Antigenic and genetic characteristics of H1N1 human influenza virus isolated from pigs in Japan

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The coexistence of swine (H1N1) and human (H3N2) influenza viruses in a swine population has been confirmed by virological and serological studies (Arikawa et al., 1982). Our previous reports also indicated that a number of pigs had detectable antibodies to swine H1N1 viruses throughout the observation period (Goto et al., 1988). The pigs were infected with human H1N1 or H3N2 virus during the human epidemics of each virus (Miwa & Goto, 1986). Evidence for the spread of Russian (H1N1) influenza viruses from humans to pigs has been described in a few countries (Alexander, 1982; Shortridge & Stuart-Harris, 1982), and there is one report of the isolation of Russian (H1N1) influenza virus from a pig in Thailand (Nerome et al., 1982) following the reappearance of H1N1 influenza virus in 1977. This communication describes the antigenic and genetic characterization of H1N1 viruses isolated from pigs in Japan.

For the isolation of virus, nasal swabs were collected (in 1.5 ml of heart infusion broth containing antibiotics) from 280 pigs, approximately 7 months of age, during the period from June to December 1992 at the abattoir in Obihiro, Hokkaido. Nasal samples were inoculated into the amniotic and allantoic cavities of 10-day-old embryonated hens' eggs. After incubation of the eggs at 35 °C for 3 days, the amniotic and allantoic fluids were harvested and tested for haemagglutinating activity with 0.5% chicken erythrocytes. Haemagglutination-inhibition (HI) (Miwa & Goto, 1986) and neuraminidase-inhibition (NI) (Nerome et al., 1982) tests were used for the identification of isolated strains using antisera to four reference type A viruses (Table 1). The nucleotide sequences of the HA gene coding for the HA1 domain and the NA gene of isolate sw/Obihiro/5/92 were shown to be closely related to those of current human H1N1 viruses. Evolutionary trees constructed from nucleotide sequences showed that the HA and NA genes of A/sw/Obihiro/5/92 were apparently on a branch cluster containing human strains isolated between 1990 and 1992.

Two strains of influenza A virus were isolated from pigs in northern Japan in 1992. Serological tests showed that the haemagglutinin (HA) and neuraminidase (NA) antigens were more closely related to those of recent human H1N1 viruses than to those of swine H1N1 viruses. The HA and NA genes of isolate A/sw/Obihiro/5/92 were shown to be closely related to those of current human H1N1 viruses. Evolutionary trees constructed from nucleotide sequences showed that the HA and NA genes of A/sw/Obihiro/5/92 were apparently on a branch cluster containing human strains isolated between 1990 and 1992.

The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession numbers: D28518 (HA genes of A/sw/Obihiro/5/92), D31949 (HA gene of A/Yamagata/32/89), D31947 (NA gene of A/sw/Obihiro/5/92), D31944 (NA gene of A/Hokkaido/11/88), D31945 (NA gene of A/Hokkaido/2/92), D31948 (NA gene of A/Yamagata/120/86) and D31950 (NA gene of A/Yamagata/32/89).

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Table 1. Antigenic analyses of the haemagglutinin and neuraminidase of influenza viruses isolated from pigs in 1992

<table>
<thead>
<tr>
<th>Test antigens</th>
<th>HI titres* with antisera to:</th>
<th>NI titres* with antisera to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NJ/76†</td>
<td>USR/77†</td>
</tr>
<tr>
<td>A/NJ/8/76 (H1N1)</td>
<td>256 &lt; 32 &lt; 32 &lt; 32 &lt; 32 &lt; 32</td>
<td>128 &lt; 4 &lt; 4 &lt; 4</td>
</tr>
<tr>
<td>A/USSR/92/77 (H1N1)</td>
<td>&lt; 32 2048 &lt; 32 &lt; 32 &lt; 32 &lt; 32</td>
<td>&lt; 4 128 32 32 32</td>
</tr>
<tr>
<td>A/Yamagata/120/86 (H1N1)</td>
<td>&lt; 32 4096 1024 1024 1024 &lt; 4</td>
<td>&lt; 4 32 128 256 256</td>
</tr>
<tr>
<td>A/Yamagata/32/89 (H1N1)</td>
<td>32 2048 4096 1024 1024 &lt; 4</td>
<td>32 64 128 256 256</td>
</tr>
<tr>
<td>A/sw/Obihiro/4/92</td>
<td>32 &lt; 32 2048 4096 1024 1024</td>
<td>32 64 128 256 256</td>
</tr>
<tr>
<td>A/sw/Obihiro/5/92</td>
<td>32 &lt; 32 1024 2048 1024 1024</td>
<td>32 64 128 256 256</td>
</tr>
</tbody>
</table>

* Values represent reciprocals of terminal serum dilution inhibiting HA and NA activities of virus antigens tested.
† Post-infection ferret sera.
‡ Hyperimmune chicken sera.
§ Hyperimmune rabbit serum.

Fig. 1. Evolutionary tree for HA (a) and NA (b) gene of the swine isolate and other H1N1 influenza viruses constructed by the neighbour-joining method on the basis of nucleotide substitutions. (a) The HA sequence of sw/Obihiro/5/92 and Yamagata/32/89 strains were determined in this study, whereas others were obtained from the literature: USSR/90/77 (Concannon et al., 1984a), Kiev/59/79 (Beklemishev et al., 1986), Singapore/6/86 (Cox et al., 1989), Taiwan/1/86 (Robertson, 1987), Yamagata/120/86 (Yamada et al., 1991), Czechoslovakia/2/88, Czechoslovakia/2/89, Texas/22/90, Singapore/6/90, Goroka/2/90, Massachusetts/1/90 and Stockholm/26/90 (Xu et al., 1993) and Finland/158/91 (Kinnunen et al., 1992). (b) The NA sequence of sw/Obihiro/5/92, Yamagata/32/89, Yamagata/120/86, Hokkaido/11/88 and Hokkaido/2/92 were determined in this study, whereas others were obtained from the literature: USSR/90/77 (Concannon et al., 1984b), Kiev/59/79 (Beklemishev et al., 1985) and Chile/1/83 (Schreier et al., 1988).

The HA gene of isolate A/sw/Obihiro/5/92 showed high nucleotide (98.2 to 98.7%) and amino acid (98.0 to 98.8%) sequence identities with those of reference H1N1 viruses isolated between 1989 and 1991. The NA gene of the isolate is closely related to the A/Hokkaido/2/92 (H1N1) strain in nucleotide (98.7%) and amino acid (98.3%) sequences. These findings show that the HA and NA genes of the sw/Obihiro/5/92 virus may be derived from strains of human H1N1 virus recently prevalent. Phylogenetic trees of HA and NA genes are presented in Fig. 1. Even though two branch clusters were observed in HA and NA trees, recent human H1N1 viruses (since 1986) appeared to have evolved in a single lineage. The HA and NA genes of the Obihiro isolate belong to a newer branch cluster containing viruses isolated between 1990 and 1992. This evolutionary relationship strongly suggests that the two viruses isolated from pigs in Obihiro, Hokkaido district, northern Japan, may be derived from current human epidemic strains.

In this study, a total of 720 sera were tested for the presence of HI antibodies to the A/sw/Obihiro/4/92 and A/sw/Obihiro/5/92 strains. The sera were obtained from 60 slaughtered pigs each month in the year 1992, at
the same abattoir from which the above-mentioned nasal swabs were collected for virus isolation. Three of 120 swine sera in May and June showed positive reactions to both strains in HI tests (data not shown), but no antibody to A/USSR/92/77 (H1N1) was observed throughout the year.

It is more likely that a human H1N1 virus was introduced into the pig population in about 1990 (see Fig. 1 a), and it stayed there with little antigenic drift. The prevalence of the Obihiro isolates among the pig population in this area was low. Evidence of the spread of human H3N2 virus from humans to pigs has been reported by many workers (Hinshaw et al., 1978; Shortridge et al., 1987), whereas the transmission of human H1N1 virus from humans to pigs is rare.

The present results are of importance in considerations of the persistence of human influenza viruses in pigs during the human inter-epidemic period. Further serological and virological studies into this problem are in progress.

References


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