Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus

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The antigenic and genetic characteristics of the haemagglutinins of influenza type B viruses isolated since 1988 during periods of both widespread activity (1990/1991) and sporadic activity (1989/1990) were examined using microneutralization tests and direct RNA sequencing. During 1989/1990, influenza B viruses representative of two distinct lineages antigenically and genetically related to either B/Victoria/2/87 or B/Yamagata/16/88 were isolated, and a minor drift variant of B/Yamagata/16/88, B/Hong Kong/22/89, was identified. In 1990/1991, B/Hong Kong/22/89- or B/Yamagata/16/88-like viruses accounted for the majority of the influenza virus isolates in most countries. Sequence analysis of the HA1 domains of representative viruses confirmed the continued existence of two main lineages among recent strains of influenza B virus and identified unique amino acid changes that could account for the altered antigenic reactivity of some variants. Sequence analysis of the HA2 domains of some of the recent influenza B viruses allowed for a comparison of the evolutionary rates and patterns between the HA1 and HA2 domains.

Influenza type B viruses have been isolated during periods of widespread influenza activity during five of the last 10 influenza seasons in the United States. During this time, each major peak of influenza B virus activity has been associated with the emergence of a new antigenic variant of the virus. The antigenic sites on the haemagglutinin (HA) of influenza type B viruses have been delineated by sequence analysis of both circulating viruses and laboratory-derived variants (Verhoeyen et al., 1983; Webster & Berton, 1981; Krystal et al., 1982, 1983; Berton et al., 1984; Berton & Webster, 1985; Hovanec & Air, 1984; Bootman & Robertson, 1988).

For recent influenza B viruses, at least two discrete lineages have co-existed since at least 1983 (Rota et al., 1990; Kanegae et al., 1990). Viruses from each of these two lineages, represented by either B/Yamagata/16/88 (B/YM/88) or B/Victoria/2/87 (B/VI/87), had as many as 27 amino acid differences between their HA1 proteins by 1988 and were distinct antigenically.

During 1989/1990, influenza B viruses antigenically related to both B/VI/87 and B/YM/88 were isolated sporadically throughout the world and altogether, influenza B viruses represented less than 1% of the total number of influenza virus isolates reported in the United States (Centers for Disease Control, 1990). Despite this limited circulation, antigenic drift variants were identified from each lineage of influenza B virus. During 1990/1991, the majority of influenza virus isolates in many countries were of type B (World Health Organization, 1991c). Although viruses closely related to both B/VI/87 and B/YM/88 were identified, 65% of these isolates were antigenically related to a drift variant of B/YM/88, B/Hong Kong/22/89 (Centers for Disease Control, 1991; World Health Organization, 1991a). In 1991/1992, influenza type A (H3N2) viruses were the predominant strain, but infrequent isolations of B/YM/88-, B/Hong Kong/22/89- and B/VI/87-like viruses have been reported (World Health Organization, 1992).

In this report, influenza B viruses representative of viruses isolated during the 1989/1990 and 1990/1991 influenza seasons were further characterized by molecular and serological methods. Recently isolated influenza B viruses were initially identified as being most closely related to B/YM/88 or B/VI/87 using haemagglutination inhibition assays (data not shown) (Palmer et al., 1976), and strains representative of recently circulating influenza B viruses were chosen for further analysis. The results of microneutralization tests indicated that drift variants had arisen among the B/YM/88-like viruses (Table 1). B/HK289, B/PN/90 and B/BK/91 showed the
greatest amount of antigenic change with an eightfold drop in titre against the antiserum to B/YM/88. The B/VI/87-like viruses were antigenically homogeneous from 1987 to 1989. Antiserum to B/BJ/87, a virus genetically and antigenically similar to B/VI/87, was used because it had a higher homologous titre than the antiserum to B/VI/87. B/IN/89 showed little cross-reactivity with any of the influenza B viruses tested. Although no other B/IN/89-like viruses were reported, this virus was chosen for further analysis because of its unique antigenic characteristics.

To characterize the influenza type B isolates further, the nucleotide and deduced amino acid sequences were determined from purified viral RNA as described previously (Rota et al., 1990) for all of the HA1 domains and some of the HA2 domains of the viruses listed in Table 1. The HA1 domains from recently cocirculating viruses varied by as many as 83 (8.7%) nucleotide and 34 (9.1%) amino acid changes. The group of B/YM/88-like viruses differed by as many as 10 amino acids in HA1 and were more genetically diverse than the recent B/VI/87-like viruses. Sequence analysis indicated that B/IN/89 is clearly related to the B/VI/87-like viruses.

Fig. 1 shows the deduced amino acid sequences for the HA1-encoding regions of the recent influenza B virus isolates compared to the HA1 sequences of either B/VI/87 or B/YM/88. The HA1s of the majority of the more recent influenza B viruses from both lineages had amino acid changes at positions 73, 197 and 199. At some amino acid residues, changes appeared to be limited to one lineage. For example, all of the recent B/VI/87-like viruses had an amino acid change (V to I) at residue number 137 whereas the majority of recent B/YM/88-like viruses had changes at positions 150, 203, 230 and 298. Viruses from both lineages had the majority of amino acid changes between positions 100 and 200; this region includes the previously proposed immunodominant region of the HA of influenza B virus (Berton & Webster, 1985). The B/YM/88-like viruses had two or three changes in the region of amino acids 200 to 300 in contrast to the B/VI/87-like viruses which had none or one. The antigenic presentation of amino acids 200 to 300 on the B/YM/88-like HAs may be affected by a nearby potential glycosylation site at positions 233 to 235 that is not present on any of the B/VI/87-like HAs.

Within the HAs of viruses from each of the main lineages, amino acid differences that could account for altered antigenic reactivity were sometimes apparent. The amino acid change that had the greatest effect on antigenicity was the glycine to arginine change at position number 137 whereas the majority of recent B/YM/88-like viruses had changes at positions 76 and 150. Although amino acid changes at positions 76 and 150 were unique to recent B/HK289-1ike influenza B viruses, the histidine to glutamic acid change at position number 122 has been observed in an earlier B/YM/88-1ike virus, B/SN/88 (Fig. 3), which was antigenically identical to B/YM/88 (Rota et al., 1990).

Previous sequence analysis has revealed that clinical specimens of influenza B virus passaged in mammalian cells retain a potential glycosylation site at amino acids 197 to 199 whereas virus passaged in eggs usually lose this site (Schild et al., 1983; Robertson et al., 1985, 1990). However, analysis of individual cDNA clones did reveal that a small number of egg-derived viruses retained this glycosylation signal (Robertson et al., 1990). Our data, which were obtained by sequencing RNA purified from egg-passaged virus, indicated that several of these viruses had retained the potential glycosylation site at amino acids 197 to 199 (Fig. 1). To determine whether this site was utilized in these viruses, the HAs of viruses (B/IN/89 and B/VI/89, B/NY/90 and B/TX/91, B/HK289 and B/PN/90) which had very similar HA1 sequences, but differed at this predicted glycosylation site, were analysed by SDS-PAGE and Western blotting. The HAs from only one pair of related viruses, B/IN/89 and B/VI/89, which differed by four amino acids showed differences in apparent Mr. This migrational difference was not observed after the HAs were

Table 1. Antigenic drift of influenza type B viruses: 1989 to 1991

<table>
<thead>
<tr>
<th>Strain</th>
<th>Neutralization titre* with post-infection ferret antiserum to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B/YM/88</td>
</tr>
<tr>
<td>B/Yamagata/16/88</td>
<td>160</td>
</tr>
<tr>
<td>B/Hong Kong/9/89</td>
<td>1280</td>
</tr>
<tr>
<td>B/Guangdong/55/89</td>
<td>640</td>
</tr>
<tr>
<td>B/Texas/1/91</td>
<td>1920</td>
</tr>
<tr>
<td>B/South Dakota/5/89</td>
<td>480</td>
</tr>
<tr>
<td>B/Texas/4/90</td>
<td>960</td>
</tr>
<tr>
<td>B/New York/3/90</td>
<td>480</td>
</tr>
<tr>
<td>B/Bangkok/163/91</td>
<td>160</td>
</tr>
<tr>
<td>B/Victoria/103/89</td>
<td>640</td>
</tr>
<tr>
<td>B/Hong Kong/22/89</td>
<td>160</td>
</tr>
<tr>
<td>B/Panama/45/90</td>
<td>160</td>
</tr>
<tr>
<td>B/Victoria/2/87</td>
<td>10</td>
</tr>
<tr>
<td>B/Beijing/2/87</td>
<td>10</td>
</tr>
<tr>
<td>B/Texas/37/88</td>
<td>10</td>
</tr>
<tr>
<td>B/Victoria/19/89</td>
<td>10</td>
</tr>
<tr>
<td>B/Paris/32/90</td>
<td>10</td>
</tr>
<tr>
<td>B/India/3/89</td>
<td>10</td>
</tr>
</tbody>
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* Harmon et al. (1988).

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Fig. 1. Changes in the deduced amino acid sequence of the HA1- and HA2-encoding regions of recent B/YM/88-like or B/VI/87-like influenza B viruses. HA1 sequences begin with the first amino acid after signal peptide cleavage and end at the HA1–HA2 cleavage site. A period indicates an amino acid deletion. See Table 1 for strain abbreviations.
treated with endoglycosidase F (Fig. 2). Therefore, the additional glycosylation site at amino acids 197 to 199 was apparently maintained and utilized in some egg-derived viruses.

The evolutionary relationships of the recent influenza B virus isolates and of several previously characterized strains are shown in Fig. 3. Within each of the two main lineages of recent influenza B virus, multiple sublineages were recognized. There are at least four sublineages among the B/YM/88-like viruses; however, only the B/HK289-like viruses showed consistent antigenic differences with polyclonal antiserum. Within the B/VI/87-like viruses, there are only two sub-branches that can be identified genetically, one related to the B/TX/88-like viruses and the other represented by B/VI/89-like viruses; however viruses from these subbranches are antigenically indistinguishable with polyclonal antiserum. Viruses that were genetically related to both B/TX/88 and B/VI/89 have been isolated in Finland during 1989/1990 (Kinnunen et al., 1992). Viruses that were genetically most closely related to either B/VI103 or B/VI/89 were isolated in Russia during 1990/1991 (M. L. Hemphill & P. A. Rota, unpublished observations). The relationships observed after the sequences of influenza B viruses isolated before 1979 were included in the analysis suggested that the branchpoint between the presently cocirculating lineages occurred between 1973 and 1979.

The nucleotide and deduced amino acid sequences of the HA2 domains of B/SU/83, B/AA/86, B/HK289 and B/VI/87 were determined and compared to those of several other influenza type B viruses. As expected, few amino acid changes were detected over 10 years (Fig. 1). However, there were as many as 24 nucleotide changes between the HA2 domains of B/VI/87 and B/HK289. Phylogenetic analysis indicated that the HA2 domains of currently circulating B/YM/88- and B/VI/87-like viruses were also on separate lineages.

For the 12-year period from 1979 to 1991, the nucleotide substitution rate for HA1 (0.236 ± 0.04%/year) was similar to the rate for HA2 (0.196 ± 0.06%/year).
year). However, the rate of amino acid change was 0-30%/year for HA1 compared to 0-056%/year for HA2. Although approximately 40% of the nucleotide changes in HA1 coded for amino acid substitutions, only 8-7% of the nucleotide changes in HA2 caused changes in the HA2 protein. The rate calculated for influenza B virus is only about 30% less than that of influenza A viruses. However, when B/Lee/40 is used as the root virus, the rate that can be calculated from our data (0-11%/year) is similar to that previously described for influenza B virus (Yamashita et al., 1988; Air et al., 1990).

Although the presence of multiple cocirculating variants of influenza B virus had been demonstrated (Lu et al., 1983; Oxford et al., 1984; Yamashita et al., 1988; Donatelli et al., 1989; Rota et al., 1990), our results indicated that two distinct lineages of influenza B virus have persisted and have caused disease over a period of at least 4 years. In the case of the type A viruses a single lineage of HA rapidly became the dominant epidemic variant (Both et al., 1983; Raymond et al., 1986; Cox et al., 1989).

The antigenic as well as genetic relationships among the currently circulating strains of influenza virus provide useful information for determining the most appropriate composition for the influenza vaccine. The complexity of the evolutionary patterns of influenza type B makes the choice of type B component difficult. Vaccines for use in 1991/1992 and 1992/1993 have been updated to include either B/PN/90 or B/YM/88 as the type B component (World Health Organization, 1991a, 1992). Continued surveillance of these viruses will be necessary to detect the emergence of new lineages of virus with altered antigenic properties and to ensure that future vaccines contain the most appropriate strain of virus.

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Short communication


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