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Genetic analysis and evaluation of the reassortment of influenza B viruses isolated in Taiwan during the 2004–2005 and 2006–2007 epidemics

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Abstract

Influenza B viruses were predominant in Taiwan during the 2004–2005 epidemic and both Victoria and Yamagata lineage viruses co-circulated. A reassortant influenza B virus that contained a Victoria lineage hemagglutinin (HA) gene and Yamagata lineage neuraminidase (NA) gene appeared first in 2002 and became predominant during the 2004–2005 epidemic. During the 2006–2007 epidemic, an influenza B outbreak occurred in Taiwan and only Victoria lineage viruses circulated. We characterized the viruses isolated in the 2006–2007 epidemic and found that the HA genes of influenza B viruses from that epidemic were highly similar to those from the 2004–2005 epidemic. We also analyzed the NA genes of isolates from the 2006–2007 epidemic and found that they all belonged to the Yamagata lineage and formed a new genetic subclade. Comparison of isolates from the 2004–2005 and 2006–2007 epidemics revealed four substitutions, N220K, E320D, K343R and E404K in NA genes. Although the HA sequences from the 2006–2007 epidemic were similar to those from the 2004–2005 epidemic, the NA sequences differed, suggesting distinct patterns of evolution of the HA and NA genes from 2004–2007 in Taiwan. This study emphasizes that the evolution of the NA genes may contribute to reemergence of influenza B viruses.

Keywords: Influenza B virus; Genetic analysis; Evolution

1. Introduction

Influenza virus is a major respiratory pathogen which causes annual worldwide epidemics and it is estimated that 250,000–500,000 deaths are directly associated with influenza epidemics every year (WHO, 2003). Currently, human influenza A (H1N1, H3N2) and influenza B viruses are the most common types circulating globally. The influenza B viruses isolated since the mid-1980s can be classified into Victoria (B/Victoria/2/87-like) and Yamagata (B/Yamagata/16/88-like) lineages, which are distinct antigenically and genetically (Kanegae et al., 1990; Rota et al., 1990). In influenza B viruses, antigenic drift in the HA gene occurs at a lower rate than in influenza A viruses; nonetheless, genetic variations, including insertions, deletions and reassortment with different lineages, are common in influenza B viruses (Air et al., 1990; Lindstrom et al., 1999; McCullers et al., 1999; Nerome et al., 1998). Previous studies indicated that the reassortment of influenza B viruses that had the HA gene from the Yamagata lineage and the NA gene from the Victoria lineage occurred first in the early 1990s (McCullers et al., 1999).

Since 2001, another reassortment of influenza B viruses has been observed in southeast Asia (Barr et al., 2003; Shaw et al., 2002) and similar studies have been reported in Taiwan and other countries (Chen et al., 2007; Chi et al., 2003; Daum et al., 2006; Lin et al., 2007; Shaw et al., 2002; Tsai et al., 2006; Xu et al., 2004). All these reassorted viruses had the HA gene from the Victoria lineage and the NA gene from the Yamagata lineage.

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According to surveillance and laboratory data, both Victoria and Yamagata lineages were isolated in the summer of 2002 and a reassortant Victoria lineage with the NA genes from Yamagata lineage was detected in Taiwan in 2002 (Lin et al., 2002; Tsai et al., 2006). During the 2004–2005 epidemic, the Yamagata and the reassortant Victoria lineages co-circulated in Taiwan. After the 2004–2005 epidemic, there were only sporadic laboratory confirmed cases of influenza B viruses from May 2005 to October 2006. During this period, the number of weekly isolates of influenza B never exceeded 20 and both lineages were detected in Taiwan.

However, the number of virus isolates and the rate of culture confirmed cases of influenza B viruses, according to the Influenza Surveillance Network, rose dramatically in November 2006 and more than 300 cases were identified as influenza B viruses in December 2006. This epidemic was the largest influenza B epidemic in Taiwan since the establishment of the Influenza Surveillance Network in 2000.

In order to clarify the genetic relationship between influenza B viruses from the 2004–2005 and 2006–2007 epidemics, we first analyzed the HA and NA genes of influenza B viruses isolated from 2004–2007. Then, we compared the isolates from the two epidemics by phylogenetic analysis and amino acid variations in important residues. We also tried to explain the possible reason for the severity of the 2006–2007 influenza B epidemic in Taiwan.

2. Materials and methods

2.1. Sample collection and cell culture

All isolates were collected through the Influenza Surveillance Network, which was coordinated by the Centers for Disease Control (CDC), Department of Health, Taiwan. A previous report described this network in detail (Shih et al., 2005). In brief, 13 virology laboratories in Taiwan collected the clinical samples for initial cell culture and identified subtypes by immunofluorescence assay (IFA). Then, these isolates were sent to CDC, Taiwan for further analyses.

2.2. PCR and DNA sequencing

We used QIAamp Viral RNA Mini Kits (Qiagen, Santa Clara, CA) to extract viral RNA from 140 μl of viral culture medium. RNA was eluted into 60 μl Buffer AVE. Then Qiagen OneStep RT-PCR Kits (Qiagen) were used to amplify the target genes. PCR amplifications of the HA and NA genes were carried out as described previously (Hoffmann et al., 2002; Nakagawa et al., 2003). PCR products were purified by Agencourt AMPure PCR purification kit (Beckman Coulter). Sequencing reactions were performed using an ABI Prism Dye Terminator III cycle sequencing kit with reaction products resolved on an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

2.3. Sequence analysis

Nucleotide sequences were aligned with other global sequences using the CLUSTALW program (Thompson et al., 1994) and these global sequences were obtained from the NCBI Influenza Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/). We also added Taiwanese strains from the 2004–2005 epidemic, as reported previously (Lin et al., 2007), for comparison. Alignment results were edited using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/) for amino acid variations. Phylogenetic analyses were carried out using Molecular Evolutionary Genetics Analysis software (MEGA, version 3.1, Kumar et al., 2004).

2.4. Sequence information

The gene accession numbers of influenza viruses from the 2004–2005 epidemic were provided previously (Lin et al., 2007). The HA and NA gene sequences of influenza viruses

Table 1
Accession numbers of sequences used in the study[a]

Isolated strain	HA gene	NA gene	Onset date	Number of isolates[b]
B/Taiwan/44/2006	EF621740	EF621726	13 May 2006	1
B/Taiwan/50/2006	EF621741	EF621727	17 July 2006	1225
B/Taiwan/1612/2006	EF621742	EF621728	18 July 2006	1225
B/Taiwan/2219/2006	EF621743	EF621729	12 June 2006	3
B/Taiwan/1677/2006[c]	EF621744	EF621730	19 June 2006	66
B/Taiwan/3729/2006	EF621745	EF621731	16 November 2006	1225
B/Taiwan/1917/2006	EF621746	EF621732	23 November 2006	1225
B/Taiwan/156/2006	EF621747	EF621733	28 November 2006	5
B/Taiwan/1336/2006	EF621748	EF621734	02 December 2006	17
B/Taiwan/7505/2006	EF621749	EF621735	03 December 2006	43
B/Taiwan/7508/2006	EF621750	EF621736	04 December 2006	18
B/Taiwan/3561/2007	EF621751	EF621737	04 January 2007	38
B/Taiwan/3808/2006	EF621752	EF621738	19 December 2006	8
B/Taiwan/2589/2007	EF621753	EF621739	04 January 2007	13

[a] The gene accession numbers of influenza viruses in 2004–2005 were described previously (Lin et al., 2007). Isolates of B/Taiwan/39/2004, B/Taiwan/70/2005 and B/Taiwan/117/2005 belongs to the largest group ($N = 1225$).

[b] Indicates the number of virus isolates which were grouped, based on the putative amino acid sequences of the HA gene (52–271 aa). For example, the largest group contains 1225 isolates with the same HA gene sequences of 52–271 aa.

[c] Belongs to the Yamagata lineage.