

Comparison of the HA genes of type B influenza viruses in herald waves and later epidemic seasons

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SUMMARY

From January 1985 to May 1991, herald strains of influenza B virus were isolated in 1987 and 1989 in Japan. In both cases, influenza epidemics caused by the same type followed in the next winter season. The HA gene sequences of the influenza B viruses isolated in Japan from 1987–91, which covers two herald waves of influenza B viruses, were analysed and located on the phylogenetic tree for influenza B viruses after the B/Singapore/64 strain. Co-circulation of at least two evolutionary lineages of the HA genes existed for influenza B viruses in Japan during the period of this study. The herald viruses in one wave (1987) were genetically close to the winter isolates and were considered to be the parental viruses for the following influenza season, while in the other wave (1989) winter isolates belonged to another lineage on which one of the herald viruses was located, but they were genetically and antigenically different from the herald viruses.

INTRODUCTION

The development of a surveillance system for virus infections in Japan makes it possible to isolate influenza viruses throughout almost all the year. For at least the last five influenza seasons (1985–90) in Japan, the influenza virus type or subtype isolated in the spring became the dominant epidemic strains of the following influenza season. This phenomenon has been called the ‘herald wave’ and was expected to forecast the virus most likely to be prevalent during the next influenza season [1, 2].

In our previous study, we analysed the HA gene sequences of the influenza A(H3N2) and A(H1N1) viruses isolated in Japan and Southeast Asia during the period of 1985–90 in order to determine whether the viruses isolated in off-seasons are genetically related to the viruses of the following epidemics [3]. The results

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showed that the spring isolates were in most cases genetically close to some of the winter isolates and they were considered to be the parental viruses for the following influenza seasons.

Analysis of the HA genes of recent influenza B viruses has shown the existence of two co-circulating evolutionary lineages [4–6], and, therefore, clearcut results should be expected for the genetic relationship between herald and the later epidemic viruses. In the present study, we compared the HA gene sequences of influenza B viruses isolated during herald waves and later epidemic seasons in the same districts in Japan during 1987–90.

MATERIALS AND METHODS

Viruses and purification of viral RNA

The strains and passage histories of the influenza B viruses used in the present study are shown in Table 1. The virus strains were mainly chosen by the following criteria: (a) viruses isolated in Nagano prefecture or Kamata area in Tokyo where we have been working on influenza surveillance, (b) vaccine strains used in Japan (IK/85₁, NS1/87₅, NS3/87₅, YG16/88₁, AI5/88₁, HK22/89), and (c) herald and epidemic strains isolated from different parts of Japan. Herald and epidemic strains isolated from the same districts were included. The viruses were isolated by us or obtained from prefectural public health laboratories. The viruses were grown in MDCK cells at 34 °C and purified as described previously [7]. Viral RNA was extracted by the procedure described by Palese and Schulman [8].

Haemagglutination-inhibition tests

Haemagglutination-inhibition (HI) tests were performed with post-infection ferret sera treated with receptor-destroying enzyme according to the procedure described by Dowdle and colleagues [9]. Ferret sera and reference strains of influenza B virus were kindly provided by Dr M. Ishida, National Institute of Health (NIH), Japan.

Nucleotide sequencing of the HA genes

Nucleotide sequences of the HA gene were determined from virion RNA by the dideoxy chain termination method [10] as described previously [7].

Oligonucleotide primers

Synthetic oligonucleotides were used as the primers. They corresponded to the nucleotide positions 50–63, 196–210, 403–22, 631–45, and 880–99, numbered according to the positive strand sequence of the HA gene of the B/Lee/40 strain [11].

RESULTS AND DISCUSSION

Influenza surveillance in Japan during 1985–91

Fig. 1 shows the chronological surveillance of influenza viruses isolated by municipal and prefectural public health laboratories throughout Japan during 1985–91 [12]. In the 7 influenza seasons in this period, there were 6 epidemics

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

Strain name	Month of specimen collection	Abbreviation	Passage history*
B/Ibaraki/2/85	January	IK/85 ₁	EX
B/Nagasaki/1/87	May	NS1/87 ₅ †	EX
B/Nagasaki/3/87	May	NS3/87 ₅ †	E3CK3 or EX§
B/Kochi/258/87	May	KI258/87 ₅ †	CK7
B/Nagasaki/8/88	January	NS8/88 ₁	CK3
B/Yamagata/16/88	January	YG16/88 ₁	EX
B/Aichi/5/88	January	AI5/88 ₁	EX
B/Kochi/94/88	January	KI94/88 ₁	CK7
H/Hong Kong/22/89	—	HK22/89	EX
B/Nagano/722/89	April	NN722/89 ₄ ‡	CK4
B/Kamata/202/89	April	KT202/89 ₄ ‡	CK3
B/Kamata/292/89	June	KT292/89 ₈ ‡	CK3
B/Nagano/80/90	January	NN80/90 ₁	CK3
B/Kamata/22/90	January	KT22/90 ₁	CK3
B/Hyogo/4/91	February	HG4/91 ₂	CK4
B/Kamata/218/91	March	KT218/91 ₃	CK3

* E, egg; CK, MDCK cells. The numbers indicate passages. X, passage number unknown.

† Herald strains 1987.

‡ Herald strains 1989.

§ B/Nagasaki/3/87 strain used as a vaccine was passaged on eggs only.

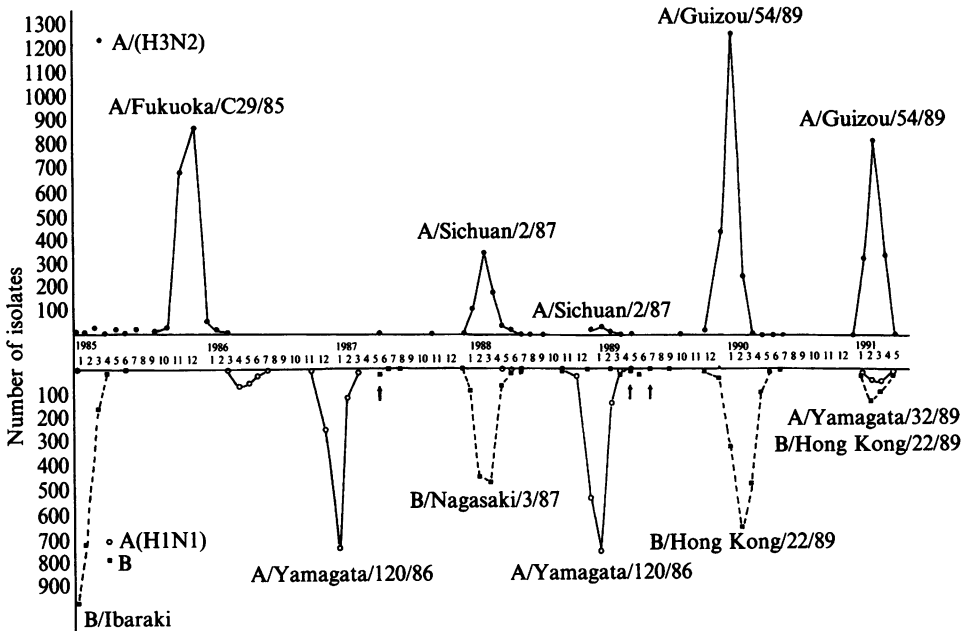


Fig. 1. Chronological sequence of isolation of influenza viruses by municipal and prefectural public health laboratories throughout Japan. The numbers of isolated viruses were reported to the National Institute of Health in Japan [12]. The main epidemic viruses in Japan are shown above each epidemic peak. Influenza B herald viruses used in the present study are shown by arrows.

Table 2. *Haemagglutination inhibition reactions of influenza B viruses isolated in Japan during 1985-91*

Virus strain	Ferret serum*						Antigenic grouping
	IK/85 ₁	NS1/87 ₅	NS3/87 ₅	YG16/88 ₁	AI5/88 ₁	HK/89	
IK/85 ₁ †	<u>240</u> †	120	60	< 10	80	< 10	IK/85 (1)
NS1/87 ₅ †	120	<u>320</u>	120	< 10	80	< 10	IK/85 (1)
NS3/87 ₅ †	10	15	<u>40</u>	< 10	< 10	< 10	NS3/87 (2)
YG16/88 ₁ †	20	30	15	<u>640</u>	40	80	YG16/88 (3)
AI5/88 ₁ †	40	120	60	< 10	<u>80</u>	< 10	AI5/88 (4)
HK22/89‡	10	30	< 10	20	40	<u>160</u>	HK22/89 (5)
NS3/87 ₅	20	15	40	< 10	< 10	< 10	NS3/87 (2)
KI258/87 ₅	10	30	40	< 10	< 10	< 10	NS3/87-like (2)
NS8/88 ₁	10	10	30	< 10	10	< 10	NS3/87-like (2)
KI94/88 ₁	10	10	20	< 10	< 10	< 10	NS3/87-like (2)
NN722/89 ₄	15	80	60	< 10	20	< 10	AI5/88-like (4)
KT202/89 ₄	10	20	10	< 10	20	< 10	Unidentified
KT292/89 ₆	< 10	< 10	< 10	480	< 10	120	YG16/88-like (3)
NN80/90 ₁	< 10	< 10	< 10	80	< 10	120	HK22/89-like (5)
KT22/90 ₁	< 10	< 10	< 10	80	< 10	100	HK22/89-like (5)
HG4/91 ₂	10	10	30	< 10	< 10	< 10	NS3/87-like (2)
KT218/91 ₃	< 10	< 10	< 10	80	< 10	120	HK22/89-like (5)

* All ferret sera were treated with receptor-destroying enzyme to inactivate non-specific inhibitors. Each titre is the mean of duplicate tests.

† Homologous titres are underlined.

‡ Egg-passaged vaccine strains.

caused by influenza A virus and 3 by influenza B virus. In the 1987-8 and 1989-90 influenza seasons, epidemics were caused by two different types of influenza viruses. Herald strains of influenza B virus were isolated in 1987 at four prefectures and in 1989 from nine prefectures, but the numbers reported to NIH, Japan, were small compared to those of winter epidemic viruses. In both cases, influenza epidemics caused by the same type followed in the winter influenza season. During this period, at least four antigenic variants of influenza A, and three of influenza B virus caused epidemics as shown above each epidemic peak (Fig. 1).

Antigenic analysis of the influenza B viruses

The results of the HI tests on influenza B viruses are shown in Table 2. Homologous titres of ferret sera against NS3/87₅ and AI5/88₁ were low, and therefore the antigenic structure was deduced from the relative HI patterns. Of the three herald strains isolated in 1987, NS1/87₅ was antigenically similar to IK/85₁, while NS3/87₅ and KI258/87₅ were antigenic variants with similar antigenic structure. The main epidemic influenza B viruses in Japan in the 1987-8 influenza season were antigenically like NS3/87₅ which is similar to the strain B/Victoria/2/87 (5). Two epidemic strains, NS8/88₁ and KI94/88₁, resembled NS3/87₅ antigenically. Two antigenic variants of influenza B virus (YG16/88₁ and AI5/88₁) were also isolated in this season. The three herald strains isolated in 1989 (NN722/89₄, KT202/89₄, and KT292/89₆) were antigenically different. NN722/89₄ was like AI5/88₁, KT202/89₄ was like NS3/87₅ or unidentified, and KT292/89₆

was like YG16/88₁. The main epidemic viruses in the 1989–90 influenza season were antigenically like HK22/89 which had drifted slightly from YG16/88₁. The two epidemic strains in the 1989–90 influenza season (NN80/90₁ and KT22/90₁) were both antigenically close to HK22/89. KT218/91₃, was isolated from a small epidemic in the 1990–1 influenza season, resembled HK22/89. HG4/91₂, one of a few isolates known so far after the 1989 herald strains which did not react with YG16/88₁ ferret serum after 1989 herald strains, was antigenically like NS3/87₅.

Nucleotide sequence changes in the HA genes of influenza B strains isolated between 1985–91 from that of SI/64

Fig. 2 shows the nucleotide changes in the HA genes of 15 influenza B strains isolated between 1985–91 in Japan from that of the B/Singapore/64 (SI/64) strain [4], along with the corresponding amino-acid changes. The nucleotide changes in the B/Hong Kong/8/73 (HK/73) [13] and B/Singapore/222/79 (SI/79) [14] strains are also included. Only the nucleotide sequences of the HA1 region are shown. The sequences of the non-coding region are not included. An evolutionary tree which shows how the HA genes of the 1985–91 influenza B strains are derived from that of SI/64 based on nucleotide changes is shown in Fig. 3. This was constructed by giving priority to mainstream changes (changes inherited by most subsequent strains) over strain-specific changes and minimizing the chance that a strain-specific change shared by two viruses occurred independently. As reported by others [4–6], two evolutionary lineages (A, B in Fig. 3) of influenza B viruses co-circulated during this period as deduced from the evolutionary tree of the HA genes. The viruses of the two lineages did not interact with each other in the HI tests (Table 2). Two evolutionary trees (a, b) can be drawn as for the separation point between evolutionary lineages A and B. IK/85₁ was located on evolutionary lineage B and YG16/88₁ on evolutionary lineage A. Herald strain NS1/87₅ was the first influenza B isolate after the 1984–5 influenza season and was located on a branch derived five mainstream changes (186, 294, 420, 485, and 565) away from IK/85₁. Two herald strains (NS3/87₅ and KI258/87₅) were located close to each other one mainstream change (948) away from NS1/87₅. Three strains of the following influenza season (NS8/88₁, KI94/88₁, and AI5/88₁) were located close together on different twigs one mainstream change (296) away from NS3/87₅ and KI258/87₅. Two 1989 herald strains (NN722/89₄ and KT202/89₄) were directed differently from other strains described so far, although they were located on evolutionary lineage B. One herald strain (KT292/89₆) was located on evolutionary lineage A, five mainstream changes (98, 99, 115, 160, and 860) away from YG16/88₁. The winter epidemic strains NN80/90₁ and KT22/90₁ were located close together, three further mainstream changes (297, 527, and 588) away from a herald strain (KT292/89₆). KT218/91₃, which was isolated from a small epidemic in the 1990–1 influenza season, was located close to the viruses of the previous season.

As discussed in relation to our studies on influenza A virus (3), when we compare the viruses isolated during a short period, the rate of the nucleotide substitution differs with the virus and it is difficult to estimate the genetic relationship among them by the number of changed bases. Therefore, in our previous study we estimated the genetic relationship among the HA genes of influenza A isolates

Base No.	SI/79	Y167/85 K1292/89 M80/90 K122/90 K1218/91 HK/73	MS1/87 K52/87 K1258/87 K58/88 K794/88 A15/88 M122/89 K1202/89 H4/91	A.A. No.	A.A. change	Base No.	SI/64	Y167/85 K1292/89 M80/90 K122/90 K1218/91 HK/73	MS1/87 K52/87 K1258/87 K58/88 K794/88 A15/88 M122/89 K1202/89 H4/91	A.A. No.	A.A. change		
81	T		C C C			571	A	---		165	N - -		
98	T	C C C C		C C C		572	A	---		165	N - -		
99	A	T T T T			7	I - T	573	C		165	N - -		
105	G	T T T T T T	T T T		13	P - S	574	A		166	K - R		
115	C	T T T T					575	A		166	K - R		
117	C	T T T T T					580	G		168	A - T		
132	T	A A A A A A					584	C		169	T - I		
160	G	A A A A		A			585	G	A A A A A A A A A				
165	G			A A	28	G - S	588	T					
186	T	A A A A A A	C C C C C C C C C				589	C					
189	C	A A A A A A					592	T	C C C C C	T	171	P - S	
213	A			G			603	A					
220	C	A A A A A A	A A A A A A A A A A				622	A	G G G G G G G G G G		182	K - E	
222	G		A A A A A A A A A A		48	Q - K	642	T					
225	C		T				652	T					
231	A	G G G G G G G G	G G G G G G G G G G G		56	N - K	657	C	T T T T T T T		192	F - V	
246	C		G G G G G G G G G G A				664	A	G	G G			
270	C	T T T T T					665	A		G			
285	A			G			667	G	A A A A A				
290	A	T T T T T			71	K - M	670	A	G	T G			
291	G			A			679	G	A A A A A				
294	T		C C C C C C C C C				680	T	A A A A A	C			
295	A	G			73	M - V	683	C	A A A A A A A A A A A A A A A				
296	T			C C C	73	M - T	684	A	C				
297	G	A A A			73	M - I	702	G	T T T T T				
305	T			C	76	I - T	708	A		G G G G G G G G G G			
320	C			T	81	A - V	729	C	T T T T T T T				
333	C			T			735	A		G G	G	G G G G	
341	A	G G G G G			88	K - R	739	A			G G		
348	T			A			747	T		C C C C C C C C C C		221	T - A
354	T	C C C C C					748	G		A			
390	C		T T T T T T T T T				753	T		A A A A A A A A A A A		224	V - I
393	A		G				763	G		A			
396	G		A A				764	G	A				
399	A			G			778	A		G G G G G G G G G G G G G		229	G - S
402	T	C C C C C C C C	C C C C C C C C C C C				788	A	G G G G G G G G G G G G G G G G		229	G - D	
420	T		C C C C C C C C C C C				793	C		A			
424	A		C C C C C C C C		116	N - H	797	A	C C C C C C C C C C C C C C C C C		237	E - G	
426	T	C	C				804	C			T		
430	A			G	118	R - G	807	C		T T T T T T T T T T T T T			
432	G	A A A A A					834	A			G G		
435	A			G			838	C		T T T T T T T T T T T		254	P - S
443	G	A A A A A	A A A A A A A A A A A		122	R - N	843	A	G G G G G G				
444	T			C C			849	A		C			
456	T	C C C C C C C C	C C C C C C C C C C C C C				850	G		A		258	G - R
463	A	G			129	T - A	859	G		A A A A A A A A A A A		261	V - I
464	C	G G G G G G		A	129	T - R	860	T	C C C C C C	C C C C C C C C C C C C C		261	V - A
477	A		G				864	T		C C C C C C C C C C T			
485	T	G G G G G G	A A A A A A A A A A A		136	I - K	874	G		A A A A A A A A A A A		266	V - I
487	G	A C C C C C C		A A A A	137	V - I	879	A	G G G G G G				
501	A		G G G G G G G G	G			891	A	G G G G G G				
514	G			A	146	V - I	896	G		C		273	W - S
521	A	G G G G G G			148	N - S	901	G		C		275	A - P
523	G	A A A A A A			149	G - R	930	G			A	286	L - F
525	G	A A A A A A A A	A A A A A A A A A A A				936	G		T			
527	A	G G G			150	K - R	948	T		C C C C C C C C A			
538	G			A	154	A - T	949	G		C		291	E - Q
540	A			T T			963	C	T T T T T				
561	A			G			980	G		C		301	G - A
562	A			G G G G	162	K - E	1011	A		G G G G G G G G G G G		312	E - A
563	A	G G G G G G			162	K - R	1013	A		C			
564	A	G G G G G G			1014		1014	G	A A A A A A A A A A A A A	A		325	K - R
564a	-		A A A A A A A A A A A		162a	- N	1052	A			G		
564b	-		A A A A A A A A A A A		162a	- N	1059	C	T T T T T				
564c	-		C C C C C C C C C C C		162a	- N	1068	G	T T T T T				
565	A	G G G G G G G	G G G G G G G G G		163	N - D	1092	G	T T T T T T T T T T T T T T T				
568	A			G	164	K - E	1095	T		C			
570	A	C C C C C C	C C C C C C C C C C C		164	K - N				C			

Fig. 2. Nucleotide changes in the HA1 region of the HA genes of 15 influenza B strains between 1985-91. The nucleotide bases are numbered according to the positive strand sequence of the B/Lee/40 HA gene [11]. Only positions which drifted from the HA gene of SI/64 are shown. Part of the strain-specific changes in SI/64 and HK/73 are not included. The position and the direction of the amino-acid changes from SI/64 are also shown in the last two columns. One-letter codes for amino acids are used. The

during several years by mainstream changes. However, in the present study of influenza B viruses, especially in evolutionary lineage B, there were fewer mainstream changes than strain-specific changes which were shared by several strains (e.g. 424, 501, 81, 105, 396, 487). Furthermore, it is possible to separate mainstream and strain-specific changes in the long term but difficult in the short term, because the selection of mainstream changes was not fully accomplished. Therefore, we judged the genetic relationship between the influenza B viruses flexibly in the present study. The three 1987 herald strains (NS1/87₅, NS3/87₅, and KI258/87₅) shared five strain-specific nucleotide changes (81, 105, 396, 424, and 501) and were genetically close, but different from a previous epidemic strain, IK/85₁. The epidemic strains of the 1987–8 influenza season (NS8/88₁, KI94/88₁, and AI5/88₁) shared two or three strain-specific changes (424, 501, and 81) with herald strains, and these were thought to be genetically close. Among the three 1989 herald strains (NN722/89₄, KT202/89₄, and KT292/89₆), NN722/89₄ and KT202/89₄ were located on evolutionary lineage B like the previous season viruses, while KT292/89₆ was located on evolutionary lineage A, as was YG16/88₁, and this was also deduced by the HI test (Table 2). The winter viruses (NN80/90₁ and KT22/90₁) were located three mainstream changes (292, 527, and 588) away from KT292/89₆ and were thought to be genetically different from herald viruses.

Two 1989 herald strains (NN722/89₄ and KT202/89₄) lacked the nucleotide changes at 424 and 501 (Fig. 3). Two American strains isolated in 1988 also lacked these changes [6]. Whether these strains form a third evolutionary lineage will become clear after analysis of worldwide isolates. HG4/91₂ is one of a few strains in Japan with an NS3/87₅-like antigenic structure isolated since 1989.

Amino-acid changes in the 1985–91 HA1 polypeptides of the influenza B strains

The evolutionary relationships of the 1985–91 HA1 polypeptide of influenza B strains based on the amino-acid changes from SI/64 are shown in Fig. 4, along with the antigenic structure determined by the HI tests. In the 1987–8 influenza season, two types of antigenic variants, YG16/88₁ and AI5/88₁, were isolated in addition to the main epidemic strains. AI5/88₁, as well as other epidemic strains, was located on evolutionary lineage B, while YG16/88₁ was located on evolutionary lineage A. The evolutionary pathway of the HA1 polypeptides correlated well with the one based on nucleotide changes. There were many common amino-acid changes between lineages A and B, especially in mainstream amino-acid changes. This may reflect the amino-acid changes selected by antibody pressure. Antigenic analysis by the HI test did not necessarily reflect the evolutionary relationships of the HA1 polypeptides of influenza B viruses and it was difficult to correlate the specific amino-acid change with a specific antigenic change. However, the HI test clearly distinguished the viruses on lineage A from lineage B.

In this study, we showed that the influenza B viruses belonging to two evolutionary lineages independently caused epidemics until 1991. Herald viruses

SI/64 [4], HK/73 [13], SI/79 [14], IK/85 [5], NS1/87 [5], YG16/88 [5], and AI5/88 [6] HA gene sequences were reported previously. The — mark in the amino-acid column indicates a deletion. The — mark in the AA change column represents an arrow (e.g. I → T at base 81).

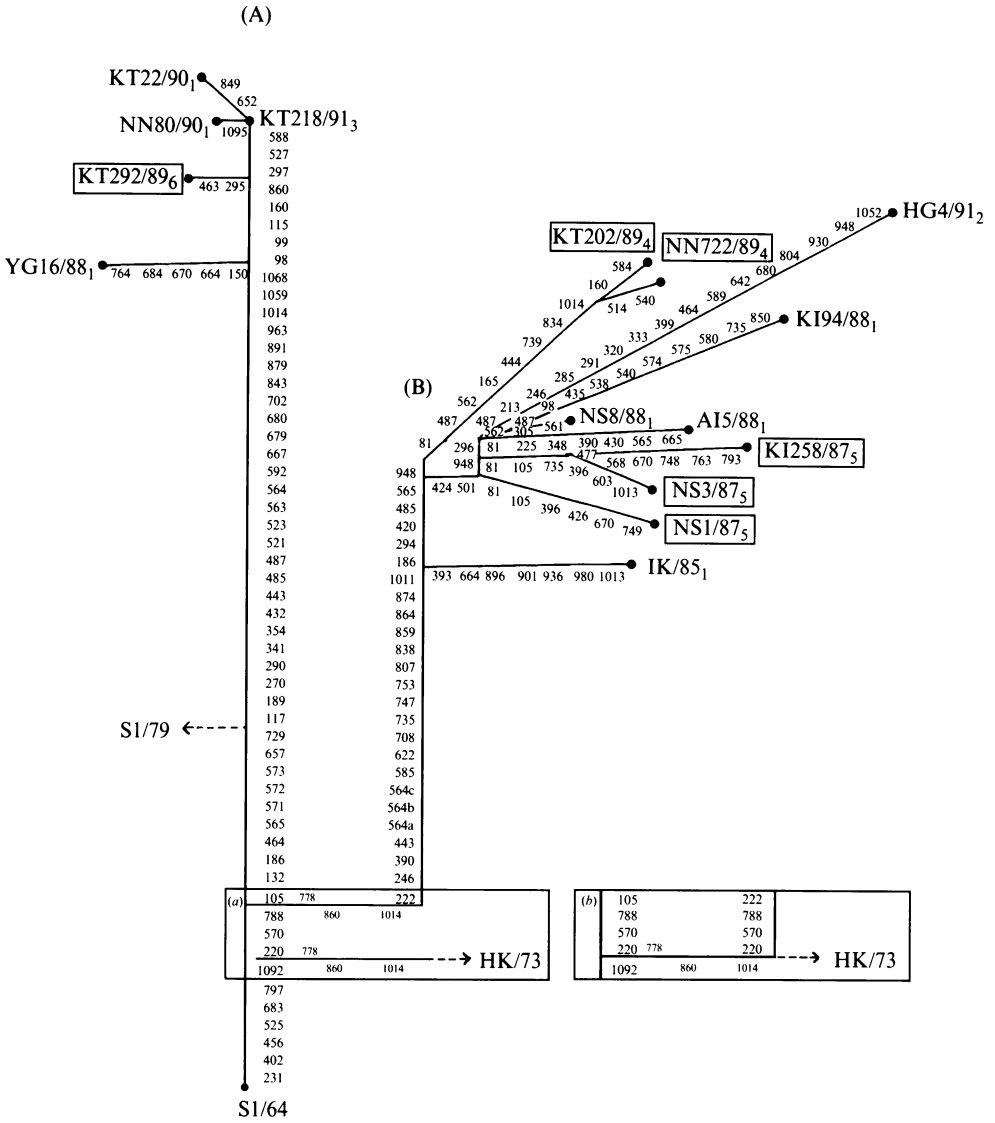


Fig. 3. The evolutionary tree for the HA genes of the influenza B strains. Numbers refer to the mainstream nucleotide changes which have become fixed in most of the subsequent strains (vertical line); or to strain-specific nucleotide changes on the side-branches. Strain-specific changes are shown only after IK/85₁ in the figure. Herald viruses are boxed. (A) and (B) indicate evolutionary lineages, and (a) and (b) show two possible separation points between lineages A and B.

in one case were genetically close to winter epidemic viruses, but in another case antigenically drifted viruses became dominant after 2 years. At present we do not know whether the viruses belonging to evolutionary lineage B on which most of the Japanese isolates during 1985–8 belonged will become a dead-end in the future. It was reported recently that in Finland influenza B strains on lineage B were isolated [15]. However, in worldwide, 96% of influenza B isolates in 1990–1 influenza season belonged to lineage A (YG16/88-like) and 4% belonged to lineage B (B/Victoria/2/87-like) [16].

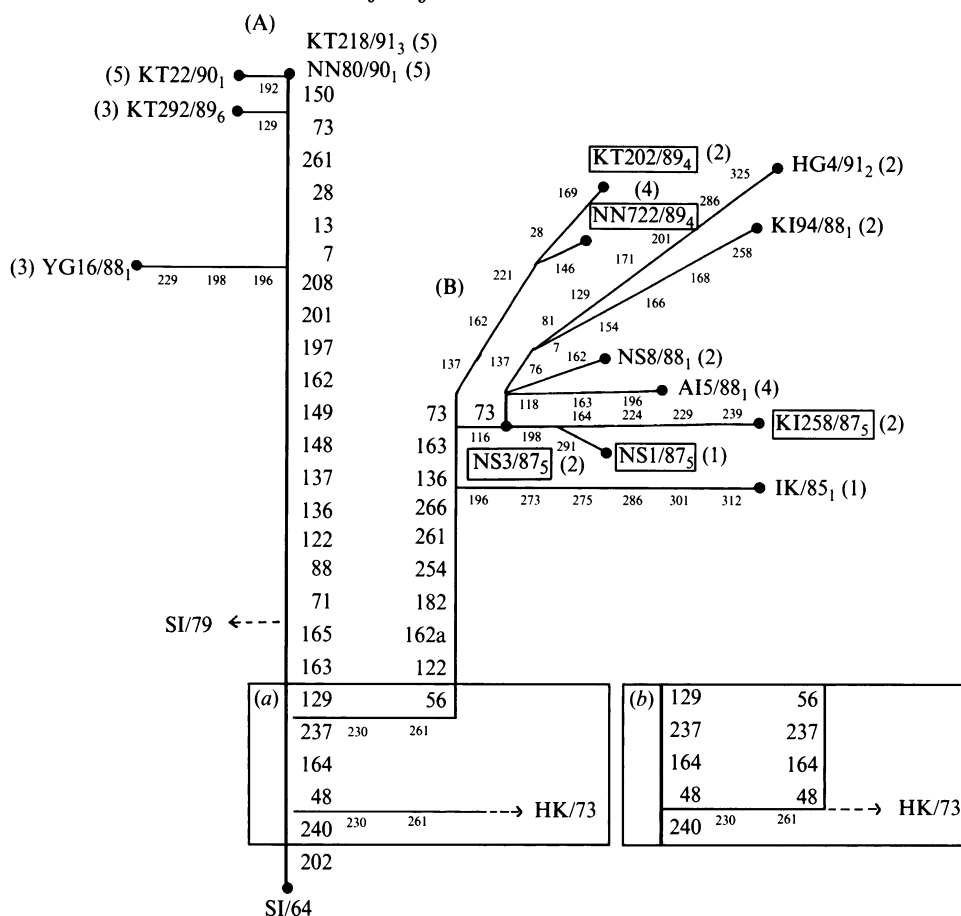


Fig. 4. The evolutionary relationships of the 1985–91 HA1 polypeptide of influenza type B strains based on the amino-acid changes from SI/64 HA1 polypeptide. Herald viruses are boxed. (A) and (B) indicate evolutionary lineages, and (a) and (b) show two possible separation points between lineages A and B. The number in parentheses after the strain shows the HI grouping according to Table 2.

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