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Evolutionary pattern of influenza B viruses based on the HA and NS genes during 1940 to 1999: origin of the NS genes after 1997

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Summary. Phylogenetic analysis was carried out for genes encoding hemagglutinin (HA) (24 new and 25 previously reported sequences) and nonstructural proteins (NS) (22 new and 14 previously reported sequences) of influenza B virus isolates obtained from 1940 to 1999. Two antigenically and genetically distinct HA lineages are presently known to exist. Divergence into these two lineages was estimated to have occurred around 1969. Phylogenetic analysis of NS genes revealed that their phylogenetic relationships were not linked to the two HA lineages but suggested that reassortment of viral genes between the viruses of two HA lineages had occurred. In addition two distinct NS lineages which were not linked to the two HA lineages were observed. Viruses isolated after 1997 formed their own lineage in combination with B/Houston/84 while other virus isolates obtained from 1973 to 1995 comprised the other NS lineage.

Introduction

The evolutionary pattern of hemagglutinin (HA) genes of influenza B viruses appears to have arisen as a result of cocirculation of multiple lineages [33]. This is in contrast to that of influenza A viruses whose evolutionary tree is marked by a single trunk with many short-lived branches [4, 22].

During the 1988/1989 influenza season, two distinct antigenic variants of influenza B viruses were detected using postinfection ferret serum [12, 24]. These variants were antigenically related to either B/Yamagata/16/88 (YA/88) or B/Victoria/2/87 (VI/87) and both groups of viruses (YA/88-like and VI/87-like groups) were in worldwide cocirculation until 1990 [12, 14, 24, 25]. The YA/88-like group was in continuous circulation until 1997, while the VI/87-like group had been only infrequently isolated since 1991 [19]. However, since 1997, the VI/87-

like group has again been circulating in Japan and China [19]. As for influenza A viruses, two subtypes (H1N1 and H3N2) viruses have been in worldwide cocirculation since the appearance of H1N1 viruses in 1977. During these past 20 years, genetic reassortment between both subtypes has occasionally occurred [2, 9, 16, 34]. Genetic reassortment among influenza B viruses has also been presumed to occur, although no direct evidence of this has been obtained. Only one report at a conference suggested the existence of genetic reassortment between circulating influenza B viruses shown by comparing the restriction enzyme digestion patterns of polymerase chain reaction (PCR) amplified gene segments [32].

To understand the evolutionary mode of influenza B viruses after their divergence into the two HA lineages, YA/88-like and VI/87-like HA lineages, we examined the nucleotide sequences of NS genes including newly determined sequences of 36 isolates from 1940 to 1999 together with those of HA genes. In this report we suggest the frequent genetic reassortment between the two HA lineages. In addition, the evolutionary relationships based on the sequence data of NS genes show the existence of two NS lineages, which were not linked to the two HA lineages. We discuss the evolutionary mode of influenza B viruses in consideration of the two NS lineages.

Materials and methods

Viruses

Following viruses were used for the sequencing of HA genes: B/Russia/69, B/Victoria/70, B/Yamagata/1/73, B/Baylor/4/78, B/Houston/18513/84, B/Aichi/14/97, and B/Aichi/8/98. Viruses used for the sequencing of NS genes included B/Nagasaki/1/87, B/Aichi/5/88, B/Yamagata/16/88, B/Mie/1/93, and B/Ohsaka/543/97. Other viruses used for the sequencing of HA and NS genes were B/Thailand/62, B/Aichi/7/76, B/Aichi/70/81, B/Aichi/21/82, B/Houston/4181/82, B/Aichi/1/84, B/Aichi/5/90, B/Aichi/1/91, B/Aichi/1/93, B/Aichi/1/94, A/Aichi/10/95, B/Aichi/4/97, B/Aichi/15/97, B/Aichi/33/97, B/Aichi/3/98, B/Aichi/5/98, and B/Aichi/20/99. B/Thailand/62, B/Russia/69, B/Victoria/70, B/Yamagata/1/73, B/Baylor/4/78, B/Houston/4181/82, and B/Houston/18513/84 were generously provided by Dr. Nakada (Yamanouchi Pharmaceutical Co., Ltd.). B/Nagasaki/1/87 and B/Ohsaka/543/97 were gifts of Dr. Okuno (Osaka Prefectural Institute of Public Health). The viruses isolated in Japan were directly isolated from clinical samples grown in MDCK cells. Other viruses were not grown in these cells, but used directly for genetic analysis. Abbreviations for all the virus strains used in this study are shown in Table 1.

Preparation of viral RNA and determination of nucleotide sequences

Viral RNA for cloning was extracted by the guanidinium thiocyanate-hot phenol method [18]. cDNA of HA and NS genes was prepared by reverse transcription of virus RNA using Moloney murine leukemia virus reverse transcriptase (Gibco BRL). Oligonucleotide primers BHA-1 (5′ AGCAGAAGCGTTGCA 3′) and BNS-1 (5′ AGCAGAAGCAGAGGATTT 3′) complementary to the 3′ terminus of the HA and NS genes, respectively, were used for the cDNA cloning. The cDNA was amplified by PCR using Taq polymerase (Takara Shuzo, Kyoto, Japan) with additional antisense primers; BHAR-1143 (5′ ACCAGCAATAGCTTCGAA 3′) for HA and BNSR-1096 (5′ AGTAGTAACAAGAGG 3′) for NS. The nucleotide sequences of these PCR products were directly determined by the dideoxy chain termination method [27] using the primers described above as well as an additional primer BNS-965

Table 1. Influenza B virus strains used in the present study

Strain	Abbreviation	Reference[a]	
		HA gene	NS gene
B/Lee/40	Lee/40	Krystal et al. (1983) (K00423)	Briedis et al. (1982) (J02096)
B/Great Lakes/54	GL/54	Yamashita et al. (1988) (M22947)	Yamashita et al. (1988) (M19797)
B/Maryland/59	MD/59	Krystal et al. (1983) (K00424)	Yamashita et al. (1988) (M19796)
B/Thailand/62	TAI/62	This report (AB027386)	This report (AB027409)
B/Singapore/64	SI/64	Yamashita et al. (1988) (M22946)	Yamashita et al. (1988) (M19795)
B/Ann Arbor/1/66	ANN/66	–[b]	DeBorde et al. (1988) (M20225)
B/Russia/69	RU/69	This report (AB027387)	Yamashita et al. (1988) (M19794)
B/Victoria/70	VI/70	This report (AB027388)	–
B/Hong Kong/73	HK/73	Hovanec et al. (1984) (M10298)	Yamashita et al. (1988) (M19792)
B/Yamagata/1/73	YA/73	This report (AB027389)	Norton et al. (1987) (M16633)
B/Aichi/7/76	AI/76	This report (AB027390)	This report (AB027474)
B/Baylor/4/78	BA/78	This report (AB027391)	Yamashita et al. (1988) (M19793)
B/Paris/1/79	PA/79	–	Yamashita et al. (1988) (M19791)
B/Singapore/222/79	SI/79	Verhoeyen et al. (1983) (K00038)	Yamashita et al. (1988) (M19790)
B/Oregon/5/80	OR/80	Berton et al. (1984) (K02713)	–
B/Aichi/70/81	AI/81	This report (AB027392)	This report (AB027475)
B/Aichi/21/82	AI/82	This report (AB027393)	This report (AB027476)
B/Houston/4181/82	HT/82	This report (AB027407)	This report (AB027477)
B/USSR/100/83	RU/83	Air et al. (1990) (X13552)	–
B/Aichi/1/84	AI/84	This report (AB027495)	This report (AB027478)
B/Houston/18513/84	HT/84	This report (AB027394)	Yamashita et al. (1988) (M19789)
B/Ibaraki/2/85	IB/85	Kanegae et al. (1990) (M36107)	–
B/Georgia/1/86	GA/86	Yamashita et al. (1988) (M22944)	Yamashita et al. (1988) (M19787)
B/Nagasaki/1/87	NA/87	Kanegae et al. (1990) (M36108)	This report (AB027479)
B/Victoria/2/87	VI/87	Rota et al. (1990) (M58428)	Yamashita et al. (1988) (M19786)
B/Aichi/5/88	AI/88	Rota et al. (1990) (M58424)	This report (AB027480)
B/Singapore/7/88	SI/88	Rota et al. (1990) (M58423)	–
B/Yamagata/16/88	YA/88	Rota et al. (1990) (M58419)	This report (AB027481)
B/Hong Kong/9/89	HK/89	Rota et al. (1992) (M65197)	–
B/Aichi/5/90	AI/90	This report (AB027395)	This report (AB027482)
B/Finland/150/90	FIN/90	Kinnunen et al. (1992) (L19642)	–
B/Finland/184/91	FIN/91	Ikonen et al. (1996) (L76317)	–
B/Aichi/1/91	AI/91	This report (AB027396)	This report (AB027483)
B/Aichi/1/93	AI/93	This report (AB027408)	This report (AB027484)
B/Finland/254/93	FIN/93	Ikonen et al. (1996) (L76318)	–
B/Guangdong/8/93	GU/93	Nerome et al. (1998) (AF050064)	–
B/Mie/1/93	MIE/93	Nerome et al. (1998) (D38643)	This report (AB027485)
B/Aichi/1/94	AI/94	This report (AB027397)	This report (AB027486)
B/Guangdong/5/94	GU/94	Nerome et al. (1988) (AF050063)	–
B/Harbin/7/94	HAR/94	Nerome et al. (1998) (AF050065)	–
B/Kagoshima/15/94	KAG/94	Nerome et al. (1998) (D38647)	–
B/Aichi/10/95	AI/95	This report (AB027398)	This report (AB027487)
B/Aichi/4/97	AI/4/97	This report (AB027399)	This report (AB027488)
B/Aichi/14/97	AI/14/97	This report (AB027400)	–

Table 1 (*continued*)

B/Aichi/15/97	AI/15/97	This report (AB027401)	This report (AB027489)
B/Aichi/33/97	AI/33/97	This report (AB027402)	This report (AB027490)
B/Beijing/243/97	BEI/97	Nerome et al. (1998) (AF050062)	
B/Ohsaka/543/97	OK/97	–	This report (AB027491)
B/Aichi/3/98	AI/3/98	This report (AB027403)	This report (AB027492)
B/Aichi/5/98	AI/5/98	This report (AB027404)	This report (AB027493)
B/Aichi/8/98	AI/8/98	This report (AB027405)	–
B/Aichi/20/99	AI/20/99	This report (AB027406)	This report (AB027494)

[a]Nucleotide sequences of HA genes and NS genes used for the phylogenetic analysis. The GenBank and EMBL accession numbers of the sequences are shown in the parentheses

[b]Sequence not available

(5′ ACCAGCAATAGCTTCGAA 3′) for the NS gene. The numbers used for nucleotides of HA and NS genes were based on the length of the HA gene of VI/87 and that of the NS gene of GL/54, respectively.

Phylogenetic analysis of HA and NS genes

For the phylogenetic analysis of HA and NS genes, the degree of nucleotide sequence homology among respective genes was calculated by using the maximum matching using GENETYX MAC [28]. Phylogenetic analysis was carried out by the neighbor-joining method [26] and the maximum likelihood method [7]. Both methods gave essentially the same phylogenetic tree topology. The results obtained by the neighbor-joining method are described in the text.

Results

Evolutionary relationships among influenza B viruses based on the nucleotide sequences of HA genes

Nucleotide sequences of the coding regions of HA1 domains of HA genes (HA1 genes) obtained from the virus isolates during 1940 to 1999 (24 new and 25 previously reported sequences) were analyzed to show the genealogy of HA1 genes. Based on the number of nucleotide substitutions, similarities among HA1 genes of 49 viruses were calculated by the neighbor-joining method and the maximum likelihood method, as described in Materials and methods. The phylogenetic tree indicates that the two main lineages, YA/88-like and VI/87-like HA lineages, continue to exist until the present time (Fig. 1).

The time of divergence into the two lineages was estimated from the nucleotide substitutions in the HA1 genes of each virus compared with substitutions in the AI/76 virus. Divergence was then estimated to have occurred around 1969 (Fig. 2).

Evolutionary relationships among NS1 genes

To ascertain whether NS genes have a similar genealogy to that of HA1 genes, we also analyzed the nucleotide sequences of coding regions for NS1 protein of NS

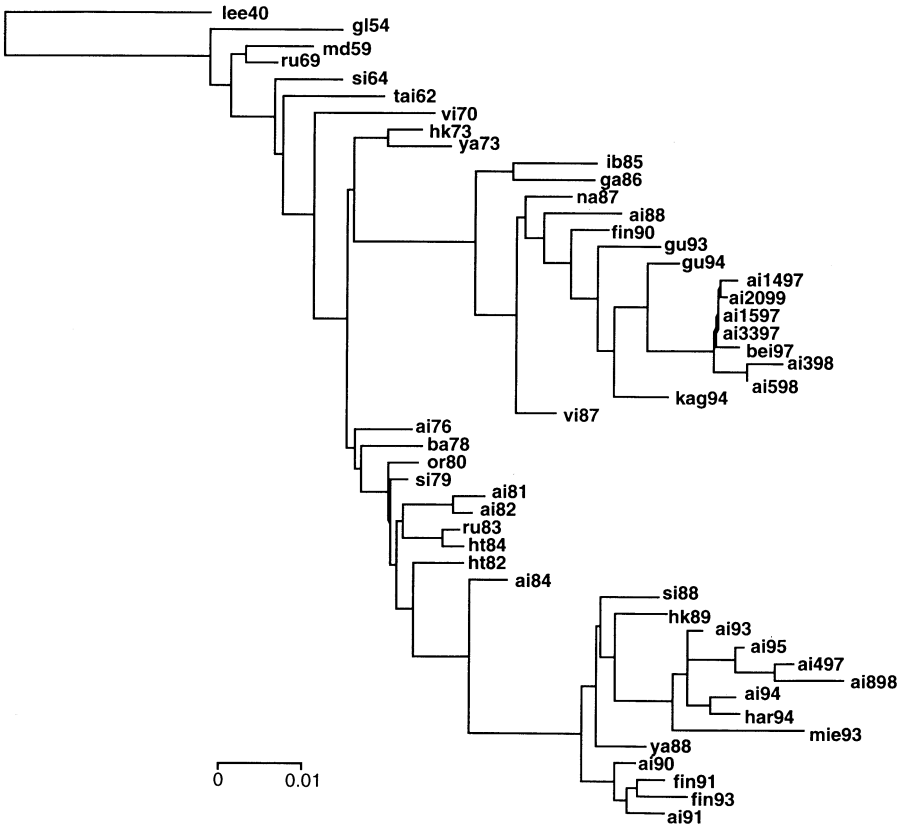

Fig. 1. The phylogenetic tree for HA1 genes of influenza B virus isolates obtained from 1940 to 1999. The dendrogram was constructed using the neighbor-joining method as described in the text. The root of the dendrogram could not be estimated by this method. Branch length represents the genetic distance

genes (NS1 genes) of 36 viruses isolated from 1940 to 1999 (22 new and 14 previously reported sequences). We performed pairwise comparisons of nucleotide and deduced amino acid sequences. The results showed that the differences in nucleotide and deduced amino acid sequences of NS1 genes were neither strictly proportional to the time period between isolations nor linked to the two HA lineages (data not shown). Especially, it is interesting that NS1 of recent isolates obtained form 1997 to 1999 were more strongly related to that of SI/64, a virus isolated more than 30 years earlier, than to those of other isolates obtained in the 1990s. The NS1 genes of viruses isolated after 1997 differed by at most 35 nucleotides from that of SI/64, but differed by at least 43 nucleotides from that of AI/95.

Based on differences in the nucleotide sequences of NS1 genes, an evolutionary analysis was performed by a method corresponding to that used for the HA1 genes. The phylogenetic tree obtained (Fig. 3) was different from that obtained for HA1 genes (Fig. 1). NS genes showed their own distinctive evolutionary pattern marked by multiple lineages which did not correlate with time and which

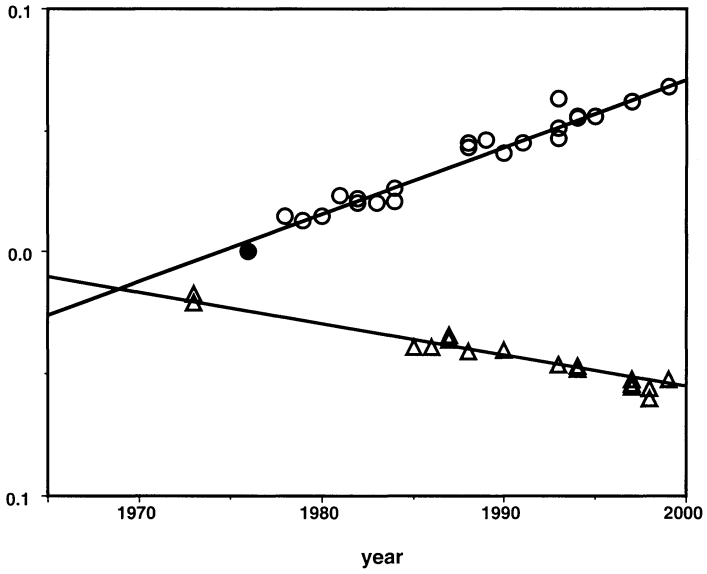

Fig. 2. The putative time for the divergence of VI/87-like (triangle) and YA/88-like (open circle) HA lineages of influenza B viruses. The time of divergence was estimated from the accumulation of nucleotide substitutions of HA1 genes of VI/87-like and YA/88-like group viruses, compared with that of AI/76 (closed circle) virus. The ordinate represents the number of nucleotide substitutions/site in HA1 genes of each virus compared to that in AI/76. The abscissa indicates the year of isolation

were not linked to the two HA lineages. Especially the lineages comparised of virus isolates during 1986 to 1995 except for HT/84 included the viruses of both two HA lineages. These differences in the genealogies of HA1 and NS1 genes suggested frequent genetic reassortment between the cocirculating YA/88-like and VI/87-like HA lineages. Moreover, genealogy of NS1 genes indicated the existence of two distinct NS lineages. Viruses isolated after 1997 formed their own lineage together with HT/84 (HT/84-like NS lineage), while virus isolates during 1973 to 1995 comprised the other lineage (HK/73-like NS lineage) (Fig. 3). Phylogenetic relationships shows that all the virus isolates of HT/84-like NS lineage were more related to SI/64 than to any virus isolates in 1980's and 1990's of HK/73-like NS lineage. We estimated the time of divergence of the two NS lineages by using a method similar to that for HA1. We calculated the number of nucleotide substitutions in the genes of each virus compared with substitutions in the HT/84 virus. In this case, the number of viruses belonging to HT/84-like NS lineage was not enough to assess the precise rate of the nucleotide substitutions per site occurred in their NS1 genes. If the nucleotide substitutions of the NS1 genes of the viruses in HT/84-like NS lineage occurred sequentially at the same rate in HK/73-like NS lineage as shown by dotted lines in Fig. 4, the time of divergence into the two lineages was estimated to be between 1937 and 1957 (Fig. 4).

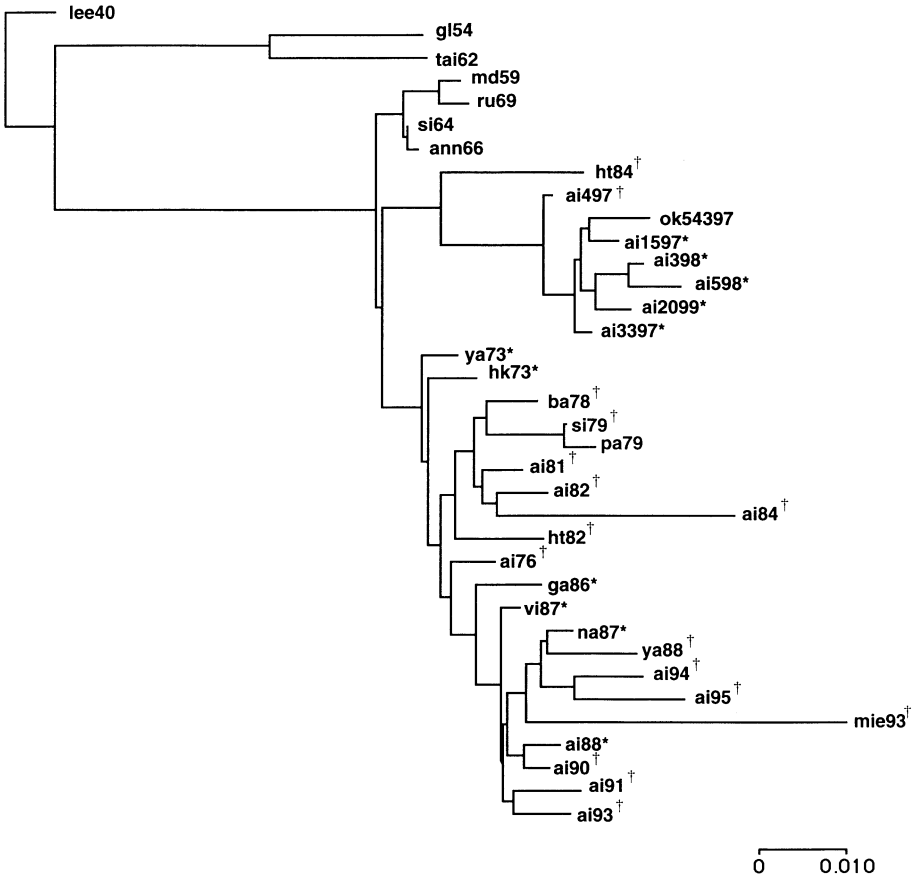

Fig. 3. The phylogenetic tree for NS1 genes of the influenza B virus isolates from 1940 to 1999. The dendrogram was constructed by a method corresponding to that used for HA1 genes. *and † indicate the viruses whose HA belong to VI/87-like and YA/88-like HA lineages, respectively. HA genes of ok54397 and pa79 were not analyzed

Discussion

In this study we determined the nucleotide sequences of HA1 genes (24 strains) and NS1 genes (22 strains) of influenza B viruses to analyze the evolutionary relationships among 49 strains (for HA1) and 36 strains (for NS1) isolated from 1940 to 1999.

As shown in the results, two HA lineages have continued to exist until 1998/99 influenza season. HA1 of both lineages differed by at most 40 amino acids (88.5% similarity between MIE/93 and AI/3/98). At present, influenza A viruses are subdivided into 15 subtypes according to their antigenic relationships in double immunodiffusion tests [31]. Among these 15 subtypes, high degrees of amino acid homology for HA1 were found between H2 and H5 (66.7%), H4 and H14 (68.5%), and H7 and H15 (71.3%) [13, 20, 23]. Therefore it is difficult to say at present that these two HA lineages of influenza B viruses are in the process of separating into two subtypes.

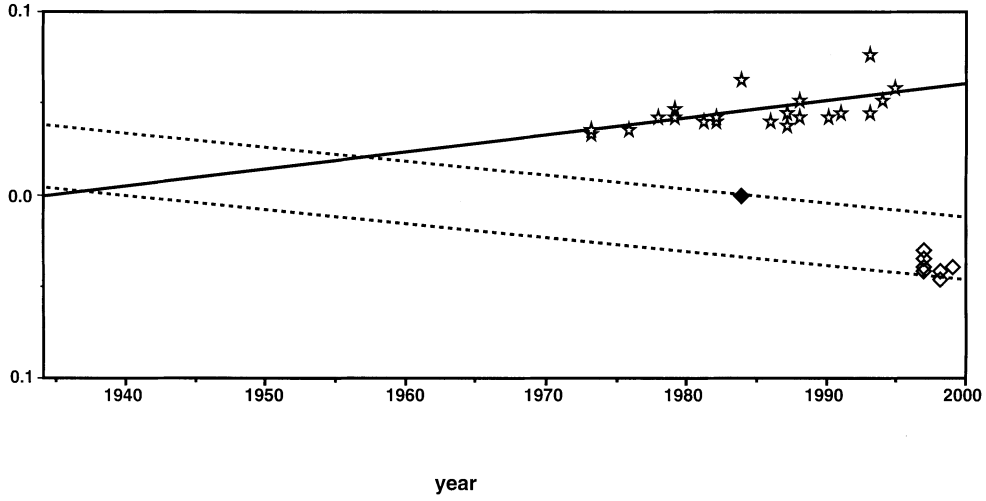

Fig. 4. The putative time for the divergence of HT/84-like (open diamond) and HK/73-like (star) NS lineages of influenza B viruses. The time of divergence was estimated from the accumulation of nucleotide substitutions of NS1 genes of the viruses shown in the text, compared with that of HT/84 (closed diamond) virus. The ordinate represents the number of nucleotide substitutions/site in NS1 genes of each virus compared to that in HT/84. The abscissa indicates the year of isolation

Evolutionary patterns of influenza B viruses were shown to be determined by the cocirculation of multiple lineages [33]. Under these conditions, genetic reassortment could occur among cocirculting lineages. A previous study, however, showed that the phylogenetic trees of HA and NS genes displayed the same genealogy for the nine isolates during 1940 to 1987, suggesting that reassortment had hardly occurred among the cocirculating lineages until 1987 [33]. A current study, however, suggested that reassortment of viral genes between the YA/88-like and VI/87-like groups has occurred since 1986. During 1986 to 1990 viruses of the two HA lineages were cocirculated worldwide [19], therefore it is reasonable to assume that the genetic reassortment between both lineages occurred during these period.

Our phylogenetic analysis based on the nucleotide substitutions of NS1 genes also indicated the existence of the HT/84-like NS lineage. HA genes of the two viruses isolated in 1984, HT/84 and AI/84, belonged to YA/88-like HA lineage, while the NS genes belonged to HT/84-like and HK/73-like NS lineages, respectively. In Asia the HT/84-like virus which appeared to be a reassortant virus between YA/88-like HA lineage and HT/84-like NS lineage has not become dominant until 1997. However, all the investigated viruses isolated in Japan after 1997 belonged to the HT/84-like NS lineage. After submission of this manuscript Lindstrom et al. reported that NS genes of GU/93 and GU/94 also belonged to the HT/84-like NS lineage [17]. HA genes of these viruses belonged to the VI/87-like HA lineage. Since the HA genes of the viruses, GU/93 and Fin/90 (VI-like HA lineage) were closely related, the reassortment of the viral genes between HT/84-

like NS lineage and VI/87-like HA lineage might occur after 1990. The questions why the NS genes of viruses of both two HA lineages have come to belong to HT/84-like NS lineage since 1997 and why the viruses of HT/84-like NS lineage have become dominant remain to be answered. Comparison of deduced amino acid sequences of NS1 proteins showed that two amino acid differences between the two NS lineages have been retained until 1999 (data not shown). However, these amino acids at positions 127 (arginnine in HT/84-like lineage, lysine or asparagine in HK/73-like lineage) and 138 (aspartic acid in HT/84-like lineage, asparagine in HK/73-like lineage) were not involved in the functional domains, nuclear localization signal or RNA binding domain [8, 30]. When we compared the growth of AI/14/97 (HT/84-like NS lineage) in MDCK cells with that of AI/95 (HK/73-like NS lineage), no significant differences were observed (data not shown). Although no differences in the growth of the viruses belonging to the two NS lineages were observed in MDCK cells, there might be a certain reason for the viruses of HT/84-like NS lineage to become dominant during circulation among individuals after 1997.

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References

1. Air GM, Gibbs AJ, Laver WG, Webster RG (1990) Evolutionary changes in influenza B are not primarily governed by antibody selection. Proc Natl Acad Sci USA 87: 3 884–3 888
2. Bean WJ, Cox NJ, Kendal AP (1980) Recombination of human influenza A viruses in nature. Nature 284: 638–640
3. Berton MT, Naeve CW, Webster RG (1984) Antigenic structure of the influenza B virus hemagglutinin: nucleotide sequence analysis of antigenic variants selected with monoclonal antibodies. J Virol 52: 919–927
4. Both GW, Sleigh MJ, Cox NJ, Kendal AP (1983) Antigenic drift in influenza virus H3 hemagglutinin from 1968 to 1980: multiple evolutionary pathways and sequential amino acid changes at key antigenic sites. J Virol 47: 52–60
5. Briedis DJ, Lamb RA (1982) Influenza B virus genome: sequences and structural organization of RNA segment 8 and the mRNAs coding for the NS-1 and NS-2 proteins. J Virol 42: 186–193
6. DeBorde DC, Donabedian AM, Herlocher ML, Naeve CW, Maassab HF (1988) Sequence comparison of wild-type and cold-adapted B/Ann Arbor/1/66 influenza virus genes. Virology 163: 429–443
7. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likehood approach. J Mol Evol 17: 368–376
8. Greenspan D, Palese P, Krystal M (1988) Two nuclear location signals in the influenza virus NS1 nonstructural protein. J Virol 62: 3 020–3 026

9. Guo Y, Xu X, Cox N (1992) Human influenza A (H1N2) viruses isolated from China. J Gen Virol 73: 383–388
10. Hovanec DL, Air GM (1984) Antigenic structure of the hemagglutinin of influenza virus B/Hong Kong/8/73 as determined from gene sequence analysis of variants selected with monoclonal antibodies. Virology 139: 384–392
11. Ikonen N, Kinnunen L, Forsten T, Pyhala R (1996) G Bank accession number L76317
12. Kanegae Y, Sugita S, Endo A, Ishida M, Senya S, Osako K, Nerome K, Oya A (1990) Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: cocirculating lineages in the sameepidemic season. J Virol 64: 2 860–2 865
13. Kawaoka Y, Yamnicova S, Chambers TM, Lvolv DK, Webster RG (1990) Molecular characterization of a new hemagglutinin, subtype H14, of influenza A virus. Virology 179: 759–767
14. Kinnunen L, Ikonen N, Poyry T, Pyhala R (1992) Evolution of influenza B/Victoria/2/87-like viruses: occurrence of a genetically conserved virus under conditions of low epidemic activity. J Gen Virol 73: 733–736
15. Krystal M, Young JF, Palese P, Wilson IA, Skehel JJ, Wiley DC (1983) Sequential mutations in hemagglutinins of influenza B virus isolates: Definition of antigenic domains. Proc Natl Acad Sci USA 80: 4 527–4 531
16. Li XS, Zhao CY, Gao HM, Zhang YQ, Ishida MY, Endo A, Nerome R, Omoe K, Nerome K (1992) Origin and evolutionary characteristics of antigenic reassortant influenza A (H1N2) viruses isolated form man in China. J Gen Virol 73: 1 329–1 337
17. Lindstrom SE, Hiromoto Y, Nishimura H, Saito T, Nerome R, Nerome K (1999) Comparative analysis of evolutionary mechanism of the hemagglutinin and three internal protein genes of influenza B virus: multiple cocirculation lineages and frequent reassortment of the NP, M, and NS genes. J Virol 73: 4 413–4 426
18. Maniatis T, Fritsch EF, Sambrook JC (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
19. Nerome R, Hiromoto Y, Sugita S, Tanaka N, Ishida M, Matsumoto M, Lindstrom SE, Takahashi T, Nerome K (1998) Evolutionary characteristics of influenza B virus since its first isolation in 1940: dynamic circulation of deletion and insertion mechanism. Arch Virol 143: 1 569–1 583
20. Nobusawa E, Aoyama T, Kato H, Suzuki Y, Tateno Y, Nakajima K (1991) Comparison of complete amino acid sequences and receptor-binding properties among 13 serotypes of hemagglutinin of influenza A viruses. Virology 182: 475–485
21. Norton GP, Tanaka T, Tobita K, Nakada S, Buonagurio DA, Greenspan D, Krystal M, Palese P (1987) Infectious influenza A and B virus variants with long carboxyl terminal deletions in the NS1 polypeptides. Virology 156: 204–213
22. Raymond FL, Caton AJ, Cox NJ, Kendal AP, Brownlee GG (1986) The antigenicity and evolution of influenza H1 haemagglutinin, from 1950–1957 and 1977–1983: Two pathways from one gene. Virology 148: 275–287
23. Röhm C, Zhou N, Süss J. Mackenzie J, Webster RG (1996) Characterization of a novel influenza hemagglutinin, H15: Criteria for determination of influenza A subtypes. Virology 217: 508–516
24. Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP Nerome K (1990) Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology 175: 59–68
25. Rota PA, Hemphill M, Whistler T, Regnery HL, Kendal A (1992) Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus. J Gen Virol 73: 2 737–2 742

26. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425
27. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5 463–5 467
28. Takeishi K, Gotoh O (1984) Computer analysis of the sequence relationships among 4.5S RNA molecular species from various sources. J Biochem 92: 1 173–1 117
29. Verhoeyen M, van Rompuy L, Min JW, Huylebroeck D, Fiers W (1983) Complete nucleotide sequence of the influenza B/Singapore/222/79 virus hemagglutinin gene and comparison with the B/Lee/40 hemagglutinin. Nucleic Acids Res 11: 4 703–4 712
30. Wang W, Krug RM (1996) The RNA-binding and effector domains of the viral NS1 protein are conserved to different extents among influenza A and B viruses. Virology 223: 41–50.
31. WHO Memorandum (1980) A revision of the system of nomenclature virus. Bull World Health Organ 58: 585–591
32. Xu X, Guo Y, Rota P, Hemphill M, Kendal A, Cox N (1993) Genetic reassortment of human influenza virus in nature. In: Hannoun C et al. (eds) Options for the control of influenza II. Excerpta Medica, Amsterdam, pp 203–207
33. Yamashita M, Krystal M, Fitch WM, Palese P (1988) Influenza B virus evolution: co-circulating lineages and comparison of evolutionary pattern with those of Influenza A and C viruses. Virology 163: 112–122
34. Young JF, Palese P (1979) Evolution of human influenza A viruses in nature: recombination contributes to genetic variation of H1N1 strains. Proc Natl Acad Sci USA 76: 6 647–6 651

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