

## Evolution of influenza B/Victoria/2/87-like viruses: occurrence of a genetically conserved virus under conditions of low epidemic activity

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Nucleotide sequence analysis of the gene region coding for the HA1 domain of the influenza B virus haemagglutinin was performed on seven field strains isolated during the 1989 to 1990 season and two field strains isolated in 1985 and 1988 in Finland. All isolates were antigenically and genetically related to B/Victoria/2/87 virus and distinct from B/Yamagata/16/88 virus. The three strains isolated at the beginning of the 1989

1990 season in Turku were almost identical to an American variant (B/Texas/37/88-B/Ohio/10/88) of the previous season, whereas the four strains isolated later in the 1989 to 1990 season in Helsinki formed a new group of heterogeneous viruses. The phylogenetic tree compiled suggests that the two branches had evolved from a common origin, probably in 1987.

The influenza B viruses isolated in different parts of the world since 1987 have belonged to two distinct evolutionary lineages, the B/Victoria/2/87-like viruses (VI/87 branch) and the B/Yamagata/16/88-like viruses (YA/88 branch), which have a common origin that can be traced back to the late 1970s (Rota *et al.*, 1990; Kanegae *et al.*, 1990). During the 1989 to 1990 influenza season, both lineages circulated in Europe, Asia and North America (WHO 1990; Centers for Disease Control, 1990). In Finland, the epidemic activity of influenza B viruses was low overall and only viruses of the VI/87 branch were isolated. To investigate the genetic variability of influenza B viruses in this novel evolutionary situation and under conditions of low epidemic activity, isolates from seven patients from two localities, Turku and Helsinki, were studied for the nucleotide sequence that codes for the HA1 domain. The strains from Turku represent the beginning of the epidemic season and the strains from Helsinki the end of it. The sequences were compared with those of two virus strains isolated in Finland in 1985 and 1988, and with previously reported sequence data.

The viral RNA was sequenced by the dideoxynucleotide chain termination method, as described by Huovilainen *et al.* (1988). The oligonucleotide primers used were: 36-GAAGGCAATAATTGTACT-53, 201-TGCAAATCTCAAAGG-215, 370-ATGCACGACAGAACA-384, 488-TTGGAACCTCAGGATCTTGC-507, 628-GGAGAAGACCAAATTACT-645, 844-GGAAAACAGGAACA-858 and 973-AAATACGGTGGATTAAA-989 (nucleotide positions are

numbered according to Rota *et al.*, 1990). Phylogenetic analysis of sequence data was performed with version 3.3 of the Phylip (Phylogeny Inference Package) software package (Joseph Felsenstein 1990, University of California, Berkeley, Ca., U.S.A.).

In a total of 345 HA1 residues there were amino acid differences at 12 (excluding amino acids 197 and 199 which are exposed to host cell-mediated selection) residues between the viruses (VI/87 branch) isolated in Finland during the three epidemic seasons over a period of 5 years. Most of the changes occurred in residues analogous to the antigenic sites of HA1 of influenza A (H3N2) viruses (Wiley *et al.*, 1981; Krystal *et al.*, 1983; Berton *et al.*, 1984; Berton & Webster, 1985; Rota *et al.*, 1990; Wilson & Cox, 1990).

B/Finland/24/85 virus isolated during the 1984 to 1985 season, when mainly B/USSR/100/83-like and B/Singapore/222/79-like viruses circulated world-wide (WHO, 1985, 1986*a*), appeared to be closely related to a more recent antigenic variant, B/Ann Arbor/1/86, which afterwards (autumn, 1986; WHO, 1986*b*) replaced B/USSR/100/83 as a component of influenza virus vaccines. Inclusion of genomic analysis in influenza surveillance systems, in addition to antigenic analysis, might make evaluation of the epidemic importance of single virus isolates which deviate antigenically from the principal epidemic virus easier and, in some situations, might expedite the decision to modify the composition of the influenza vaccine.

The strains from Turku (B/Finland/145/91, B/Fin-

	10	30	50	70	90
SN/222/79 E	DRICTGITSSNSPHVVKTATQGEVNVTVGVIPLTTTPTKSHFANLKGTKTRGKLCPNCLNCTDL	DVALGRPKCMGT	IPSAKASILHEVKPVTSGCFPI	MHD	
Fin/24/85 E			K		
Fin/56/88 M			K		Y
Fin/145/90 M			K		
Fin/146/90 M			K		
Fin/146/90 ME			K		
Fin/147/90 M			K		
Fin/148/90 ME			K	T	
Fin/149/90 M			K	T	
Fin/150/90 M			K	T	
Fin/151/90 E			K	T	

	110	130	150	170	190
SN/222/79 E	RTKIRQLPNLLRGYENIRLSTRNVINAERAPGGPYIIGTSGSCPNTNGNGFFATMAWAVPKDN--KTATNPLTVEVPYICTKGEDQITVWGFHSDTETQ				
Fin/24/85 E		H T V		N NN	F D
Fin/56/88 M		H T KV		NDNN	E N
Fin/145/90 M		H T K	I	NDNN	E N
Fin/146/90 M		H T K	I	NDNN	E N
Fin/146/90 ME		H T K	I	NDNN	E S
Fin/147/90 M		H T K	I	NDNN	E N
Fin/148/90 ME	H	H K K		NDNN S	E N A
Fin/149/90 M	H	H K K		NDNN S	E N
Fin/150/90 M	H	H K K		NDNN S	E N
Fin/151/90 E	H	H K K		NDNN S	E N I

	210	230	250	270	290
SN/222/79 E	MVKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYVMVQKPGKGTIVYQRGVLLPQKVVWCASGRSKVIGKSLPLIGEADCLHEKY				
Fin/24/85 E		A	S	T I	X
Fin/56/88 M		A	S	T I	X
Fin/145/90 M	A	A	S	T I	X
Fin/146/90 M	A	A	S	T I	X
Fin/146/90 ME	A	A	S	T I	X
Fin/147/90 M	A	A	S	T I	X
Fin/148/90 ME		A	S	T I	X
Fin/149/90 M		A	S	T I	X
Fin/150/90 M		A	S	T I	X
Fin/151/90 E		A	S	T I	X

	310	330
SN/222/79 E	GGLNKSHPYVTGEHAKAIGNCPIWVKTPKLANGTKYRPPAKLLKER	
Fin/24/85 E		
Fin/56/88 M		
Fin/145/90 M		
Fin/146/90 M		
Fin/146/90 ME		
Fin/147/90 M		
Fin/148/90 ME		
Fin/149/90 M		
Fin/150/90 M		
Fin/151/90 E		

Fig. 1. Amino acid sequence comparison of the HA1 domains of influenza B virus HA genes (Finland is abbreviated Fin throughout). Amino acids different from B/Singapore/222/79 (SN/222/79; Verhoeven *et al.*, 1983) are indicated. Amino acids difficult to interpret are marked X. Passing history: E and M refer to isolation and further propagation in embryonated eggs and MDCK cell cultures, respectively.

land/146/90 and B/Finland/147/90) did not differ from each other in their deduced HA1 amino acid sequence when the residues exposed to host-mediated selection, 197 and 199, were excluded. This was also characteristic of the strains isolated in Helsinki (B/Finland/148/90,

B/Finland/149/90, B/Finland/150/90 and B/Finland/151/90). There were six amino acid differences between the viruses isolated from Turku and those isolated from Helsinki. The specific amino acid changes between the Finnish isolates from the three epidemic seasons are

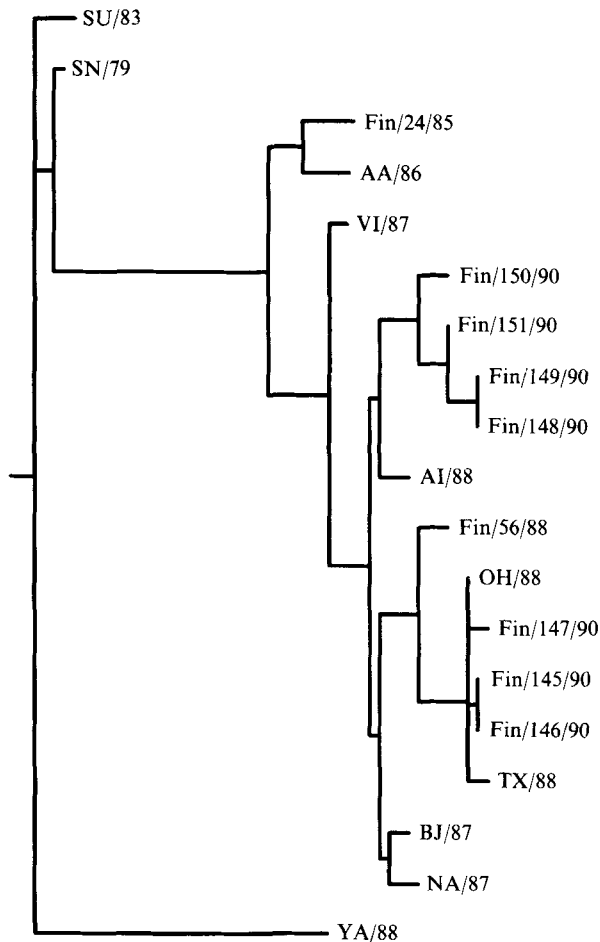


Fig. 2. Evolutionary tree for recent influenza B viruses based on 1029 nucleotides of the HA1 domain. The following sequences, published by Rota *et al.* (1990) if not otherwise specified, are included: B/Singapore/222/79 (SN/79; Verhoeven *et al.*, 1983), B/USSR/100/83 (SU/83), B/Ann Arbor/1/86 (AA/86), B/Victoria/2/87 (VI/87), B/Beijing/1/87 (BJ/87), B/Nagasaki/1/87 (NA/87; Kanegae *et al.*, 1990), B/Aichi/5/88 (AI/88), B/Texas/37/88 (TX/88), B/Ohio/10/88 (OH/88) and B/Yamagata/16/88 (YA/88). Six nucleotides of the entire HA1 which are difficult to interpret and six nucleotides exposed to host-mediated selection are excluded. The horizontal lines represent the mutational distance.

shown in Fig. 1. Five amino acid changes were recorded between the 1984 to 1985 and the 1987 to 1988 viruses, and four to six changes between the 1987 to 1988 and 1989 to 1990 viruses.

The phylogenetic tree compiled in the present study (Fig. 2) suggests that the influenza B viruses isolated in Helsinki at the end of the long epidemic season of 1989 to 1990 were not descendants of the older virus variant which circulated at the beginning of the season in Turku. Instead, two previously separate variants of influenza B virus seem to have reached Finland independently.

The strains from Turku were closely related to B/Finland/56/88, a strain from the 1987 to 1988 season,

and were almost identical to a variant isolated during the 1988 to 1989 season in the U.S.A. At its lowest, the difference was one silent nucleotide change from G of the B/Ohio/10/88 strain (Rota *et al.*, 1990) to A of the B/Finland/145/90 and B/Finland/146/90 strains at position 312. There were two amino acid changes that were unique to the American variant, B/Ohio/10/88–B/Texas/37/88 cluster (Rota *et al.*, 1990), when the HA1 sequences of 20 virus strains from 1979 to 1988 in the VI/87 branch were compared (EMBL databases, 20 June 1990, complemented with data of Dayan *et al.*, 1990; Kanegae *et al.*, 1990; Rota *et al.*, 1990; the present study). These changes at residues 146 (from Val to Ile) and 222 (from Thr to Ala), as well as the only unique silent nucleotide change (from G to A at position 165), were also characteristic of the new viruses from Turku. The strains isolated in Helsinki and three strains described recently (P. Rota, personal communication), B/India/3/89, B/Victoria/19/89 and B/Paris/329/90, lacked these amino acid markers.

The strains from Helsinki were more heterogeneous than those from Turku. In a comparison similar to that referred to above, each of these strains as well as B/Victoria/19/89 (P. Rota, personal communication) differed from the previously described viruses of the VI/87 branch by two unique amino acid changes at residues 129 (from Thr to Lys) and 172 (from Pro to Ser). There were also four unique silent nucleotide changes (at positions 258, 645, 705 and 936) common to at least three of the strains isolated in Helsinki.

Examples of intraepidemic heterogeneity of influenza B viruses have been reported (Lu *et al.*, 1983; Donatelli *et al.*, 1989; Rota *et al.*, 1989), but many details are still poorly understood, e.g. the extent of variation due to the heterogeneity of the viruses initiating the outbreak in an area, as compared to the extent of variation that arises during the outbreak. In our study both elements were obviously represented. The chance of isolating more deviant variants, among which may be conserved viruses from previous epidemics still surviving through a small number of infection chains, may be greater during a period of low epidemic activity. An example of this may be the conserved influenza B viruses, genetically almost identical to a variant from the previous epidemic season in another continent, which circulated in Turku in 1990.

Conserved viruses may serve as an important genetic reservoir for influenza B virus evolution, for new variants of epidemic significance rarely evolve from the previous epidemic variants (Yamashita *et al.*, 1988; Kanegae *et al.*, 1990; Rota *et al.*, 1990), which can be seen as dead ends. Genomic conservation is a frequent phenomenon in influenza C viruses (Buonagurio *et al.*, 1985) and in avian influenza A viruses (Kida *et al.*, 1987). The extreme example of conservation in human influ-

enza A viruses is the reappearance of the H1N1 subtype virus after an interval of more than 20 years in 1977 (Raymond *et al.*, 1986). It is unclear whether the genomic conservation associated with the ordinary antigenic drift can be explained solely by transmission of the virus in human communities whose low population density restricts the development of an epidemic and whose immunity does not create, for a period of several years, a barrier against the re-occurrence of an older variant. To solve this problem, it may be sensible to direct attention to viruses in periods of low epidemic activity and in outlying areas. Further studies of the genetic variability of influenza B viruses in Europe in 1989 to 1990 are under way in our laboratory.

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