Human influenza A (H1N2) viruses isolated from China

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Reassortant influenza A viruses bearing H1 haemagglutinin and N2 neuraminidase were isolated from humans in China between December 1988 and March 1989. As primary isolation of influenza A (H1N2) viruses from humans had not been reported previously, it was of interest to determine the genetic origin of these virus isolates. The haemagglutinins of the H1N2 viruses were antigenically and genetically related to those of H1 viruses isolated worldwide since 1986, and the neuraminidases of these viruses were antigenically and genetically related to those of recent H3N2 viruses.

Partial sequencing of each gene segment of three of the H1N2 viruses revealed that all gene segments except that encoding the haemagglutinin gene were derived from virus of the H3N2 subtype. Sequence differences amongst the neuraminidase, nucleoprotein and non-structural genes of these three H1N2 reassortant viruses as well as the isolation of reassortants in seven laboratories over a 4 month period make it unlikely that the H1N2 viruses are laboratory artefacts. The spread of these reassortant viruses to other countries has not yet been documented.

Introduction

In 1977, influenza A (H1N1) viruses reappeared in the human population after a 20 year absence and caused world-wide epidemics, primarily among persons under the age of 25 years (Kendal et al., 1979a, b; Stuart-Harris et al., 1985). These influenza A (H1N1) viruses have continued to circulate and during several periods have co-circulated with influenza A (H3N2) viruses, providing an opportunity for genetic reassortment to occur during mixed infections. Indeed, it has been reported previously that influenza A viruses that were reassortants between H3N2 and H1N1 viruses were identified in single isolates (Yamane et al., 1978; Kendal et al., 1979b; Nishikawa & Sugiyama, 1983). However, the possibility that reassortment actually occurred during virus isolation from a specimen containing both subtypes could not be ruled out. On the other hand, the wide circulation in some countries from 1978 to 1981 of reassortant influenza A (H1N1) viruses that derived genes encoding the replication complex from the H3N2 subtype has been well documented (Young & Palese, 1979; Nakajima et al., 1981; Bean et al., 1980; Cox et al., 1983). Although the co-circulation of the two subtypes of influenza A has continued, reports that H1N1–H3N2 reassortant viruses were isolated from humans after 1982 have not appeared in the literature.

Between December and March of the 1988 to 1989 influenza season, 19 strains of influenza A (H1N2) virus were isolated from sporadic cases by seven laboratories in six Chinese cities (Guo et al., 1990). Here we describe in detail the antigenic and genetic properties of these viruses.

Methods

Viruses. Nineteen strains of influenza A (H1N2) virus were isolated between December 1988 and March 1989 in the six Chinese cities of Harbin, Beijing, Baoding, Shanghai, Chengdu and Xianfeng. Isolates were obtained from patients ranging in age from 2 months to 55 years who visited Chinese health clinics for upper respiratory tract infections. Although no major influenza epidemic activity occurred, influenza B, influenza A (H1N1) and influenza A (H3N2) viruses were isolated during the same period.

The influenza A (H1N2) viruses chosen for in-depth analysis were A/Harbin/1/88, A/Harbin/1/89 and A/Xianfeng/3/89 isolated in December 1988, January 1989 and March 1989, respectively. A/Beijing/1/86, A/Taiwan/1/86 and A/Sichuan/4/88 viruses are viruses of the H1N1 subtype, whereas the A/Sichuan/2/87 and A/Beijing/57/89 viruses are strains of the H3N2 subtype. All viruses were grown in the allantoic cavity of 9- to 11-day-old embryonated eggs, pelleted and then purified as described previously (Cox & Kendal, 1984).

Antigenic analysis. Strain-specific antisera were prepared in ferrets or chickens by using methods described previously (Kendal et al., 1982). Haemagglutination inhibition tests using post-infection ferret sera treated with receptor-destroying enzyme were done as described previously (Kendal et al., 1979a). Neuraminidase (NA) inhibition tests using chicken antisera raised against selected virus strains and against the reassortant virus A/equine/Prague/1/56 (H7)–A/Bangkok/1/79 (N2) were also carried out as described previously (Henry et al., 1973).

RNA sequencing. Virion RNA was extracted from purified virus as described previously (Palese & Schulman, 1976). RNA sequence analysis was performed by the dideoxynucleotide chain termination
method, using synthetic oligodeoxynucleotide primers and reverse transcriptase (RT) essentially as described (Cox et al., 1986a), except that 10 μCi of [35S]dATP (Amersham; sp. act. 1000 Ci/mmol) for each 60 μl reaction was used and RT incubations and chase were done at 42 °C for 20 min. Four of the primers used for sequencing the HA1 domain of the H1 subtype HA gene have been described previously (Cox et al., 1989). In addition, primers 6, 666 and 819 corresponding to H1 HA sequences 5' d(AAGCAGGGGAAAATAAAA), 5' d(GAAAATGCTTATGTCTCT) and 5' d(GGAAATCTAATAGCGCCA), respectively, were utilized. Partial gene sequences were obtained for the three polymerase (PB2, PB1 and PA) genes, the nucleoprotein (NP) gene, the NA gene, the matrix or membrane (M) protein gene and the non-structural (NS) protein gene. Primers for partial sequencing were as follows: primer 28, 5' d(ATGGAAAGATAAAA) for the PB2 gene; primer 13, 5' d(CAAACCATTTGAATGG) for the PB1 gene; primer 452, 5' d(CACACATCCACA) for the PA gene; primer 888, 5' d(TGGACCTGCCGT) for the NP gene; primer 7, 5' d(AGCAGGAGTGAAG) for the NA gene; primer 8, 5' d(GAAAGATGAGTCTTCTAA) for the M gene and primer 596, 5' d(ACAGTTCGAGTC) for the NS gene.

Results

Antigenic analysis of H1N2 viruses

Haemagglutination inhibition tests were carried out with the three H1N2 viruses (A/Harbin/1/88, A/Harbin/1/89 and A/Xianfeng/3/89), two H1N1 viruses (A/Taiwan/1/86 and A/Sichuan/4/88), ferret antiserum to these two H1N1 viruses and chicken antiserum to the H3N2 virus, A/Shanghai/11/87. The results shown in Table 1 are representative of a larger sample and demonstrate that each of the three H1N2 viruses were strongly inhibited by post-infection ferret sera raised against the two H1N1 viruses, but were not inhibited by antiserum raised against a recent H3N2 strain. These results demonstrate that the haemagglutinins of the H1N2 viruses are related to those of H1N1 viruses isolated since 1986. In a more extensive experiment, additional H1N2 viruses were tested and all were found to be antigenically similar (data not shown).

NA inhibition tests were also carried out using the three H1N2 viruses, A/Beijing/1/86 (an H1N1 virus that is closely related to A/Taiwan/1/86), A/Sichuan/2/87 (H3N2) and A/Beijing/57/89 (H3N2). The results shown in Table 2 demonstrate that the NA activity of the A/Beijing/1/86 (H1N2) virus was strongly inhibited by chicken antiserum raised against the two H3N2 viruses, but was not inhibited by antiserum raised against N1 NA. Similar results (data not shown) were obtained for the A/Beijing/1/88 (H1N2) and A/Xianfeng/3/89 (H1N2) viruses.

These antigenic analyses revealed that the H1N2 viruses have haemagglutinins related to those of the A/Taiwan/1/86 (H1N1) reference virus and NAs that are strongly inhibited by antiserum to N2 NAs.

<table>
<thead>
<tr>
<th>Reference antigen</th>
<th>Ferret antiserum</th>
<th>H3N2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Taiwan/1/86†</td>
<td>2560§</td>
<td>160</td>
</tr>
<tr>
<td>A/Sichuan/4/88†</td>
<td>5120</td>
<td>5120§</td>
</tr>
<tr>
<td>A/Beijing/1/88‡</td>
<td>3840</td>
<td>960</td>
</tr>
<tr>
<td>A/Beijing/1/88‡</td>
<td>2560</td>
<td>640</td>
</tr>
<tr>
<td>A/Xianfeng/3/89‡</td>
<td>2560</td>
<td>320</td>
</tr>
<tr>
<td>* Reference chicken antiserum to A/Shanghai/11/87 (H3N2). † H1N1. ‡ H1N2. § Homologous titres.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. NA inhibition reactions of influenza A (H1N1), (H1N2) and (H3N2) viruses

<table>
<thead>
<tr>
<th>Reference antigen</th>
<th>Chicken antiserum</th>
<th>H7N2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Sichuan/2/87†</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>A/Beijing/57/89‡</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>A/Beijing/1/86‡</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A/Beijing/1/88§</td>
<td>640</td>
<td>320</td>
</tr>
<tr>
<td>* H7N2, A/equine/Prague/1/56 (H7):A/Bangkok/1/79 (N2). † H3N2. ‡ H1N1. § H1N2.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genetic analysis of H1N2 viruses

The nucleotide and deduced amino acid sequences of the HA gene HA1 domain of the three H1N2 viruses were determined and compared with each other and with the HA sequences of H1N1 reference strains published previously (Cox et al., 1989), as well as with unpublished sequences. The HA1 sequences of the three H1N2 viruses differed from each other by seven nucleotides and by four amino acids. The deduced amino acid sequences of the H1N2 viruses and the H1N1 strains A/Taiwan/1/86 and A/Sichuan/4/88 are shown in Fig. 1. There are 10 amino acid changes between the HA genes of the H1N2 viruses and the A/Taiwan/1/86 virus; four of these 10 changes were also present in A/Sichuan/4/88 (H1N1), a virus isolated in China in February 1988. Thus, the amino acid sequences of the HA1 domains of the H1N2 viruses are related, but distinguishable from the HA sequence of a 1986 reference virus and are most closely related to that of a 1988 H1N1 virus isolated from China.

Partial gene sequences were obtained for the NA genes of the three H1N2 viruses and the H3N2 virus
Influenza A (H1N2) viruses

Fig. 1. The deduced amino acid sequences of the HA gene HA1 region of the three H1N2 viruses compared with sequences of two H1N1 reference strains (A/Taiwan/1/86 and A/Sichuan/4/88). The amino acid sequences are numbered to correspond to the numbering for the H3 subtype according to the alignment of Winter et al. (1981) with additional amino acid residues present in the H1 subtype sequence marked by an asterisk.

Fig. 2. Partial nucleotide sequences of NA genes of three H1N2 viruses compared with the sequence of H1N1 [A/USSR/90/77 (Concannon et al., 1984)] and H3N2 [A/Bangkok/1/79 (Martinez et al., 1983) and A/Sichuan/2/87] reference strains. The sequences not yet determined are marked by an asterisk.
Table 3. Comparison of partial gene sequences of the H1N2 viruses with sequences of H1N1 and H3N2 reference viruses

<table>
<thead>
<tr>
<th>RNA segment (encoded protein)</th>
<th>Position of nucleotides sequenced</th>
<th>Number of nucleotide differences between H1N1 and H3N2 sequence</th>
<th>Number of nucleotides in H1N2 gene sequence corresponding to H1N1 or H3N2 sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (PB2)</td>
<td>53–292</td>
<td>22</td>
<td>A/Harbin/1/88 H1N1* H3N2† Unique‡ A/Harbin/1/89 H1N1* H3N2† Unique‡ A/Xianfeng/3/89 H1N1* H3N2† Unique‡</td>
</tr>
<tr>
<td>2 (PB1)</td>
<td>46–270</td>
<td>27</td>
<td>1 21 6 1 21 6 1 21 6</td>
</tr>
<tr>
<td>3 (PA)</td>
<td>478–683</td>
<td>17</td>
<td>1 16 0 1 16 0 1 16 0</td>
</tr>
<tr>
<td>5 (NP)</td>
<td>923–1170</td>
<td>25</td>
<td>2 23 2 2 23 2 2 23 3</td>
</tr>
<tr>
<td>7 (M1 + M2)</td>
<td>51–283</td>
<td>12</td>
<td>1 11 0 1 11 0 1 11 0</td>
</tr>
<tr>
<td>8 (NS1 + NS2)</td>
<td>621–866</td>
<td>25</td>
<td>2 22 3 2 22 3 0 24 5</td>
</tr>
</tbody>
</table>

* A/Taiwan/1/86. ‡ A/Sichuan/2/87. † Nucleotides different from both * and †.

Between nucleotide positions 50 and 360, there were 203 nucleotide differences between the previously published NA gene sequences of the A/Bangkok/1/79 (H3N2) (Martinez et al., 1983) and A/USSR/90/77 (H1N1) (Concannon et al., 1984) reference viruses. However, the nucleotide sequences of the three H1N2 viruses NA genes were identical throughout this region (Fig. 2). The H1N2 viruses had the same nucleotide as the reference H3N2 viruses A/Bangkok/1/79 or A/Sichuan/2/87 in 200 or 202 of the 203 differing positions, respectively, but had the same nucleotide as the H1N1 reference virus A/USSR/90/77 in only three of these positions (Fig. 2). In addition, the H1N2 NA sequences had the same nucleotide as the A/Sichuan/2/87 sequence in two positions that did not differ between A/USSR/90/77 and A/Bangkok/1/79. Only one nucleotide difference was observed between the NA sequence of the H1N2 viruses and that of the A/Sichuan/2/87 (H3N2) virus. Thus, the NA genes of these H1N2 viruses encoded an N2 NA closely related to that of the A/Sichuan/2/87 (H3N2) virus.

Partial gene sequencing was also undertaken for the other six RNA segments of each of the three H1N2 viruses. These sequences were compared with those obtained for the H1N1 reference strain A/Taiwan/1/86 and the H3N2 reference strain A/Sichuan/2/87. The number and position of nucleotides that differed between the gene sequences of the H1N1 and H3N2 reference strains were determined; for each of these positions, it was observed whether the H1N2 virus genes had nucleotides corresponding to those of the H1N1 or H3N2 sequence. In addition, the nucleotide changes unique to the H1N2 genes were noted. Table 3 summarizes the results of partial gene sequencing for RNA segments 1 (PB2), 2 (PB1), 3 (PA), 5 (NP), 7 (M) and 8 (NS) of the three H1N2 viruses. These results demonstrate that all three H1N2 viruses derived each of these RNA segments from a virus of the H3N2 subtype. In addition, nucleotide differences were detected between the NP and NS genes of the H1N2 viruses (Table 3). One nucleotide difference was observed between the NP gene sequences and two nucleotide differences were observed between the NS gene sequences of the A/Xianfeng/3/89 virus and the corresponding sequences of the two viruses isolated in Harbin.

Discussion

Although reassortant H3N1 and H1N2 influenza A viruses have been reported previously (Yamane et al., 1978; Kendal et al., 1979b; Nishikawa & Sugiyama, 1983), each report was based on a single isolate or set of isolates from one individual that had both the H1N1 and H3N2 subtypes present. In each of these cases the emergence of reassortants during laboratory isolation could not be ruled out.

Here we documented the isolation of influenza A (H1N2) viruses from several locations in China, first in northeast China (Harbin) and 4 months later in a city over 1600 km to the southwest (Xianfeng). Influenza A
viruses of the H1N2 subtype had not been isolated previously in any laboratory in China, thus minimizing the possibility that this observation resulted from laboratory contamination (Guo et al., 1990).

According to antigenic and genetic analyses, the H1N2 viruses were reassortants between viruses related to H1N1 and H3N2 viruses that had recently circulated. The observed sequence differences are to be expected for independent isolates of viruses of the same subtype (Young et al., 1979; Ortin et al., 1980; Cox et al., 1983). Eleven nucleotide differences (out of a total of 2744 nucleotides sequenced) were observed between sequences of the A/Harbin/1/88 and the A/Xianfeng/3/89 viruses that were isolated 4 months apart. It can be estimated that the genomes of these two viruses vary by 0.4%, a value within the range expected for viruses of the same genotype isolated within a year of each other (Cox et al., 1983). Viruses that have been isolated as a result of laboratory contamination would be expected to show less variation than that seen between these two viruses (Cox et al., 1986b).

The reassortment event documented here would not be expected to confer an increased epidemic potential on the H1N2 reassortants compared with their parents because significant numbers of individuals in the population had already developed immunity to these antigens from previous infection (Y. J. Guo, unpublished results). Accordingly, although H1N2 viruses were isolated from sporadic cases in China for 4 months, there were no reports of influenza outbreaks or epidemics caused by these viruses; the spread of these reassortant viruses to other countries has not been documented.

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References


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