Antigenic Characteristics and Genome Composition of a Naturally Occurring Recombinant Influenza Virus Isolated from a Pig in Japan

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SUMMARY

We performed antigenic analysis of the haemagglutinin and neuraminidase subunits of a recombinant virus (A/swine/Kanagawa/2/78) isolated from a pig in Japan in 1978, using a series of monoclonal antibodies to H1 (Hswl) haemagglutinin and N2 neuraminidases of H2N2 and H3N2 viruses. Results obtained in haemagglutination inhibition tests with five monoclonal antibodies to the haemagglutinin of A/N J/8/76 (H1N1) revealed that the haemagglutinin of three H1N1 and the recombinant viruses were indistinguishable from that of A/N J/8/76. The neuraminidase of A/swine/Kanagawa/2/78 was found to be antigenically similar to A/Kumamoto/22/76 (H3N2, A/Victoria/3/75-like strain). The oligonucleotide maps of the entire RNAs of H1N1, H1N2 and H3N2 viruses showed that A/swine/Kanagawa/2/78 (H1N2) virus was more similar to swine (H1N1) virus than to A/Kumamoto/22/76 (H3N2) virus. Radioactive cDNA was prepared by reverse transcription of the recombinant virus RNA using a dodecadeoxyribonucleotide primer and used in DNA–RNA hybridization experiments. The results obtained in molecular hybridization based on blotting procedures showed that all cDNA segments except gene 6 hybridized efficiently with RNAs of swine (H1N1) influenza virus. The sixth cDNA segment was homologous to the corresponding RNA segment of H3N2 virus. The genetic relatedness of A/swine/Kanagawa/2/78 (H1N2) with either A/swine/Kanagawa/4/78 (H1N1) or A/Kumamoto/22/76 (H3N2) was clearly established by hybridization between the cDNA segment probes and viral RNA. It was concluded that the neuraminidase gene of A/swine/Kanagawa/2/78 (H1N2) was derived from a human H3N2 virus, while the seven other genes were from a swine H1N1 virus.

INTRODUCTION

The frequent transmission of H3N2 influenza viruses from man to swine has been documented by genome and antigen characterization of numerous H3N2 virus variants isolated from the above two hosts (Kundin, 1970; Shortridge et al., 1976, 1979; Hinshaw et al., 1978; Shortridge & Webster, 1979; Kanai et al., 1981; Nerome et al., 1981; Nakajima et al., 1982). On the other hand, in a large-scale serological and virological survey conducted by research workers in several countries it was shown that swine (H1N1, formerly HswlN1) virus different from the 'classical' strain has been rapidly circulating in the swine population of many areas of the world (Kendal et al., 1977; Hinshaw et al., 1978; Shortridge & Webster, 1979; Yamane et al., 1979; Pensaert et al., 1981; Sugimura et al., 1980; Nerome et al., 1982a).
The prevalence of swine (H1N1) influenza virus in the swine population of Japan allows us to explore the possibility that a recombinant virus may appear between recent swine (H1N1) virus and H3N2 virus already present in pigs. Recent virological surveillance showed that out of many swine influenza virus isolates in Japan, two viruses were found to be recombinants possessing haemagglutinin subunits related to A/NJ/8/76 (H1N1, formerly Hsw1N1) and N2 neuraminidase subunits similar to recent H3 influenza viruses from man (Sugimura et al., 1980; Nerome et al., 1982 a, b). In the present study we describe detailed immunological characterization of the haemagglutinin and neuraminidase subunits of a recombinant (H1N2) virus using monoclonal antibodies to the haemagglutinin of A/NJ/8/76 (H1N1) and N2 neuraminidases of different H2 and H3 influenza viruses. These studies also examine the genome composition of the above recombinant virus using molecular hybridization with DNA complementary to A/swine/Kanagawa/2/78 viral RNA.

**METHODS**

*Viruses.* The following human H3N2 influenza viruses were used in the present study: A/Aichi/2/68, A/Tokyo/1/72, A/Tokyo/6/73 (A/Port Chalmers/1/73-like strain), A/Ehime/2/74, A/Kumamoto/22/76, and A/Tokyo/1/77 (A/Texas/1/77-like strain). In addition, the following viruses were employed: A/swine/Kanagawa/2/78 (H1N2), A/NJ/8/76 (H1N1), A/swine/Niigata/1/77 (H1N1), A/swine/Niigata/2/77 (H1N1), A/swine/Kanagawa/4/78 (H1N1), A/swine/Shizuoka/1/78 (H1N1). The viruses were grown in the allantoic cavity of 11-day-old embryonated hens' eggs. For genetic analysis, virus was concentrated by centrifugation at 44,000 g for 1.5 h in a Beckman 21 rotor and was banded twice in linear sucrose gradients, 20 to 50% (w/w), at 64,000 g for 2 h using a SW27 rotor.

*Monoclonal antibodies.* The methods for producing monoclonal antibodies to the haemagglutinin of A/NJ/8/76 and the N2 neuraminidase of reference strains of H2N2 and H3N2 viruses by hybridoma cells were essentially similar to those of Köhler & Milstein (1976). Two strains of myeloma cells, P3/X-36/Ag 8 and Sp 2/0/Ag 14 were employed in hybridoma preparations (Webster et al., 1983).

*Serological tests.* Haemagglutination and haemagglutination-inhibition (HI) tests were performed in reduced volume using 0.5% chicken red blood cells. Neuraminidase titration and neuraminidase-inhibition (NI) tests were done according to the methods recommended by a WHO Expert Committee (1973).

*Oligonucleotide mapping.* Purified RNA from egg-grown virus was digested with ribonuclease T1 (Sankyo Co., Ltd.) and the 5' ends of the resulting oligonucleotides were labelled with [γ-32P]ATP (Amersham International) using T4 polynucleotide kinase (Boehringer) by the methods described previously (Nakajima et al., 1978; Billeter et al., 1974). Two-dimensional separation of oligonucleotides was done as described previously (Billeter et al., 1974; De Wachter & Fiers, 1972).

*Preparation of DNA complementary to A/swine/Kanagawa/2/78 (H1N2) RNA.* cDNA was synthesized by transcribing the RNA of the recombinant virus into 32P-cDNA with avian myeloblastosis virus reverse transcriptase (Life Sciences, St Petersburg, Fla. U.S.A.) and a dodecadeoxyribonucleotide primer (AG-CAAAAG-CAGG; Collaborative Research, Inc.) in the presence of [α-32P]dCTP (Amersham International) according to methods described previously (Davis et al., 1981).

*DNA—RNA hybridization on nitrocellulose paper.* DNA—RNA hybridization by blotting procedures on nitrocellulose paper was done as described previously (Southern, 1975; Thomas, 1980). For hybridization, total viral RNA was fractionated on a 1.4% agarose gel at 100 V for 2.5 to 3 h. Fractionated RNAs were transferred from the gel to nitrocellulose paper, and hybridized with 32P-DNA complementary to total RNA of the recombinant virus. Unhybridized DNA was removed from the paper by washing at least six times at different temperatures under stringent conditions (Thomas, 1980) and the remaining radioactive cDNA, which hybridized with corresponding RNA segments was detected by autoradiography.

*Liquid DNA—RNA hybridization.* cDNA segments corresponding to each of the RNA segments were separated by 7 M-urea—4% polyacrylamide gel electrophoresis. After determination of the migration patterns of cDNA by autoradiography, fractions were eluted from the gel by shaking gently in buffer [0.5 M-NH4CH3CO2, 0.01 M-Mg(CH3CO2)2, 0.1 mM-EDTA] and were used in quantitative molecular hybridization. For hybridization, 6 ng of isolated 32P-cDNA was mixed with different concentrations of viral RNA and hybridized at 70°C for 5 min and 50°C for 3 h under stringent conditions [50% formamide, 0.04 M-PIPES (pH 6.5), 1 mM-EDTA, 0.4 M-NaCl] according to methods described previously (Avvendimento et al., 1980). After hybridization for the time indicated, the reaction mixtures were treated with S1 nuclease (1000 U/ml; Miles Laboratories) at 45°C for 30 min to remove the unhybridized radioactive cDNA probes.
Swine influenza virus characterization

Table 1. Antigenic characterization of the haemagglutinin of swine isolates using monoclonal antibodies to the haemagglutinin of A/NJ/8/76

<table>
<thead>
<tr>
<th>Test viruses</th>
<th>Antiserum* to A/NJ/8/76</th>
<th>Monoclonal antibodies</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>6/1a</td>
</tr>
<tr>
<td>A/NJ/8/76 (H1N1)</td>
<td>2560</td>
<td>6400</td>
</tr>
<tr>
<td>A/swine/Niigata/1/77 (H1N1)</td>
<td>2560</td>
<td>6400</td>
</tr>
<tr>
<td>A/swine/Niigata/2/77 (H1N1)</td>
<td>1280</td>
<td>6400</td>
</tr>
<tr>
<td>A/swine/Shizuoka/1/78 (H1N1)</td>
<td>1280</td>
<td>6400</td>
</tr>
<tr>
<td>A/swine/Kanagawa/2/78 (H1N2)</td>
<td>640</td>
<td>6400</td>
</tr>
<tr>
<td>A/swine/Kanagawa/4/78 (H1N1)</td>
<td>640</td>
<td>6400</td>
</tr>
</tbody>
</table>

* Ferret serum.
† Less than 100.

Table 2. Analysis of the neuraminidase protein of a recombinant swine influenza virus using monoclonal antibodies to different strains of H2N2 and H3N2 influenza viruses

<table>
<thead>
<tr>
<th>Antibody to Homologous Clones</th>
<th>Aichi NI titre</th>
<th>Tokyo 1/72</th>
<th>Tokyo 6/73</th>
<th>Ehime 2/74</th>
<th>Tokyo 2/75</th>
<th>Tokyo 2/78</th>
<th>swine/Kanagawa 22/76</th>
<th>Kumamoto 1/77</th>
<th>Tokyo 1/77</th>
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</tr>
<tr>
<td>S10/1</td>
<td>700</td>
<td>+</td>
<td>+</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
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<td>−</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>S25/4</td>
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<tr>
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<td>+ (128)</td>
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<td>+ (256)</td>
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<td>−</td>
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<td>+ (256)</td>
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<td>−</td>
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</tr>
<tr>
<td>88/2</td>
<td>200</td>
<td>−</td>
<td>+</td>
<td>+ (8)</td>
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<td>+ (512)</td>
<td>+ (64)</td>
<td>+ (128)</td>
<td>+ (256)</td>
</tr>
</tbody>
</table>

* Less than 4. For comparison, the table also shows NI titre to heterologous viruses isolated after 1973.

RESULTS

Antigenic characterization

Haemagglutinin

To determine the antigenic relatedness between the above recombinant virus and other swine (H1N1) isolates in Japan, representative strains were analysed in HI tests with five different monoclonal antibodies to the haemagglutinin of A/NJ/8/76 and ferret infected serum. Results obtained in HI tests with ferret serum showed that the isolates including the recombinant virus from Japan were indistinguishable from A/NJ/8/76. Five monoclones to the haemagglutinin of A/NJ/8/76 could clearly differentiate between representative strains of swine (H1N1) viruses from Japan. Haemagglutination of four strains was inhibited by all monoclones tested, indicating that these strains were identical with A/NJ/8/76 at the five different epitopes (Table 1). However, the A/swine/Shizuoka/1/78 strain did not react with two monoclones (117/2, 72/3) showing that this virus differs from the former isolates at two sites. HI testing of the recombinant virus with five monoclones revealed identical patterns of reactivity with A/NJ/8/76 and the above-mentioned three strains (A/swine/Niigata/1/77, A/swine/Niigata/1/78, A/swine/Kanagawa/4/78).
Neuraminidase

The neuraminidase antigen of the recombinant virus was characterized in detail using 20 monoclonal antibodies to the N2 neuraminidase of different H2N2 and H3N2 strains. Four monoclonal antibodies to neuraminidases of A/Tokyo/3/67 (H2N2) inhibited the neuraminidase activity of early Hong Kong viruses, such as A/Aichi/2/68 and A/Tokyo/1/72 (A/England/42/72-like strain), but failed to react with those of viruses in and after 1973 (Table 2). Of the 20 monoclones tested, 11 monoclonal antibodies to three strains of H3N2 virus, designated A/Port Chalmers/1/73, A/Victoria/3/75, and A/Texas/1/77, revealed significant cross-reactions among different H3N2 viruses from 1973 to 1977, showing that the neuraminidases of these viruses are different from each other at several antigenic determinants. The above results indicated that these monoclones could clearly differentiate between early and late strains of H3N2 virus which have been prevalent in man since 1968. As shown in Table 2, the reaction with monoclonal antibodies indicated that the recombinant and A/Kumamoto/22/76 viruses gave similar patterns of reactivity with all monoclonal antibodies, with the exception of one (88/2). This antibody inhibited the neuraminidase activity of A/Kumamoto/22/76 and failed to react with A/swine/Kanagawa/2/78, indicating that the neuraminidases differ from each other at one of the sites examined.

Genome analysis

Oligonucleotide mapping of viral RNAs

Although detailed antigenic analyses of a recombinant and other viruses revealed that A/swine/Kanagawa/2/78 virus contained H1 haemagglutinin identical to A/N1/8/76, and N2 neuraminidase closely related to A/Kumamoto/22/76, the RNA homology was not known. These results led us to compare the genomes of the three viruses by oligonucleotide mapping. Fig. 1 shows the oligonucleotide maps of the RNAs of A/Kumamoto/22/76 (H3N2), A/swine/Kanagawa/4/78 (H1N1) and A/swine/Kanagawa/2/78 (H1N2) viruses. Migration of oligonucleotides of A/swine/Kanagawa/4/78 (Fig. 1c) and A/swine/Kanagawa/2/78 (Fig. 1e) were essentially similar, while they were greatly different from A/Kumamoto/22/76 (Fig. 1a). In order to compare the oligonucleotide spots produced by these viruses more precisely, electrophoresis of mixtures of two of the viral RNA digests was conducted. To determine the degree of homology between these viruses, about 50 large oligonucleotide spots were selected. Results obtained by co-electrophoresis and close examination with A/Kumamoto/22/76 and A/swine/Kanagawa/2/78 (Fig. 1a, b, e) showed that only 11 out of 49 spots were common to the two viruses (Fig. 1f, closed circle). As can be seen in Fig. 1c, nine oligonucleotides (indicated by arrows) of A/swine/Kanagawa/4/78 were not present in digests of A/swine/Kanagawa/2/78 virus. However, migration profiles analysed by co-electrophoresis of oligonucleotides of the above two viral RNAs (H1N1, H1N2) showed that A/swine/Kanagawa/2/78 was genetically closer to A/swine/Kanagawa/4/78, according to the considerable number of common spots (32 out of 54 spots), than to A/Kumamoto/22/76. These results suggested that a large proportion of the RNA segments of the recombinant virus was derived from swine (H1N1) virus.

DNA–RNA hybridization on nitrocellulose paper

DNA–RNA hybridization was performed according to the blotting procedures described by Thomas (1980) to determine the derivation of RNA segments of the above recombinant virus other than the haemagglutinin and neuraminidase genes. For this purpose, DNA complementary to A/swine/Kanagawa/2/78 RNA was synthesized as described in Methods. The electropherogram presented in Fig. 2(a) indicates that reverse transcription in the presence of the dodecadoxyribonucleotide primer yielded eight cDNA segments. Fig. 3(a) shows RNA migration patterns of these viruses before transfer to nitrocellulose paper. These RNAs were transferred from agarose gels to nitrocellulose paper and DNA–RNA hybridization was carried out under stringent conditions using radioactive 32P-DNA complementary to A/swine/Kanagawa/2/78. As can be seen in Fig. 3(b), all RNA segments of the homologous virus (K-2) hybridized completely with the cDNA probes mentioned above. RNA segments 1 + 2 + 3, 4, 5, 7, and 8 of A/swine/Kanagawa/4/78 (K-4) were essentially of similar homology to the cDNA.
Swine influenza virus characterization

Fig. 1. Comparison of the oligonucleotide maps of the RNAs between a recombinant (H1N2) and two other (H1N1, H3N2) viruses. (a) RNA from A/Kumamoto/22/76 (H3N2). (b) Mixture of RNAs from A/Kumamoto/22/76 and A/swine/Kanagawa/2/78 (H1N2). (c) RNA from A/swine/Kanagawa/4/78 (H1N1); arrows pointing to left represent the spots present in A/swine/Kanagawa/4/78 but absent in A/swine/Kanagawa/2/78. (d) Mixture of RNAs from A/swine/Kanagawa/4/78 and A/swine/Kanagawa/2/78. (e) RNA from A/swine/Kanagawa/2/78. (f) Diagram of oligonucleotide maps of A/swine/Kanagawa/2/78; closed circles represent the spots detected in oligonucleotide maps of A/Kumamoto/22/76; the remaining spots, except two, were common to those of A/swine/Kanagawa/4/78; the two spots indicated by arrows were not detected in the H1N1 and H3N2 viruses used in the test. X and B are the position of dye markers xylene cyanol FF and bromophenol blue.

segments of the corresponding recombinant viral RNA, whilst segments 6 of both viruses were different from each other. On the other hand, RNA segment 6 of A/Kumamoto/22/76 virus (KUMA) hybridized efficiently with the cDNA probe of the recombinant virus. This preliminary hybridization test strongly suggests that only gene 6 of A/swine/Kanagawa/2/78 (H1N2), which may code for the neuraminidase, derives from H3N2 virus.

Liquid DNA–RNA hybridization

In order to characterize the genome composition of the above-mentioned recombinant virus more accurately, DNA–RNA hybridization was also done using isolated cDNA probes to the individual segments. Before use, the cDNA was examined to determine if DNA segments that
Fig. 2. Fractionation of synthetic DNA complementary to A/swine/Kanagawa/2/78 viral RNA and determination of molecular size of eluted cDNA segments. For hybridization tests, cDNA prepared as described previously (Davis et al., 1981) was separated by 7 M-urea-polyacrylamide gel electrophoresis at 250 V for 40 h. (a) Separated cDNA segments were eluted from the gel using buffer as described in Methods. In order to determine molecular size, the seven eluted cDNA segments were subjected to electrophoresis on a 1.4% alkaline agarose gel and autoradiographed. (b) Standard molecular size markers were phage λ DNA fragments digested with HindIII. cDNA segments were numbered 1 + 2 to 8. K-cDNA is the DNA complementary to A/swine/Kanagawa/2/78 viral RNA.

were synthesized under these conditions were full length. The lengths of the eluted DNA segments were 2.3 (segments 1, 2), 2.2, 1.7, 1.6, 1.5, 1.0, and 0.8 kilobases based on mol. wt. markers, HindIII-digested phage λ DNA and HaeIII-digested φX174 RF DNA (data not shown) (Fig. 2b). While seven cDNA segments could be separated on 4% polyacrylamide gel electrophoresis, the slowest moving band could not be resolved. However, two diffuse bands at the top of the gel could be partially separated by prolonged electrophoresis. These results were very similar to those obtained in RNA analysis of the A/N1/8/76 virus reported by Palese & Schulman (1976a, b) and swine (H1N1) isolates from Japan (unpublished data). On the basis of RNA and cDNA migration patterns, the slowest moving band at the top of the gel probably consists of a mixture of P1 and P2 genes. Since the three large cDNA segments could not be well resolved, they were separated into two fractions (P1 + P2, P3) and used in the hybridization. For hybridization, 6 ng of isolated 32P-cDNA segments was mixed with increased concentrations of viral RNA and hybridized under stringent conditions. In these experiments, considerable homology was seen between cDNA segments 1 + 2 of the A/swine/Kanagawa/2/78 (H1N2) and A/swine/Kanagawa/4/78 (H1N1) viruses even under stringent conditions, while RNA from the H3N2 virus failed to show significant hybridization (Fig. 4). Similar hybridization tests with the individual cDNA segments indicated that there was close base sequence homology between the swine and recombinant viruses. Seven of the RNA segments in the recombinant virus were homologous with the swine influenza virus while one segment (RNA 6) was homologous with the H3N2 strain.
Swine influenza virus characterization

Fig. 3. Molecular DNA–RNA hybridization on nitrocellulose paper. (a) RNA migration patterns of
the isolates before transfer from agarose to nitrocellulose paper. The RNAs were fractionated on 1.4%
agarose at 100 V for 3 h and stained with 0.05% ethidium bromide in EB buffer (40 mM-Tris–HCl,
20 mM-sodium acetate, 2 mM-EDTA) for 60 min. K-4 RNA was from A/swine/Kanagawa/4/78
(H1N1), K-2 RNA from A/swine/Kanagawa/2/78 (H1N2) and KUMA RNA from
A/Kumamoto/22/76 (H3N2). RNA segments were numbered 1 to 8. (b) Hybridized 32P-labelled DNA
complementary to RNA of A/swine/Kanagawa/2/78; arrows pointing to right show segment 6. The
methods for hybridization were essentially similar to those described by Thomas (1980). RNAs
transferred to nitrocellulose paper were hybridized in low salt buffer (2 × saline sodium citrate) and the
paper was washed under stringent conditions. Hybridized 32P-cDNA segments were detected by
autoradiography.

DISCUSSION

Results obtained in hybridization tests demonstrated that the fourth gene of the recombinant
virus had complete base sequence homology with a recent swine isolate. As shown in Table 2,
the neuraminidase antigen of the recombinant virus appeared to be very similar to that of
A/Kumamoto/22/76 and direct DNA–RNA hybridization also supports this finding. The
hybridization tests showed that there was more homology between gene 7 of H1N1 and the
recombinant virus, and the degree of homology between them was different from that of H3N2
virus. Segment 8, which codes for the non-structural proteins, hybridized to a varying degree
depending on the concentration of RNA being tested, but the extent of hybridization with the
H1N1 virus was greater than that of H3N2 virus. These results clearly indicate that only the
neuraminidase gene of the recombinant virus was derived from H3N2 virus and all of the
remaining genes were from swine (H1N1) influenza virus and were in agreement with the
evidence obtained by direct DNA–RNA hybridization on nitrocellulose paper.

Recently, several research workers have reported that genetic relatedness between the genes
of different subtypes or variants of a subtype could be differentiated on the basis of the extent of
RNA–RNA homology (Scholtissek et al., 1978; Sriram et al., 1980; Bean et al., 1980). The
present study also demonstrates the utility of DNA–RNA hybridization for genome analysis of
influenza viruses from man or animals.
Fig. 4. Comparison of base sequence homology of the recombinant virus using specific cDNA–RNA hybridization. To investigate accurately the derivation of the recombinant virus, A/swine/Kanagawa/2/78 RNA segments, DNA–RNA hybridization was performed under stringent conditions (Avvendimento et al., 1980). Six ng of isolated cDNA segments was used in hybridization experiments and increased concentrations of viral RNA were used in the tests. The extent of hybridization was monitored by removing the unhybridized portion of 32P-DNA by digestion with S1 nuclease. The nuclease-resistant fraction was precipitated by adding an equal volume of 20% trichloroacetic acid and 50 μg of tRNA at 0 °C for 2 to 3 h. The radioactivity was determined by counting in a scintillation spectrophotometer. RNAs were from A/swine/Kanagawa/2/78 (○), A/swine/Kanagawa/4/78 (●), and A/Kumamoto/22/76 (▲).

Antigenic and genome analyses of A/swine/Kanagawa/2/78 strongly suggest that the recombinant virus found in Japan in 1978 may have been generated by genetic reassortment in swine between a swine (H1N1) influenza virus and an A/Kumamoto/22/76 (H3N2)-like strain which had been prevalent in man in 1976.

The finding of a recombinant virus in swine provides suggestive evidence that swine may play a role in the appearance of future recombinant virus for human and lower animals. On the basis of characterization of H3N2 viruses (Kundin, 1970; Shortridge et al., 1976, 1979; Hinshaw et al., 1979).
al., 1978; Nerome et al., 1981; Nakajima et al., 1982), swine (H1N1) (Hinshaw et al., 1978; Kendal et al., 1977; Yamane et al., 1979; Nerome et al., 1982a), and a Russian (H1N1) virus (Nerome et al., 1982b), it seems that a variety of influenza A viruses have co-circulated in the swine population. Antigenic and genetic characterization of the H3N2 virus which suddenly appeared in man in 1968, demonstrated that the haemagglutinin of this virus may have originated from an avian influenza virus and that the remaining structural genes were derived from human Asian (H2N2) virus which was prevalent in humans in the years 1957 to 1967 (Scholtissek et al., 1978; Laver & Webster, 1973; Fang et al., 1981).

Furthermore, Pensaert et al. (1981) indicated that avian influenza virus may be transmitted to swine, suggesting its ability to cross the species barrier from birds to mammals. As we report here, ecological studies on influenza viruses which are widely distributed in the swine population may provide evidence about the origin of new pandemic viruses in man.

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