Arch Virol (1994) 134: 17–28

Archives of Virology

Origin and evolutionary pathways of the H1 hemagglutinin gene of avian, swine and human influenza viruses: cocirculation of two distinct lineages of swine virus

Y. Kanegae[1], S. Sugita[1], K. F. Shortridge[2], Y. Yoshioka[1], and K. Nerome

[1] Department of Virology I, National Institute of Health, Shinjuku- ku, Tokyo, Japan
[2] Department of Microbiology, University of Hong Kong, Queen Mary Hospital Compound, Hong Kong

Accepted August 23, 1993

Summary. The nucleotide sequences of the HA1 domain of the H1 hemagglutinin genes of A/duck/Hong Kong/36/76, A/duck/Hong Kong/196/77, A/sw/North Ireland/38, A/sw/Cambridge/39 and A/Yamagata/120/86 viruses were determined, and their evolutionary relationships were compared with those of previously sequenced hemagglutinin (H1) genes from avian, swine and human influenza viruses. A pairwise comparison of the nucleotide sequences revealed that the genes can be segregated into three groups, the avian, swine and human virus groups. With the exception of two swine strains isolated in the 1930s, a high degree of nucleotide sequence homology exists within the group. Two phylogenetic trees constructed from the substitutions at the synonymous site and the third codon position showed that the H1 hemagglutinin genes can be divided into three host-specific lineages. Examination of 21 hemagglutinin genes from the human and swine viruses revealed that two distinct lineages are present in the swine population. The swine strains, sw/North Ireland/38 and sw/Cambridge/39, are clearly on the human lineage, suggesting that they originate from a human A/WSN/33-like variant. However, the classic swine strain, sw/Iowa/15/30, and the contemporary human viruses are not direct descendants of the 1918 human pandemic strain, but did diverge from a common ancestral virus around 1905. Furthermore, previous to this the above mammalian viruses diverged from the lineage containing the avian viruses at about 1880.

Introduction

On the basis of serological surveillance of humans [2, 16, 30], it is generally accepted that a virus similar to the classic swine H1N1 influenza virus may have been the causative agent of the 1918 pandemic. Since then, a large number of its possible descendants have been isolated from swine populations in different

countries [11], these are characterized by a minor degree of antigenic drift which contrasts a considerable degree of antigenic drift exhibited by human influenza A viruses [32]. Similarly, following the isolation of human H1N1 viruses in the 1930s [31], numerous antigenic variants were involved in influenza outbreaks [12, 15]. The origin of these antigenic variants in relation to the above swine virus has been investigated [1, 6, 8, 29]. In a large-scale virological survey [9, 10] it was shown that natural infection with influenza viruses possessing a subtype H1 hemagglutinin (HA) antigen occur in a variety of avian species.

Also the complete nucleotide sequence of the H1 HA gene from the avian strain A/duck/Alberta /35/76 (H1N1) was recently determined [3]. As a result, a relatively low degree of nucleotide sequence homology among avian, swine and human viruses suggests that they diverged, however not in recent years, from a common ancestral virus. In addition, the evolutionary relationship of the NP genes among the viruses from mammals and a wide variety of nonmammals has been examined by several individuals [1, 6–8, 29]. In two papers [8, 29] it has been suggested that the classic swine virus isolated in 1930 evolved derived directly from the virus associated with the 1918–1919 outbreaks of human influenza. However, the exact origin of the 1918 pandemic strains and H1N1 viruses isolated from humans and pigs in the 1930s is still uncertain. The present study was initiated to define the origin and evolutionary pathways of the H1 HA genes of avian, swine, and human influenza viruses.

Materials and methods

For comparison, the complete nucleotide sequences of the HA1 domain of the HA genes of A/duck/Hong Kong/36/76 (H1N1; dkHK76), A/duck/Hong Kong/196/77 (H1N1; dkHK77), A/sw/North Ireland/38 (H1N1; swNI38), A/sw/Cambridge/39 (H1N1; swCAM39) and A/Yamagata/120/86 (H1N1; YMG86) were determined. In addition to the above strains, the nucleotide sequences of the HA genes of A/sw/Iowa/15/30 (H1N1; swIOWA30), A/sw/Illinois/1/63 (H1N1; swILL63), A/sw/Hong Kong/1/74 (H1N1; swHK74) and A/sw/Ehime/1/80 (H1N2; swEHM80), which were determined in our previous study [33], were also used in the phylogenetic analyses. Evolutionary relationships among the nucleotide sequences of these viruses were analyzed, along with those of previously reported HA genes [23]. All viruses were grown in 11-day-old embryonated hen's eggs and purified as described previously [20].

The cDNA was synthesized from the viral RNA template with a 12-mer oligonucleotide universal primer. Double stranded cDNA was inserted into the plasmid pBR322 or pUC118, and the recombinant plasmids were then transformed into *Escherichia coli* HB101. The nucleotide sequences of the HA genes were determined by the dideoxy method [17]. In addition to the above method, the direct RNA sequencing technique was also employed in this study [25]. A total of 29 hemagglutinin genes, including those previously published, were used in the phylogenetic analyses. The Nei-Gojobori method was used to estimate the number of synonymous (silent) and nonsynonymous (amino acid-changing) substitutions [19]. Because this computer simulation's estimation of the evolutionary rates of synonymous and nonsynonymous substitutions are quite accurate. Phylogenetic trees were constructed by the neighbor-joining (N-J) method [24]. Each branch length shown in the tree was estimated by the principle of minimum evolution.

Results

Analyses of the nucleotide and deduced amino acid sequences of the HA genes

Nucleotide sequences of the HA genes of dkHK76 (D00838), dkHK77 (D00839), swNI38 (D00840), swCAM39 (D00837) and YMG86 (D00841) were determined and deposited in the DNA Data Bank of Japan (DDBJ). The deduced amino acid sequences of the HA1 domains of the HA gene of these five strains were aligned with those of the previously sequenced HA genes of swine and human viruses (Fig. 1).

Analyses of the amino acid sequences showed that residues 125–195, which correspond to the antigenic sites Sa, Sb, Ca1 and Ca2 [4], were highly conserved among avian viruses. This is in sharp contrast to the pattern of amino acid substitutions in swine and human viruses, which varies greatly. In agreement with a previous report [3], there were five potential glycosylation sites on the HA1 domain of dkHK76 and dkHK77 viruses. The degree of amino acid similarity at positions 125–195 of swine and avian viruses was apparently higher (swILL63/dkHK76:76.1% – swIOWA30/dkHK77:85.9%) than that between human and avian viruses (USSR77/dkHK76:56.3% – WSN33/dkALB76:74.7%). Although the predicted protein structure of the HA1 region of swNI38 and swCAM39 was very similar to that of human strain WSN33, one amino acid deletion at position 127 was observed in swCAM39 and WSN33. In the latter strains, residues 130–135 and 221–225, which are thought to be on the surface of the receptor-binding pocket sides, were substituted at four positions.

The nucleotide and predicted amino acid sequence homologies were further illustrated when pairwise comparisons among three avian, six swine and two human viruses were made. These results are summarized in Table 1. Comparison of the avian virus HA genes revealed extreme similarity, with homology between pairs of nucleotide sequences ranging from 87.2% to 98.4%. It is interesting to note that the degree of nucleotide and amino acid sequence homology between dkHK76 and dkHK77 (98.4%, 97.6%) is apparently higher than that of the former two strains and dkALB76 (87.7%, 92.9%) in spite of being isolated in nearly the same period, thus reflecting their different geographic origin. This result is compatible with the previous report in that the H4 HA genes are divided into two groups based on their geographical differences [5]. On the basis of the nucleotide sequence homology, H1 HA genes presented in Table 1 can be segregated into three host groups; avian, swine and human genes.

With the exception of two swine isolates (swNI38, swCAM39), H1 HA genes showed the highest nucleotide and amino acid sequence homology within the group. Interestingly, the nucleotide sequences of swNI38 and swCAM39 viruses showed higher homology (87.4%–97.6%) with that of human viruses (WSN33, PR834, USSR77, YMG86) than that (79.1%–83.1%) within the swine virus group. The extreme similarity among the HA genes of the above two swine strains and human WSN33 led eventually to the suggestion that the former swine viruses may have been introduced from a human virus which had been prevalent in the 1930s.

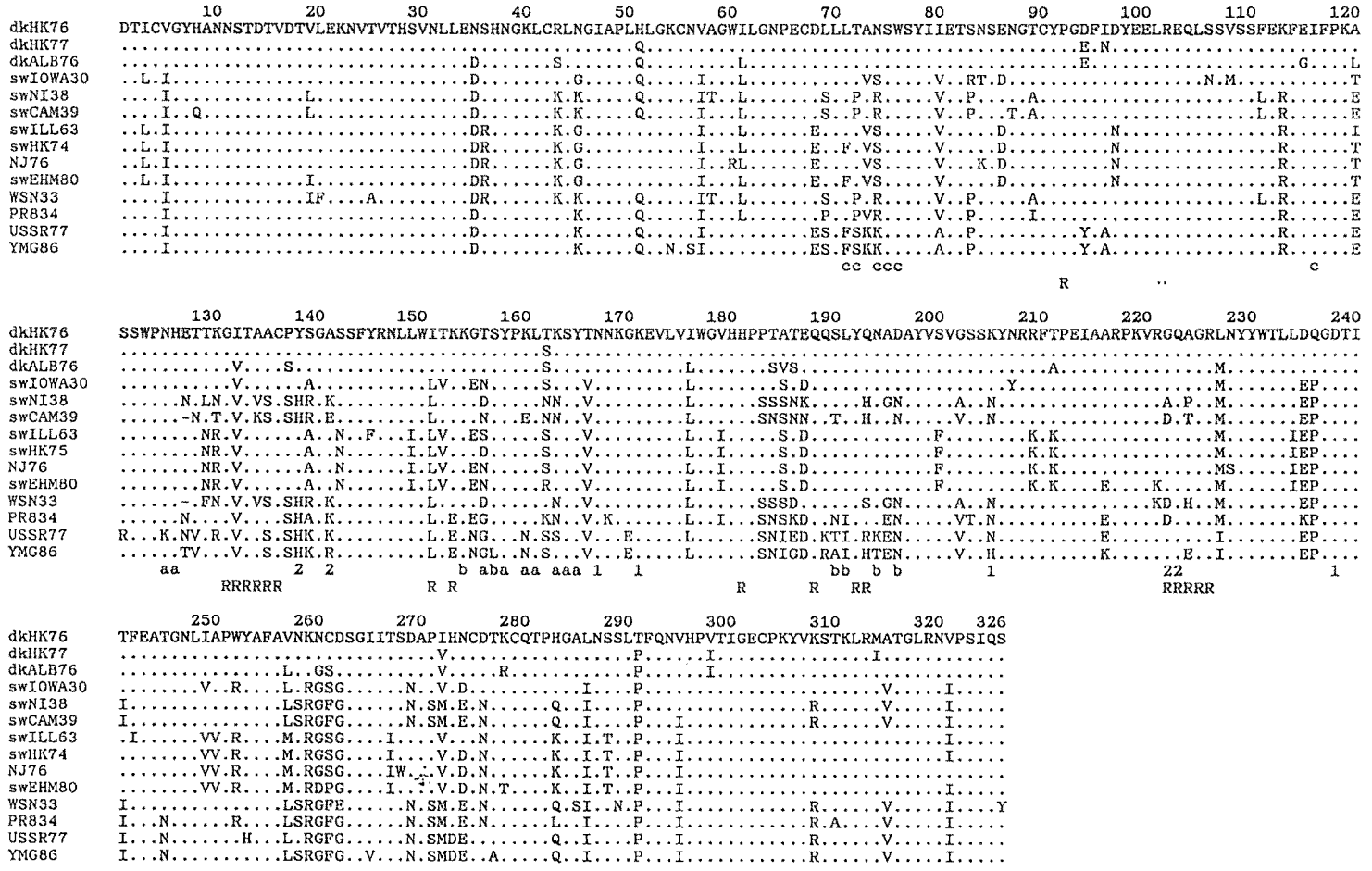

Fig. 1. Comparison of the amino acid sequences of the HA1 domain of H1 HA of avian, swine and human influenza viruses. The sequences were aligned with that of avian strain dkHK76. Amino acid differences from the latter virus are also shown. In addition to the nucleotide sequences described in the text, those of the previously sequenced HA genes of A/NJ/11/76 (H1N1; NJ76), A/WSN/33 (H1N1; WSN33), A/PR/8/34 (H1N1; PR834), A/USSR/ 90/77 (H1N1; USSR77) and A/duck/Alberta/35/76 (H1N1; dkALB76) are also compared here

Table 1. Pairwise comparison of the nucleotide and amino acid sequence homologies among the H1 haemagglutinin genes of avian, swine and human influenza A viruses

Strain	% Homology[a]													
	dkHK76	dkHK77	dkALB76	swIOWA30	swNI38	swCAM39	swILL63	swHK74	NJ76	swEHM80	WSN33	PR834	USSR77	YMG86
dkHK76	***	98.4	87.2	77.0	74.5	74.7	76.1	76.2	76.3	74.9	73.8	74.6	73.3	71.8
dkHK77	97.6	***	87.9	76.7	73.5	73.7	75.5	75.8	75.7	74.6	72.7	73.4	72.5	71.0
dkALB76	92.9	94.2	***	77.8	74.6	74.5	75.9	76.7	76.3	76.0	74.0	73.3	72.5	71.4
swIOWA30	86.2	86.2	87.1	***	85.7	85.4	90.2	88.0	88.0	86.7	84.4	85.5	82.1	80.9
swNI38	79.5	78.8	81.3	83.1	***	97.1	82.1	80.6	80.1	79.1	97.6	93.7	88.5	87.5
swCAM39	78.5	77.9	80.1	82.5	94.2	***	82.2	80.4	80.2	79.4	97.5	93.5	88.4	87.4
swILL63	82.2	81.6	82.8	89.6	79.1	78.5	***	96.1	95.5	94.0	81.2	82.1	78.5	77.5
swHK74	82.5	81.9	83.1	90.2	80.1	79.1	97.9	***	98.4	97.0	79.7	80.3	78.0	76.6
NJ76	81.6	81.0	82.2	89.9	78.2	77.9	96.9	97.6	***	96.2	79.1	80.1	77.4	76.2
swEHM80	81.0	80.1	81.0	88.7	78.2	77.9	96.0	97.2	96.0	***	78.7	79.3	77.4	76.1
WSN33	77.6	76.7	79.1	80.4	94.5	91.4	77.6	78.5	76.7	77.6	***	92.3	87.7	86.8
PR834	78.5	77.9	79.8	84.4	89.0	89.0	80.4	80.4	79.5	79.8	86.8	***	89.9	88.8
USSR77	76.7	77.0	77.6	80.1	85.0	85.6	76.1	77.0	75.2	75.8	82.5	87.4	***	95.8
YMG86	76.1	76.4	77.0	79.5	84.1	85.0	74.9	75.8	73.9	74.2	81.6	85.9	93.6	***

[a] Numbers of nucleotide (upper right half) and amino acid (lower left half) homologies (%) between HA1 domain of haemagglutinin genes

Evolutionary analysis of the HA genes by the N–J method

The host-specific feature and possible co-circulation of the distinct lineages in the swine population led us to analyze the evolutionary patterns of the H1 HA genes of avian, swine and human viruses by the N–J method. This method was reported to minimize the total number of nucleotide substitutions required to

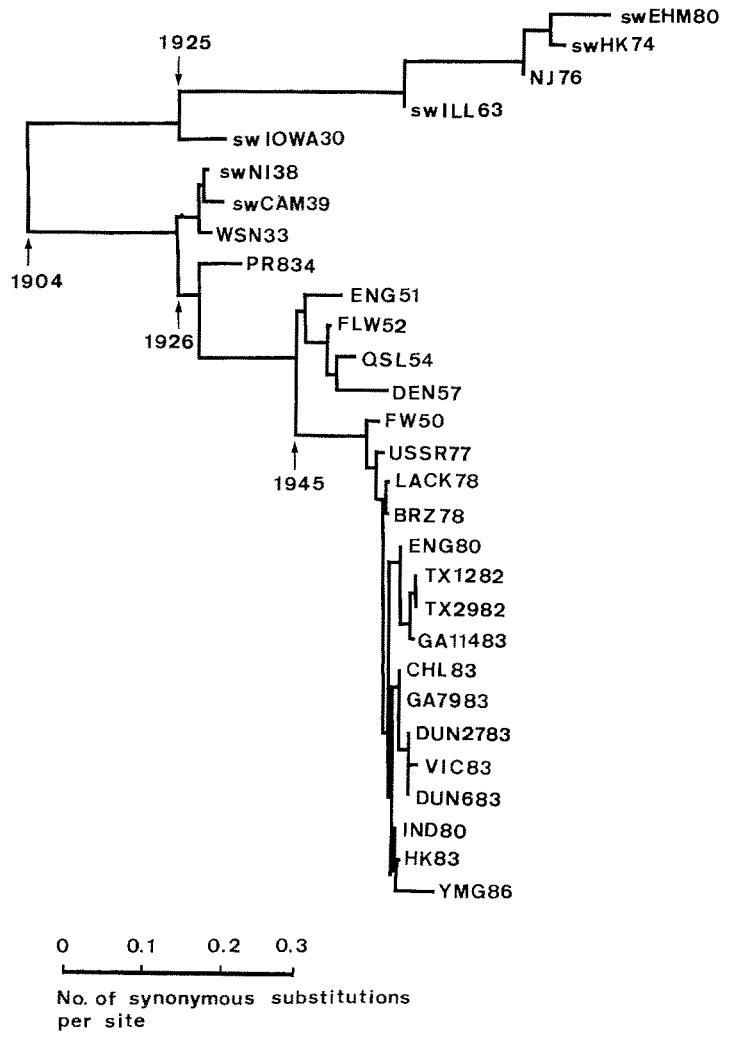

Fig. 2. Phylogenetic tree for the H1 HA gene of swine and human influenza viruses. The tree was constructed by the N–J method [22], using the number of synonymous substitution [17]. The length of each branch was estimated by the principle of minimum evolution to minimize the total number of nucleotide substitution for a tree. The following H1N1 strains were also included here: ENG51, A/England/1/51; FLW52, A/Fort Leonard Wood/1/52; GSL54, A/Gueensland/34/54; DEN57, A/Denver/1/57; FW50, A/Fort Warren/1/50, USSR-77, A/USSR/90/77; LACK78, A/Lackland/3/78; BRZ78, A/Brazil/11/78; ENG80., A/England/333/80; TX1282, A/Texas/12/82; TX2982, A/Texas/29/82; GA11483, A/Georgia/114/83; CHL83; A/Chile/1/83; GA7983, A/Georgia/79/83; DUN2783, A/Dunedin/27/83; VIC83, A/Victoria/7/83; DUN683, A/Dunedin/6/83; HK83, A/Hong Kong/32/83

construct a phylogenetic tree. The results of phylogenetic analyses of 29 influenza virus HA nucleotide sequences are expressed as an evolutionary tree rooting from a putative origin indicated by an arrow (1904). The putative origin was deduced theoretically from the evolutionary rates of swine and human lineages. As seen in Fig. 2, within mammalian viruses, HA genes are clearly divided into two major lineages and one minor lineage, one including five swine strains, the second one including two swine and twenty-two human viruses, and the third minor one having been derived from the second branch cluster. As already described in the preceding section, the phylogenetic tree constructed from synonymous substitutions revealed that two swine strains, swNI38 and swCAM39, are apparently on the human evolutionary lineage.

The pandemic influenza virus, which is related antigenically to the swine viruses, is thought to have appeared first in man in 1918. The phylogenetic tree suggests that the ancestral strain of these viruses had already branched before appearance of the above pandemic strain in man, pointing in two directions, human and swine pathways. The branch giving rise to the HA gene of swILL63 strain diversed from the branch containing the "classic" swine strain swIOWA30 in the mid-1920s, with the swine virus then appearing to evolve in a single lineage. In agreement with the evidence based on pairwise comparison of the nucleotide sequence, two swine viruses isolated between 1938 and 1939 are closest evolutionally to the human WSN33 virus, belonging to the same branch cluster. It was interesting to determine that the H1N1 (formerly A prime) viruses, which were prevalent during the 10-year period from 1946 to 1956, had previously divided from the lineage containing the PR834 strain. This result is compatible with previous reports in which NP genes were analyzed from an evolutionary point of view [1, 6, 8, 29]. Furthermore, in agreement with previous papers [18, 26], the tree clearly shows that USSR77 virus originates from the FW50 strain. Since 1977 the tree for the HA genes of H1N1 virus suggests of the sequential evolution of a common lineage, however, YMG86 isolated in Japan originated from a HK83 isolated. The present study defined the evolutionary position of the YMG86 virus that was prevalent during the 1986–1987 outbreak in Japan.

Using the evolutionary pathways of the mammalian virus genes, we tried to determine the evolutionary relationship between nonmammalian and mammalian virus H1 HA genes. Because the avian and mammalian HA genes are distantly related, phylogenetic analysis was conducted with a model tree constructed from the nucleotide substitutions at the third codon position. The evolutionary pathways of mammalian viruses as seen in the tree (Fig. 3) are very similar to those obtained from the analysis of synonymous substitutions. It was shown that the HA genes of avian and mammalian viruses may have diverged considerably earlier than the appearance of the possible ancestral strains of mammalian virus genes in the early 1900s. According to the tree presented in Fig. 3, the divergence among the H1 subtypes of HA genes might have occurred during the last 120 years (1870). The construction of the phylogenetic tree allows us to estimate long-term evolutionary rates for HA genes in different lineages.

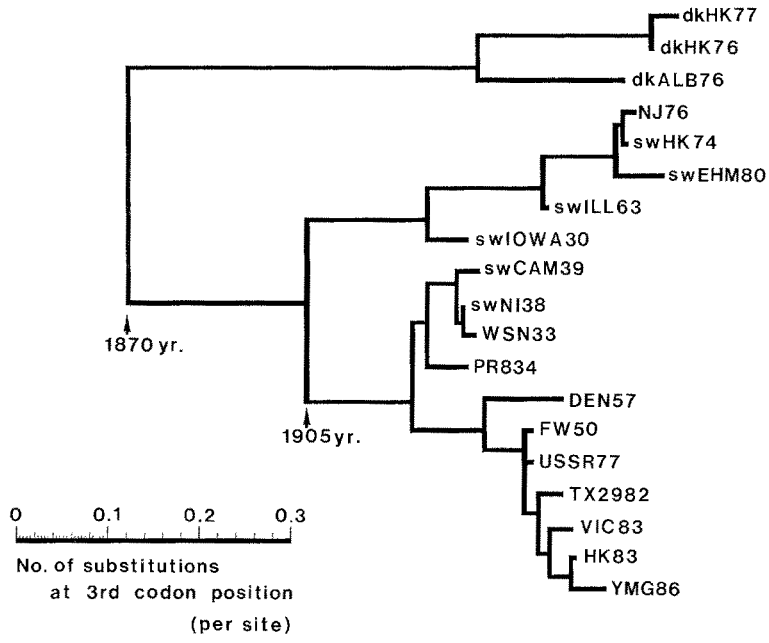

Fig. 3. Evolutionary pathways of H1N1 avian, swine and human influenza viruses based on the mutational distances calculated between HA1 domains. The phylogenetic tree was constructed by the N–J method, using the number of nucleotide substitution at the third codon position

The evolutionary rates were obtained by plotting the year of virus isolation versus the branch length to the ancestor of each lineage (data not shown). We estimated the rate of nucleotide substitution in the synonymous site for the HA gene of swine and human viruses isolated between 1933 and 1956 at 0.00946 and 0.00847/site/year, respectively, which are nearly the same as that reported by Sugita et al. [33]. However, the evolutionary rate of recent H1N1 viruses seems to be slightly slower than that of the above human H1N1 viruses isolated before 1957.

Discussion

In the present study, we defined three host-dependent evolutionary pathways of H1 subtypes of avian, swine and human influenza viruses. To date, a direct precursor of the classic swine strain swIOWA30 has been considered to be the causative virus of the great pandemic of human influenza in 1918–1919 [32]. Coupled with this speculation, it is of particular interest that a 1918–human pandemic virus may have entered the pig population at the time of the Spanish influenza outbreak/epidemic [29]. This is consistent the evidence, which suggests the possible existence of a common ancestor of the 1918 human and 1930–classical swine viruses around 1918 [8]. Nevertheless, in a serological surveillance of humans, it was shown that antibodies reacting with swIOWA30 virus could be detected in a high proportion of sera collected from persons born

between 1900 and 1920 [2, 16, 30], suggesting the existence of swIOWA30-like virus during the above period. This is clearly compatible with our phylogenetic analysis indicating that swine and human H1N1 viruses may have been derived from an ancestral virus which was prevalent in the early 1900s. This also supports recent evidence showing that swine and human H1N1 viruses isolated in the 1930s may have been derived from a virus which appeared before the outbreak of 1918–1919 human influenza [7, 8, 29].

Evolutionary rates of human, swine and avian viruses have also been analyzed by Gorman et al. [8] and Schultz et al. [29]. It was shown that a common ancestor of human and swine H1N1 viruses might have appeared shortly before or at the time of the pandemic. These results are, however, in disagreement with our conclusion concerning the ancestral node: a common ancestor to the 1918 human pandemic strain and swine H1N1 viruses was estimated to exist around 1904. This difference may be due to the gene species and methods used in the analysis of evolutionary rates. As already suggested by several authors [1, 6, 8, 29], our analysis also shows the existence of a common avian virus ancestor from which human and swine H1N1 viruses have diverged previous to the appearance of the 1918-human pandemic and classical swine viruses.

In contrast to human and avian viruses, the evolution of HAs in swine viruses is divided into two lineages, coinciding with the result obtained in pairwise comparison of their nucleotide sequences. Although five swine viruses belonging to the swine lineage evolved in a single lineage, swNI38 and swCAM39 apparently belong to human lineage. In agreement with previous evidence, of the frequent inter-species transmission of human and swine viruses as reported by Gorman et al. [8] and others [1, 29], our phylogenetic tree constructed from synonymous substitutions by N–J method indicates that two swine viruses isolated from swine belong to human lineage, suggesting the transmission of human virus to the swine population in the 1930s. In earlier studies, a comparison of evolutionary patterns of influenza B viruses [13, 35], showed the presence of multiple evolutionary pathways. Unlike the mechanism by which influenza B viruses evolve, the existence of two distinct lineages in the swine population might be attributable to the transmission of human epidemic virus into the swine population.

Recently, even though Gammelin et al. [6] and Gorman et al. [7] reported that human and swine virus NP genes share a common ancestor, NPs from the oldest nonavian viruses are much more closely related to that of avian viruses. In the above studies, the NP gene can be divided into five lineages, suggesting host-dependent evolution after introduction from an ancestral avian virus. Coupled with this evidence, the ubiquitous characteristic of genes coding for surface glycoproteins in the avian species may reflect its potential role as a gene pool [14]. This hypothesis is supported by the NP genes involvement in determining host range [28, 34]. Similarly, swine is understood to be the major host reservoir for human A influenza virus [10], and it was shown that HA and neuraminidase (NA) genes evolve as a single lineage [21, 33].

The present study first demonstrates that two distinct H1 HA lineages, as distinguished by the phylogenetic tree, were involved in influenza outbreak in

swine populations in the 1930s. Evidence has been presented in a previous study [22] that avian influenza virus can be transmitted to swine, suggesting its unique nature to cross the host barrier from birds to mammals. Recent characterization of hemagglutinin genes of H1 influenza virus field isolates from swine and birds supports the above evidence based on experimental infection in pigs [27]. In an earlier study [20], we also characterized a reassortant (H1N2) virus, which contains seven RNA segments from swine (H1N1) virus and the remaining NA gene from human H3N2 virus, demonstrating the natural occurrence of reassortment between swine H1N1 and human H3N2 viruses [20]. It is consistent with previous reports that H1N1 viruses circulating in the swine population may be derived from an avian virus gene pool [8, 29], and that the NP gene of human viruses are closely related to those of swine and avian viruses [1]. Our results also led to suggest that swine have played a potential role as intermediates for the introduction of avian influenza virus genes into human viruses.

References

1. Altmüller A, Kunerl M, Müller K, Hinshaw VS, Fitch WM, Scholtissek C (1991) Genetic relatedness of the nucleoprotein (NP) of recent swine, turkeys and human influenza A virus (H1N1) isolates. Virus Res 22: 79–87
2. Andrewes CH, Laidlaw PP, Smith W (1935) Influenza: observation on the recovery of virus from man and on the antibody content of human sera. Br J Exp Pathol 16: 566–582
3. Austin FJ, Kawaoka Y, Webster RG (1990) Molecular analysis of the haemagglutinin gene of an avian H1N1 influenza virus. J Gen Virol 71: 2471–2474
4. Caton A, Brownlee CG, Yewdell JW, Gerhard W (1982) The antigenic structure of influenza virus A/PR/8/34 hemagglutinin (H1 subtype). Cell 31: 417–427
5. Donis RO, Bean WJ, Kawaoka Y, Webster RG (1989) Distinct lineages of influenza virus H4 hemagglutinin genes in different regions of the world. Virology 169: 408–417
6. Gammelin M, Altmüller A, Reinhardt U, Mandler J, Harley VR, Hudson PJ, Fitch WM, Scholtissek C (1990) Phylogenetic analysis of nucleoproteins suggests that human influenza A viruses emerged from a 19th-century avian ancestor. Mol Biol Evol 7: 194–200
7. Gorman OT, Bean WJ, Kawaoka Y, Webster RG (1990) Evolution of the nucleoprotein gene of influenza A virus. J Virol 64: 1487– 1497
8. Gorman OT, Bean JB, Kawaoka Y, Donatelli S, Guo Y, Webster RG (1991) Evolution of influenza A virus nucleoprotein genes: implications for the origin of H1N1 human and classical swine viruses. J Virol 65: 3704–3714
9. Hinshaw VS, Webster RG, Rodringue R (1981) Influenza A viruses: combination of hemagglutinin and neuraminidase subtypes isolated from animals and other sources. Arch Virol 67: 191–201
10. Hinshaw VS, Webster RG (1982) The natural history of influenza A viruses. In: Beare AS (ed) Basic and applied influenza research. CRC Press, Boca Raton, pp 79–104
11. Hinshaw VS, Alexander DJ, Aymard M, Bachmann PA, Easterday BC, Hannoun C, Kida H, Lipkind M, Mackenzie JS, Nerome K, Schild GC, Scholtissek C, Senne DA, Shortridge KF, Skehel JJ, Webster RG (1984) Antigenic comparisons of swine influenza-like H1N1 isolates from pigs, birds and humans: an international collaborative study. Bull World Health Organ 62: 871–878
12. Isaacs A, Gledhill AW, Andrews CH (1952) Laboratory studies with special reference to the European outbreak of 1950-51. Bull World Health Organ 6: 287–315

13. Kanegae Y, Sugita S, Endo A, Ishida M, Senya S, Osako K, Nerome K, Oya A (1990) Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: Cocirculating lineages in the same epidemic season. J Virol 64: 2860–2865
14. Kawaoka Y, Chambers TM, Sladen WL, Webster RG (1988) Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247–250
15. Kendal AP, Noble GR, Skehel JJ, Webster RG (1978) Antigenic similarity of influenza A (H1N1) viruses from epidemics in 1977–1978 to "Scandinavian" strains isolated in epidemics of 1950–1951. Virology 89: 632–636
16. Masurel N (1969) Serological characteristics of a "new" serotype of influenza A virus: the Hong Kong strain. Bull World Health Organ 41: 461–468
17. Mizusawa S, Nishimura S, Seela F (1986) Inprovement of the chain termination method of DNA sequencing by use of deoxy-7- deazaguanosine triphosphate in place of dGTP. Nucleic Acids Res 14: 1319–1324
18. Nakajima K, Desselberger D, Palese P (1978) Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. Nature 274: 334–339
19. Nei M, Gojobori T (1986) Simple methods for estimating the number of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 34: 418–426
20. Nerome K, Sakamoto S, Yano N, Yamamoto T, Kobayashi S, Webster RG, Oya A (1983) Antigenic characteristics and genome composition of a naturally occurring recombinant influenza virus isolated from a pig in Japan. J Gen Virol 64: 2611–2620
21. Nerome K, Kanegae Y, Yoshioka Y, Itamura S, Ishida M, Gojobori T, Oya A (1991) Evolutionary pathways of N2 neuraminidase of swine and human influenza A viruses: Origin of the neuraminidase gene of two reassortants (H1N2) isolated from pigs. J Gen Virol 72: 693–698
22. Pensaet MM, Ottis K, Vandeputte J, Kaplan MM, Bachmann PA (1981) Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. Bull World Health Organ 59: 75–78
23. Reymond FL, Caton AJ, Cox NJ, Kendal AP, Brownlee GG (1986) The antigenicity and evolution of influenza H1 hemagglutinin, from 1950–1959 and 1977–1983: two pathways from one gene. Virology 148: 275–287
24. Saitou M, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425
25. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitions. Proc Natl Acad Sci USA 14: 5463– 5467
26. Scholtissek C, Von Hoyningen V, Rott R (1978) Genetic relatedness between the new 1977 epidemic strains (H1N1) of influenza and human influenza strains isolated between 1947 and 1957 (H1N1). Virology 89: 613–617
27. Scholtissek C, Bürger H, Bachmann PA, Hannoun C (1983) Genetic relatedness of hemagglutinin of the H1 subtype of influenza A viruses isolated from swine and birds. Virology 129: 521–523
28. Scholtissek C, Bürger H, Kistner O, Shortridge KF (1985) The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. Virology 147: 287–294
29. Schultz U, Fitch WM, Ludwig S, Mandler J, Scholtissek C (1991) Evolution of pig influenza viruses. Virology 183: 61–73
30. Shope RE (1938) Serological evidence for the occurrence of infection with human influenza virus in swine. J Exp Med 67: 739–748
31. Smith W, Andrewes CH, Laidlaw PP (1933) A virus obtained from influenza patients. Lancet 1: 66–68
32. Stuart-Harris CH, Schild GC (1976) Influenza viruses of lower animals and birds. In:

Stuart-Harris CH, Schild GC (eds) Influenza, the viruses and the diseases. E. Arnold, London, pp 78–83
33. Sugita S, Yoshioka Y, Itamura S, Kanegae Y, Oguchi K, Gojobori T, Nerome K, Oya A (1991) Molecular evolution of hemagglutinin genes of H1N1 swine and human influenza A viruses. J Mol Evol 32: 16–23
34. Synder MH, Buckler-White AJ, London WT, Tierney EL, Murphy BR (1987) The avian influenza virus nucleoprotein gene and a specific constellation of avian and human virus polymerase genes each specify attenuation of avian-human influenza A/Pintail/79 reassortant viruses for monkeys. J Virol 61: 2857–2863
35. Yamashita M, Krystal M, Fitch WM, Palese P (1988) Influenza B virus evolution: co-circulating lineages and comparison of evolutionary pattern with those of influenza A and C viruses. Virology 163: 112–122

Authors' address: Dr. K. Nerome, Department of Virology I, National Institute of Health, 23-1, Toyama 1-chome, Shinjuku, Tokyo 162, Japan.

Received May 15, 1992