Evolutionary characterization of recent human H3N2 influenza A isolates from Japan and China: novel changes in the receptor binding domain

Brief Report

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Summary. Recent human H3N2 influenza viruses isolated in Japan and China were characterised from an evolutionary point of view. They appeared to have divided into three minor branch clusters, including 1992–1993, 1993–1994 and 1994–1995 isolates. It was of particular interest to reveal that in addition to amino acid substitutions in the antigenic sites of the HA molecule, amino acid changes occurred at position 226 of the receptor binding site from lysine or glutamine to isoleucine in all strains belonging to the 1994–1995 branch cluster. This is the first evidence of human H3N2 influenza isolates, or any other influenza HA serotypes, to contain a conserved amino acid residue other than lysine or glutamine at this key position.

Since the first appearance of human H3N2 viruses in China in 1968, a number of antigenic variants have been isolated in many parts of China [12, 20, 24]. Indeed, a number of vaccine strains for Hong Kong influenza which have been recommended by WHO have been Chinese isolates [29–34]. The WHO recommended virus for 1994–1995 epidemic season, A/Johannesburg/33/94 (H3N2), is an exception to this, however, even this virus is antigenically indistinguishable from

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an earlier Chinese strain, A/Guangdong/25/93 [35]. In the present study, sequence and phylogenetic analyses were done on the HA of Japanese and Chinese human H3N2 influenza strains to determine the evolutionary relationship between isolates of the above two countries from the same epidemic season.

Phylogenetic analysis was done on the HA1 domain of the HA gene by constructing an evolutionary tree as described previously [10, 19] (Fig. 1). Virus strains used for analyses in the present study and their abbreviations are shown in the legend to Fig. 1. A total of 31 strains were used in phylogenetic analysis of the HA gene. Twenty-seven of their HA genes were sequenced in this study. In agreement with previous reports [1, 2, 7, 9, 23, 24], human H3N2 viruses have essentially evolved in a single lineage, although recent strains since 1991 tended to form several branch clusters. The evolutionary tree showed, for example, that Bei/352/89-like viruses such as Was/15/91, Bra/2/91, and Shi/2/91 are phylogenetically similar, belonging to the same branch cluster. Bei/352/89-like viruses circulated in the human population of Japan until the latter part of the 1992–1993 epidemic season before being displaced by Bei/32/92-like viruses. Bei/32/92-like viruses, which were isolated in China as early as 1990, were subsequently isolated in many parts of the world and found to cocirculate with Bei/352/89-like viruses in the same epidemic season [6, 7, 33]. Bei/32/92-like viruses, therefore, appear to have evolved independently of circulating epidemic strains for some time before becoming the predominant epidemic strain. Indeed, the evolutionary tree revealed that the Bei/32/92-like lineage appears to have diverged from a putative virus around 1989. Viruses from 1993–1995 were divided further into two branch clusters which distinguished the most recent Japanese and Chinese strains of 1994–1995 from the previous strains of 1993–1994. Japanese and Chinese epidemic strains of the same epidemic season were generally clustered very close to one another which demonstrated that if new antigenic variants are isolated in China there is likely very little time before they are also isolated in Japan.

Analyses of predicted amino acid sequences revealed isolate Ban/139/90 contained seven potential asparagine-linked glycosylation sites located at positions 22, 38, 63, 126, 165, 246, and 285. Nine sequences (Sic/2/87, Bei/352/89, Shi/2/91, Was/15/91, Bra/2/91, Bei/32/92, Tia/33/92, Yok/73/92 Kit/159/93 Aic/69/94, Heb/19/95, Aki/1/95 and Sen/373/95) contained an eighth potential glycosylation site at position 8, while seven of the strains (Sha/9/93, Heb/12/93, Gua/25/93, Gua/28/94, Aki/1/94, Sen/384/94 and Heb/41/94) contained a ninth potential glycosylation site at location 276. It has been demonstrated that glycosylation of position 63 can sterically hinder binding of monoclonal antibodies to antigenic site C [22]. Thus, the presence of a carbohydrate sidechain bound to an asparagine residue near, or in, an antigenic site appears to prevent binding of neutralizing antibodies. As mentioned above, Sha/9/93, Heb/12/93, Gua/25/93, Gua/28/94, Aki/1/94, Sen/384/94 and Heb/41/94 contain a potential glycosylation site at amino acid position 276, which is located in antigenic site C. Glycosylation of this residue, therefore, could effectively mask this site from neutralizing antibodies.