the

future, it is reassuring that the two later occurring pandemics were considerably milder than the one in 1918. The emergence of the 1968 H3N2 virus, for example, did not cause any more casualties than the inter-pandemic H3N2 epidemics of the seventies.<sup>32</sup>

## Virulence Factors of Influenza Viruses

As mentioned above, one of the factors that limit the spread of influenza viruses in the human population is the specific immunity built up during earlier infections with the same subtype. The ability to perform antigenic drift, therefore, is obviously a virulence factor of influenza viruses circulating in man. Nothing is known about the mechanisms that form the basis of the generally higher pathogenicity of subtype A (H3N2) virus over type B viruses, of type B viruses over the present sub-type A (H1N1) viruses, or of subtype A (H1N1)

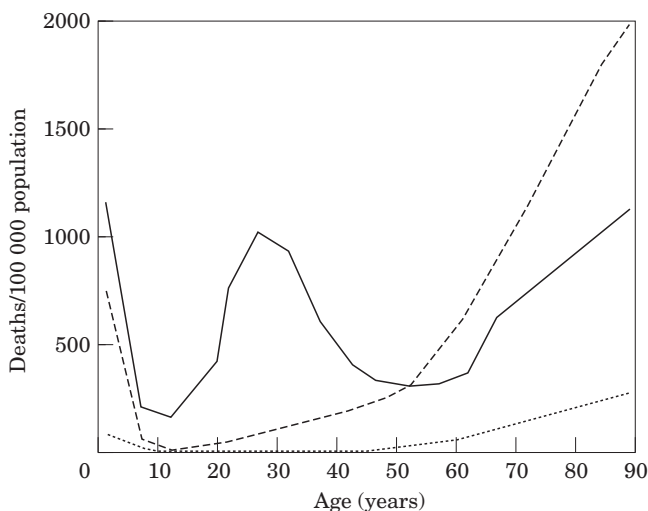


Figure 5. Deaths from pneumonia and influenza in the U.S.A. by age during the pandemic years 1892 (Massachusetts only), 1918, and 1957. Adapted from Dauer and Serfling.<sup>29</sup> (---)1892; (—) 1918; (.....) 1957.

Table II. Morbidity and mortality during influenza pandemics, directly or indirectly attributable to influenza.\*

Pandemic	Subtype	Infection	Disease	Mortality worldwide	Mortality per 100 000	
					U.S.A.	The Netherlands
1918–20	H1N1	50% of the population	25% of the population	$40\text{--}50 \times 10^6$	622	600
1957–58	H2N2	40–50% of the population	25–30% of the population	$> 1 \times 10^6$	39	50
1968–70	H3N2	?	?	Less than in 1957/58	23	30

\*Mortality is given as excess mortality; this is the difference in mortality seen during winters with and without significant influenza activity. Table derived from Potter 1998,<sup>13</sup> Simonsen, Schonberger *et al.*,<sup>30</sup> and de Jong, Rimmelzwaan, and Osterhaus.<sup>10</sup>

viruses circulating before 1957 over those circulating after 1977.

There is still no explanation for the extremely high fatality rate of the 1918 H1N1 virus. Hope for an explanation has been raised by the gradually successful attempts to elucidate the nucleotide sequences of the RNA fragments of this virus, which have been partially recovered from preserved pieces of tissue from victims of the great pandemic.<sup>33</sup> Two features associated with enhanced pathogenicity have drawn particular attention. First, the high virulence of some avian influenza viruses – caused by replication outside the respiratory and enteric tracts, the conventional sites of replication in the bird – was found to be associated with the insertion of multiple basic amino acids (at least four) near the cleavage site between the HA1 and HA2 parts of the HA molecule.<sup>34</sup> This cleavage is needed to render the virus particle infectious. The insertions mentioned make the unsplit HA susceptible to cleavage not only by the trypsin-like enzymes which are only present in the usual target organs of avian influenza viruses, but also by the proteolytic enzymes which are ubiquitously present. The presence of the multiple basic amino acid sequences, therefore, renders the virus infectious for many organs, including heart and brain, and explains its high lethality. No insertion was found, however, at the cleavage site of the 1918 H1N1 virus.<sup>33,35</sup>

The second feature associated with enhanced pathogenicity was discovered in the H1N1 virus strain A/WSN/33. This strain was derived from A/WS/33 virus by a large number of passages in mouse brain and emerged as highly pathogenic for mice. It was shown to lack the multiple basic amino acid sequence near the cleavage site of nascent HA. The explanation of its high pathogenicity was found in the NA, which was shown to have acquired a lysine residue.<sup>36</sup> This mutation endowed NA with the ability to bind host plasminogen, which, after activation to plasmin, is capable of cleaving HA in the way referred to above. The 1918 H1N1 virus, however, does not show this mutation.<sup>37</sup>

### Pandemic Warnings

Pandemics are unpredictable. In fact, they may never occur again, although most virologists consider this unlikely. Since 1968, there have been ‘warnings’ that the series of pandemics can continue. In 1976, for example, the outbreak of swine influenza A(H1N1) virus among American military recruits in New Jersey, U.S.A., reminded the world of the great H1N1 pandemic of 1918. Health authorities reacted by planning, and partly performing, the vaccination of all Americans with vaccine prepared from the swine virus involved.<sup>38</sup> Only the

statistically significant association with a small number of cases of Guillain–Barré syndrome ended the massive vaccination campaign. The causative virus was an unchanged ‘classical’ swine virus, which was antigenically closely related to the 1918 virus.<sup>39</sup> Today, we know that such a virus is unable to spread in humans. Retrospectively, however, it was still a worrying situation, since the swine virus co-circulated with a conventional influenza A(H3N2) virus among the recruits. This could have resulted in the emergence of a reassortant with the HA (and perhaps also the NA) of the swine virus and the internal proteins of the human virus. Such a virus would have had pandemic potential.

In 1997, the infection of 18 individuals with an unchanged avian influenza A(H5N1) virus, six of whom died, was another sign that the threat of an influenza pandemic still exists.<sup>1,2,28</sup> It was clear that the direct source of these infections was an epidemic of fowl plague in Hong Kong, caused by the same H5N1 virus. Seroepidemiological investigations showed that man-to-man transmission was very rare.<sup>40</sup> Nevertheless, the 1976 risk of reassortment with a human influenza virus had returned. Fortunately, no significant human influenza activity occurred in Hong Kong at the time of the series of human infections with H5 virus at the end of 1997. In view of the impending influenza epidemic season (February up to and including July – Lim, personal communication), the health authorities of Hong Kong ordered destruction of all poultry destined for consumption. This completely halted the series of human infections with H5 virus. This success proved that the direct source of the infections had exclusively been the poultry, and also that no overt man-to-man transmission persisted in the area.

Because the H5 viruses were revealed as potentially pandemic viruses, their structure, direct origin, reservoir, and control have become the subject of extensive investigations. With regard to their structure and direct origin, the HA1 part (this is the immunologically relevant part of HA, numbering 330 amino acid residues) of four human 1997 Hong Kong H5N1 viruses was found to differ from that of three chicken 1997 Hong Kong H5N1 viruses by only one to five amino acids.<sup>41</sup> No single amino acid change separated the chicken and human viruses. The multiple basic amino acid sequence at the haemagglutinin cleavage site, which is associated with high pathogenicity in poultry,<sup>42</sup> was retained in the human Hong Kong viruses, which, in line with this feature, displayed the same high lethality for chickens.<sup>2,43</sup>

It is unlikely that domestic fowl was the reservoir for the Hong Kong chicken H5N1 viruses, since the fatality rates in these animals were too high to make long-term

circulation of these viruses possible. Therefore, wild birds in Hong Kong are being examined for the presence of influenza viruses which have genes similar to the 1997 Hong Kong H5N1 viruses. Preliminary results indicate that, in a postulated reassortment event, an A/Goose/Guandong/1/96 (H5N1)-like influenza virus donated the HA gene<sup>44</sup> and an A/Quail/Hong Kong/G1/97 (H9N2)-like influenza virus the seven other genes, including the NA gene.<sup>45</sup>

### Factors in the Development of Pandemic Strains

All influenza viruses adsorb to the host cell by means of specific binding between the receptor-binding pocket at the surface of the virus HA and an oligosaccharide at the surface of the cell, which carries a terminal sialic acid residue. Influenza viruses of different species, however, may differ in their receptor specificity. Most avian influenza viruses preferentially attach to sialyloligosaccharides projecting from the cell membrane in which the sialic acid residue is bound to a galactose residue by an  $\alpha 2,3$ -linkage (SA $\alpha 2,3$ Gal). In contrast, human influenza viruses preferentially bind SA $\alpha 2,6$ Gal.<sup>46</sup> Epithelial cells in the human trachea, one of the virus replication sites in man, contain SA $\alpha 2,6$ Gal, but not SA $\alpha 2,3$ Gal. Conversely, epithelial cells in duck intestine, where avian influenza viruses multiply, contain SA $\alpha 2,3$ Gal, but not SA $\alpha 2,6$ Gal.<sup>46</sup> Interestingly, epithelial cells in pig trachea contain both SA $\alpha 2,3$ Gal and SA $\alpha 2,6$ Gal, which explains the high susceptibility of this animal to both human and avian influenza viruses. Although the SA-Gal linkage situation is much more complicated than described above,<sup>6</sup> before 1997 these findings were supposed to give a satisfactory explanation for the fact that an avian influenza virus was never isolated from a human influenza patient. It was unexpected, therefore, that in 1997 in Hong Kong an unchanged avian influenza virus was found replicating efficiently in the human respiratory tract. The virus pneumonia of the first victim, for example, was extensive, and the tracheal aspirate from this 3-year-old boy induced a cytopathic effect in 50% of the cells of the primary MDCK cell culture within 2 days (Lim, personal communication). This is a short time, even for currently circulating human influenza viruses. The H5N1 virus from Hong Kong did not, however, start an epidemic, nor did the 1976 New Jersey unchanged swine H1N1 virus that infected several hundred people and killed one soldier,<sup>38</sup> or the 1986 unchanged swine H1N1 virus that caused severe pneumonia in a young healthy farmer.<sup>24</sup> These observations indicate that it is not suboptimal replication in the human host, but inability of efficient

transmission which is the decisive factor in the development of pandemic strains.

It is probable that the efficiency of transmission mainly depends on the structure of the two surface proteins HA and NA, and, with emerging pandemic strains, is not acquired at the reassortment event. It must be built up by a process of sequential mutations. This may explain the years that elapse between the introduction of an avian virus in the human population and the occurrence of a pandemic. Nucleotide sequence analyses have indicated that the appearance of the common ancestor of human and classic swine H1N1 influenza viruses was between 1905 and 1914, while the pandemic was in 1918<sup>47</sup> and that the transfer of the avian H3 gene to human viruses was around 1965, while the pandemic was in 1968.<sup>48</sup> Optimal adaptation of novel influenza viruses to circulation in humans, however, appears not to have been achieved at the time of the pandemic. This was revealed by the finding that human influenza virus genes have a greatly increased mutation rate when compared to those in water-fowl, not only involving the genes coding for surface proteins, but also those coding for internal proteins.<sup>49</sup>

The lack of transmission of animal influenza viruses in man may be due to several factors. For example, although a virus may be shed in the respiratory tract in large amounts, it must also be dispersed into the air in particles small enough to remain floating sufficiently long to land in the airways of another person. One of the functions of NA is to break down the mucoproteins in the mucus layer of the respiratory tract. This breakdown lowers the viscosity of the mucus and promotes the formation of small droplets during coughing and sneezing. It is conceivable that avian NA, targeted at avian sialyloligosaccharides, is not able to efficiently split the mucoproteins in the human respiratory tract. Another example is that, once nebulized, the virus is subject to damaging influences like surface tension, oxidation, and dehydration. These factors can inactivate influenza viruses in aerosols, especially at low relative humidity.<sup>11</sup> Studies have shown that avian influenza viruses are mainly transmitted by the faecal-oral route via water and are not, therefore, constructed to resist the forces of aerosolization.<sup>6</sup> In fact, transmission experiments in chickens have confirmed that the 1997 Hong Kong viruses are spread by faecal-oral transmission, rather than by aerosol.<sup>50</sup>

### The Subtype that may Cause the Next Pandemic

It is not known whether all the theoretically possible 135 influenza virus subtypes are capable of establishing

themselves in the human population. HA subtypes H1, H2, and H3 have colonized man and should be regarded first when considering the possible cause of the next pandemic. Serological evidence has shown that H1, H2, and H3 circulated before 1933, the year of the first isolation of influenza virus (Fig. 3). They can, therefore, 'recycle'. H3 was re-introduced in 1968 and has circulated since. Although the recent H3 viruses do not show any cross-neutralization with the 1968 H3 virus (Table I), there is probably sufficient 'priming' immunity (i.e. immunity due to 'priming' by an infection with a strain of the same subtype, while the formed antibodies have waned to undetectable levels) in the population to prevent re-emergence of subtype H3. After an absence of only 20 years, H1 has also been able to resume persistent circulation in man, in spite of the effective humoral and priming immunity of people more than 20 years old. Presumably no significant antibody levels to the 1918 H1N1 virus are present in the human population, because in people born after 1930 no significant antibodies can be demonstrated to classical A/Swine viruses,<sup>51</sup> which are supposed to be antigenically closely related to the virus of the Spanish Influenza. Man, however, may have sufficient priming immunity to subtype H1 to prevent the return of the 1918 virus. This immunity might be challenged in the near future. Ironically, although scientific progress in the influenza field has resulted in a better control of the disease, it may also form a threat. The ongoing clarification of the nucleotide sequences of the RNA of the 1918 virus will eventually enable virologists to re-create the virus.<sup>52</sup> This would carry with it the risk of escape of the virus from the laboratory.

These considerations leave H2 as a possible candidate for causing the next pandemic. Since H1 reappeared after only 20 years, the 30-year absence of H2 may have weakened the priming immunity sufficiently to allow its re-introduction. Other influenza virus subtypes have also been isolated from mammals and have proven capable of multiplying in this kind of host. H7, for example, has circulated for a decade in horses, while H4 and H7 have been isolated from seals, H10 from minks, H13 from a whale, and H5 and H9 from human influenza patients in Hong Kong in 1997 and 1999, respectively. Nevertheless, no persistent circulation of these subtypes has been demonstrated.

### The Impact of Future Pandemics

When considering the possibility of a repetition of the events of 1918 in the modern western society, one should realize that the socio-economic situation then was much poorer than it is today. The low level of medical

care – especially the absence of antibiotics that can reduce lethality from secondary bacterial infections – most probably contributed to the high mortality of the pandemic. In line with the marked socio-economic and medical progress made during the 20th century, the three pandemics of this century decreased successively in their impact in terms of morbidity as well as mortality (Table II). However, the actual reasons for this encouraging decline are unknown and may have been partly due to intrinsic properties of the causative viruses. Moreover, antibiotics may be of little value in the treatment of haemorrhagic primary influenza pneumonia, which was frequently observed during the Spanish Influenza pandemic and in most cases led to death within 2–3 days after hospital admission.<sup>13</sup>

### The Control of Future Pandemics: Vaccination

Because of the lack of time to produce an adequate vaccine in sufficiently large amounts, vaccination was not a significant factor in the control of past influenza pandemics. Today, however, vaccination is the cornerstone of the control of influenza in interpandemic seasons. Vaccines protect 30–90% of the vaccinees against complications of and death from influenza.<sup>53,54</sup> Production, in chicken eggs, of the annual influenza vaccine for the risk groups takes 6–8 months, measured from the recognition of a new variant.<sup>55</sup> This time period is difficult to shorten, because the supply of eggs cannot be expanded at short notice. Because of this problem and other reasons, researchers are currently investigating the possibility of growing influenza virus for vaccine purposes in mammalian cell culture.<sup>56</sup> The greater flexibility of this technique is important in view of the pandemic situation. Moreover, the problem of high pathogenicity of a pandemic strain for chicken embryos – as seen during the preparations for a vaccine against the 1997 Hong Kong H5N1 virus<sup>3</sup> – can be evaded.

The advent of new adjuvants,<sup>57</sup> antigen-presentation forms such as iscoms,<sup>58</sup> and antigen-delivery systems like virosomes<sup>59</sup> may reduce the amount of antigen required to induce protective immunity and, therefore, may shorten the production time for the desired number of vaccine doses. Relevant research is also targeted at the development of live influenza vaccines,<sup>60</sup> virus vectors (e.g. adenovirus, vaccinia virus, and Semliki Forest Virus<sup>61</sup>) and influenza virus DNA vaccines.<sup>62</sup> These vaccines have the additional advantage of being cheaper, which is important for developing countries. Unfortunately, the protection of third world populations is receiving little attention. This is a deplorable situation,

because these very people are presently living under the same circumstances that contributed greatly to the severe consequences of the 1918 Spanish Influenza pandemic.

Pandemic influenza virus strains are not the only strains that present problems for a timely vaccine production. Sometimes a new virus variant emerges after the formulation of the recommendations of the WHO for the vaccine strain composition for the next season. For example, a widely deviant antigenic drift variant of A (H1N1) was observed in mid-1986 and similar variants of A (H3N2) arose in mid-1992 and mid-1997. Consequently, there was a gross mismatch between vaccine strain and major epidemic strain in the corresponding seasons in the northern hemisphere. Evidence has been published that such a mismatch affects the serological and clinical efficacy of the vaccine.<sup>63,64</sup> It is possible to extend the time-span between the recognition of novel influenza viruses and the start of the epidemic or pandemic by increasing the intensity of influenza virus surveillance among man, pigs, and birds worldwide. Such surveillance is especially important in South-east Asia, where the two latter pandemic strains, as well as the most of the antigenic drift variants, originated.

### The Control of Future Pandemics: Antivirals

In pandemic circumstances, or when a major antigenic influenza virus variant arises, antiviral drugs like amantadine and rimantadine<sup>65</sup> and the recently developed influenza virus neuraminidase inhibitors zanamivir and oseltamivir<sup>66-69</sup> will play a more important role than vaccines, because they are generally broadly active against all influenza A virus subtypes and antigenic variants. Like vaccines, these agents are not completely effective. Protection from laboratory-proven influenza is approximately 50–90%<sup>65,70</sup> in inter-pandemic periods. When administered within 48 h after the appearance of the first symptoms, they ameliorate disease and reduce the duration of illness by 1–2 days.<sup>65,71,72</sup> Early commencement of treatment is essential; zanamivir is only active when treatment is started within 30 h after disease onset.<sup>71,73</sup> These restrictions imply that antivirals can only be (valuable) adjuncts to vaccines.<sup>74</sup> Moreover, their efficacy, or at least that of amantadine, is partly dependent on pre-existing antibodies and is, therefore, expected to be less during pandemics.<sup>65,75</sup>

The use of amantadine and rimantadine has been limited because of the considerable frequency of adverse neurological and gastrointestinal effects and the rapid emergence of resistant strains. In fact, these resistant

strains are already circulating among individuals who have not been treated with the drugs. These properties are especially undesirable for large-scale use during pandemics. In contrast, treatment with one of the neuraminidase inhibitors in clinical trials has been associated with no, or only minor, extra side-effects compared with control subjects and did not lead to the emergence of resistant strains. It is still too early to know whether these favourable features of neuraminidase inhibitors will persist in large-scale routine practice. Naturally circulating influenza virus variants with borderline sensitivity to zanamivir have already been found.<sup>76</sup> This may form a larger problem than the development of resistant mutants during treatment.

### Summary and Conclusions

Novel influenza viruses continuously emerge in the human population. Three times during the present century, an avian influenza virus subtype crossed the species barrier, starting a pandemic, and establishing itself for one to several decades in man. As the 1997 H5N1 event in Hong Kong indicated, the occurrence of another pandemic in the near future cannot be excluded. Sufficient vaccine may not be available to ameliorate the consequences of such an event, because of a shortage of time. During interpandemic periods, important antigenic drift variants sometimes arise at a point of time when, with the current state of the technique, production of a correspondingly adapted vaccine is also impossible. We may be able to solve these problems by increasing influenza surveillance and by adopting new ways of vaccine composition, production, formulation, presentation, and delivery. The recently developed anti-neuraminidase antivirals should only be considered as (valuable) adjuncts to vaccines.

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