Jpn. J. Infect. Dis., 59, 2006

Laboratory and Epidemiology Communications

Sequence Characteristics of HA Gene in Influenza Type A (H1N1) Virus Isolated during the 2005 - 2006 Season in Aichi Prefecture, Japan

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Communicated by Ichiro Kurane

(Accepted May 12, 2006)

In Aichi Prefecture, the first influenza outbreak of the 2005 - 2006 influenza season occurred from November to December 2005 in the northeast area (1). As of March 26, 2006, we had obtained a total of 182 influenza virus isolates from the respiratory specimens collected through the surveillance program in Aichi Prefecture. Of these, 70 (39%) were influenza AH1 virus, 106 (58%) were influenza AH3 virus, and 6 (3%) were influenza B virus. The percentage of AH1 virus isolates in Aichi was somewhat higher than that in the entire country (22%, Infectious Disease Surveillance Center, National Institute of Infectious Diseases [NIID], Tokyo, Japan. Available online at <http://idsc.nih.go.jp/disease/influenza/inf-keiho/YR05/flu12/jmap12.html>.). In the present report, we describe antigenic and genetic aspects of AH1 isolates obtained in the 2005 - 2006 season in Aichi Prefecture.

Throat swab specimens were collected from influenza patients as sentinels in the influenza surveillance, and were inoculated onto Madin-Darby Canine Kidney (MDCK) cells. Influenza virus was identified by hemagglutination inhibition (HI) test using culture supernatant of infected MDCK cells as the hemagglutinin and type-specific sera against influenza viruses (provided from the NIID). Viral RNA was extracted from culture supernatants with High Pure Vial RNA Kit (Roche Applied Science, Penzberg, Germany). The complete coding sequence of the HA1 gene was amplified by One-step RT-PCR Kit (Invitrogen, Carlsbad, Calif., USA). The primers used were 5′-gcaaaagcaggggaaaataa-3′ and 5′-ttgatctgcagcatagccag-3′, which yield a 1,177-bp amplicon. Direct sequencing of the amplified fragment was performed with a Model-4200 automated DNA sequencer (Li-cor, Lincoln, Nebr., USA). The NA subtype of each isolate was determined using type-specific primers (2). All 14 AH1 isolates were proved to be of the N1 subtype.

Out of the 70 AH1 isolates identified so far in the 2005 - 2006 season, we determined nucleotide sequences of the HA1 gene of 14 isolates. The phylogenetic tree of the isolates is shown in Fig. 1. The isolates formed two clusters in the 2005 - 2006 season (Fig. 1). The isolates in the early season (1) are clustered into a group (cluster *b* in Fig. 1) including Aichi/168/2005, the first isolate of the season in our institute (isolated from a specimen collected on November 17). The identity among the isolates in cluster *b* was 99.1 - 100%, without changes in the predicted amino acid sequences. A/New Caledonia/20/99, one of the 2005 - 2006 season vaccine strains, also belonged to cluster *b*, and the Aichi isolates in cluster *b* exhibited 98.1 - 98.4% nucleotide sequence identity to this vaccine strain. In contrast, the isolates in cluster *a* were genetically more distant from A/New Caledonia than were those in cluster *b*, showing 97.4 - 97.5% identity.

A comparison of the predicted amino acid residues between each of the Aichi isolates and A/New Caledonia/20/99 is shown in Fig. 2. Amino acid sequences were 100% identical among isolates within each of two clusters. Aichi/168/2005 and Aichi/21/2006 (isolated from the specimen collected on January 13) are shown as representative strains of clusters *b* and *a*, respectively, in Fig. 2. Amino acid numbering corresponds to the number of HA1 in the H3 subtype, as reported previously (3). A total of 5 amino acid changes from A/New Caledonia/20/99 were detected in the HA1 protein of Aichi/168/2005-like viruses, including V169A, N190D, W255R, Y256F, and V317A (not shown in Fig. 2). In contrast, Aichi/21/2006-like viruses contained 11 predicted amino acid

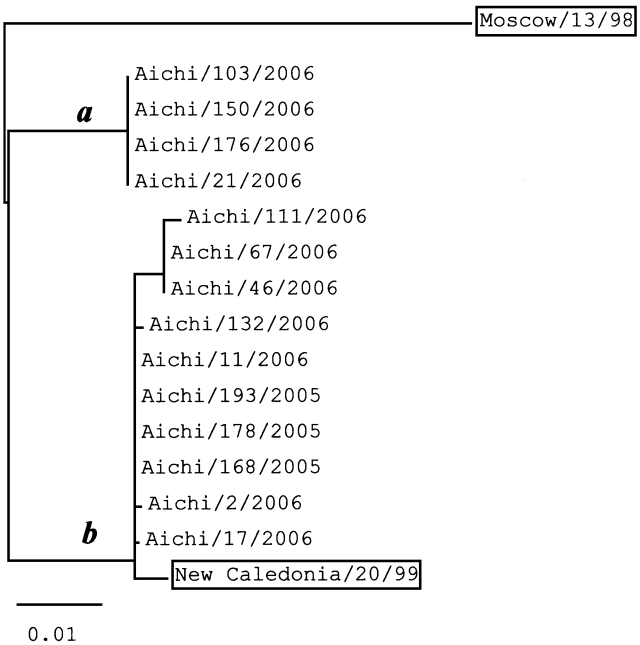

Fig. 1. Phylogenetic tree based on the nucleotide sequences of the HA1 region of the H1 genes. The tree was generated by the N-J method. The Aichi isolates in the 2005 - 2006 season and reference strains (A/New Caledonia/20/99, A/Moscow/13/98) were analyzed. Two clusters are indicated by *a* and *b*.

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Fig. 2. The predicted amino acid sequence of the HA1 region of the Aichi isolates compared with A/New Caledonia/20/99. Amino acid numbering corresponds to H3 subtype, with additional amino acid residues present in the H1 subtype sequence indicated by an asterisk. Residue substitutions are shown using one-letter amino acid code, and a dot (.) indicates amino acid residues homologous to those of A/New Caledonia/20/99.

changes from A/New Caledonia 20/99, and only three of them were common with Aichi/168/2005-like viruses (V169A, N190D, W255R). The HA1 polypeptide has been proposed to contain five antigenic sites, based on a study of the three-dimensional structure (4). It has been proposed that a new drift variant with epidemiologic importance generally had four or more amino acid substitutions located in more than one of the antigenic sites (5). Among the 11 amino acid changes in the HA1 protein of Aichi/21/2006-like viruses, at least two (V133T and K144E) were located in site A, which forms the center of the most obvious antibody-binding sites (4). Moreover, K81R was in antigenic site Cb, which is located at the bottom of the HA1 globular head (6,7). This substitution also affects oligosaccharide attachment. These substitutions in different antigenic sites could potentially alter antibody-binding properties of the viruses.

The antigenic analysis further confirmed the results of the sequence analysis. The antigenicity of the isolates was compared to that of A/New Caledonia/20/99, A/Moscow/13/98 (H1N1), and A/New York/55/2004 (H3N2). The results of the representative strains are shown in Table 1. Aichi/168/2005-like viruses showed HI titers of 1:160 for anti-A/New Caledonia serum (homologous titer 1:160), 1:10 for anti-A/Moscow serum, and less than 1:10 for anti-A/New York serum (data not shown). In contrast to Aichi/168/2005-like viruses, Aichi/21/2006-like viruses showed a substantial decrease in titers, with 1:40 for anti-A/New Caledonia serum. The results indicated that the Aichi/168/2005-like viruses were antigenically similar to A/New Caledonia, whereas Aichi/21/2006-like viruses were antigenically distinct from A/New Caledonia.

In the early stage of the 2005 - 2006 influenza season, from November to December 2005, most viral isolates showed HI titers within a twofold difference from the homologous titer for A/New Caledonia (1). However, about 40% of AH1 isolates showed fourfold lower HI titers to anti-A/New Caledonia serum than homologous A/New Caledonia after January 2006. It is possible that these isolates with the lowered titers were Aichi/21/2006-like virus.

From the 2000 - 2001 to the 2005 - 2006 influenza seasons, the World Health Organization recommended A/New Caledonia/20/99 as the H1N1 vaccine component for both the southern and northern hemispheres. However, our finding emphasizes the importance of vigilance against the emergence of influenza drift variants which may make the previously recommended influenza vaccine ineffective.

Table 1. Antigenic analysis of the AH1 isolates

Antigens	Antisera to	
	A/New Caledonia/20/99	A/Moscow/13/98
Reference viruses		
A/New Caledonia/20/99	160	–
A/Moscow/13/98	–	320
2005 - 2006 season isolates		
A/Aichi/168/2005	160	10
A/Aichi/17/2006	160	10
A/Aichi/21/2006	40	<10
A/Aichi/103/2006	40	<10

–, not tested.

Nucleotide sequence accession number: The nucleotide sequences of the HA1 gene can be found in GenBank under the indicated accession numbers as follows: A/Aichi/168/2005, AB243744; A/Aichi/2/2006, AB255389; A/Aichi/11/2006, AB255390; A/Aichi/17/2006, AB255391; A/Aichi/21/2006, AB255392; A/Aichi/46/2006, AB255393; A/Aichi/67/2006, AB255394; A/Aichi/103/2006, AB255395; A/Aichi/111/2006, AB255396; A/Aichi/132/2006, AB255397; and A/Aichi/150/2006, AB255398.

This article appeared in part in the Infectious Agents Surveillance Report (IASR), vol. 27, p. 12, 2006.

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