

Antigenic and genetic analyses of influenza type B viruses isolated in Russia, 1987–91

M. L. HEMPHILL¹, P. A. ROTA¹, V. T. IVANOVA², A. N. SLEPUSHKIN²
AND A. P. KENDAL^{1*}

¹*Influenza Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA*

²*The D. I. Ivanovsky Institute of Virology, Gamaleya Street 16, Moscow, 123098 Russia*

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SUMMARY

Four influenza type B viruses isolated in Russia during periods of relatively low (1987–8) or high (1990–1) influenza B activity were characterized antigenically using a microneutralization assay. These isolates were antigenically similar to contemporary reference strains from either of two separate lineages represented by B/Victoria/2/87 and B/Yamagata/16/88. The evolutionary relationships of the variable portion of the haemagglutinin (HA1) genes of these viruses were determined by comparison with influenza B HA1 sequences previously obtained. The Isolate B/USSR/2/87, collected during the 1987–8 influenza season, was found to be closely related to viruses on the B/Victoria/2/87 lineage that circulated during the 1988–9 influenza season in the United States. Sequence analysis of the isolates from the 1990–1 influenza season demonstrated co-circulation of viruses from both the B/Victoria/2/87 and B/Yamagata/16/88 lineages in Russia, confirming the antigenic analysis.

Significant levels of infection caused by influenza type B virus were detected worldwide in two of the four influenza seasons from 1987 to 1990. During this time, influenza vaccination trials which compared live attenuated and inactivated influenza vaccines were conducted in Russia [1]. The characterization of influenza B viruses isolated in Russia during these trials would be helpful in assessing vaccine efficacy in these studies.

During the 1987–8 influenza season, localized outbreaks of influenza B occurred throughout the world. Antigenic analysis identified variants that were different from previously prevalent strains. The majority of these isolates, including those from Russia, were shown to be similar to the reference strain B/Victoria/2/87.

* Present address: WHO European Regional Office, Copenhagen, Denmark.

Less frequently isolated were viruses similar to a minor antigenic variant, B/USSR/2/87, which was isolated in December 1987 in Moscow [2, 3].

During the 1988–9 influenza season, significant numbers of influenza type B virus isolations were made in the United States, but were isolated less frequently elsewhere. The majority of isolates from the epidemic in the United States were antigenically closely related to B/Victoria/2/87. During that season, viruses similar to a distinct antigenic variant of influenza B, B/Yamagata/16/88, were isolated in several Asian countries where they co-circulated with B/Victoria/2/87-like viruses [4]. Sequence analyses of the HA1 domains of the haemagglutinin (HA) genes of these viruses have demonstrated that two distinct evolutionary lineages of influenza B have co-existed since at least 1983, and that currently circulating strains are related antigenically and genetically to either B/Victoria/2/87 or B/Yamagata/16/88 [5, 6].

Antigenic drift of influenza type B virus is thought to occur in a manner similar to that of type A virus, by the accumulation of point mutations in the HA1 domain of the HA gene as a result of immune pressure [7–12]. Here we describe the antigenic characterization and sequence analysis of the HA of influenza type B viruses isolated in Russia during a period of relatively low influenza B activity (1987–8) and a period of relatively high influenza B activity (1990–1).

Influenza B viruses included in this study were: B/USSR/2/87 (Accession Number M58413), B/Moscow/1/90, B/Moscow/2/90 (Accession Number M76983), and B/Novgorod/21/91 (Accession Number M76984). Table 1 lists the viruses examined in this study, and their dates of collection. All isolates were passaged 4–5 times in 10- to 11-day-old embryonated hens' eggs prior to analysis.

The antigenic characteristics of these viruses were determined using a previously described microneutralization assay with post-infection ferret serum [13]. Similar results were obtained using haemagglutination inhibition assays (data not shown). Post-infection ferret serum to B/Beijing/1/87, a virus that is antigenically and genetically similar to B/Victoria/2/87 [5], was used in the microneutralization test because it produced serum with a higher homologous titre than the B/Victoria/2/87 virus. Since the majority of viruses on the B/Yamagata/16/88 lineage from the 1990–1 season were closely related antigenically to the variant B/Hong Kong/22/89, ferret antiserum to the reference strain B/Hong Kong/22/89 was also included [14]. The reference viruses used in this assay were B/Beijing/1/87, B/Victoria/2/87, B/Yamagata/16/88 [5], and B/Hong Kong/22/89 [15]. Post-infection ferret sera clearly differentiated the recent B/Victoria/2/87-like viruses from the B/Yamagata/16/88-like viruses in microneutralization tests (Table 1). Both B/Moscow/1/90 and B/Moscow/2/90 were characterized as B/Yamagata/16/88-like because each displayed a high neutralization titre to the B/Yamagata/16/88 ferret antiserum. Both B/USSR/2/87 and B/Novgorod/21/91 were characterized as B/Victoria/2/87-like because they reacted with the B/Beijing/1/87 ferret antiserum but not with the B/Yamagata/16/88 ferret antiserum.

To further characterize B/USSR/2/87, B/Novgorod/21/91 and B/Moscow/2/90, the nucleotide and deduced amino acid sequences of the HA1 domains of the HA genes of these viruses were determined from purified viral RNA as described previously [5]. The nucleotide changes in the HA1 genes of the isolates from

Table 1. Antigenic analysis of influenza B viruses isolated in Russia, 1987-91

Viruses	Date of collection	Neutralization titre* with post-infection ferret antiserum to reference viruses		
		BJ/87	YM/88	HK/89
Reference				
Beijing/1/87 (BJ/87)	3/87	640	10	30
Victoria/2/87	3/87	160	10	10
Yamagata/16/88 (YM/88)	4/88	80	> 2560	320
Hong Kong/22/89 (HK/89)	11/89	40	640	640
Victoria/2/87-like				
USSR/2/87	12/87	80	10	10
Novgorod/21/91	2/91	320	10	10
Yamagata/16/88-like				
Moscow/1/90	12/90	80	> 2560	160
Moscow/2/90	12/90	40	> 2560	80

* Neutralization titres are reported as the reciprocal of the highest serum dilution that inhibited virus growth. Homologous reactions are in bold type.

Russia compared to the reference strains B/Victoria/2/87, B/Beijing/1/87, and B/Yamagata/16/88 are shown in Figure 1. Figure 2 shows the deduced amino acid sequences for the HA1-encoding regions of the isolates from Russia compared to the sequences of either B/Victoria/2/87 or B/Yamagata/16/88.

The two reference viruses, B/Victoria/2/87 and B/Yamagata/16/88, differ from each other by 24 amino acids and 65 nucleotides. Recent Russian viruses from each lineage (B/Novgorod/21/91 and Moscow/2/90) differ from each other by 29 amino acids and 81 nucleotides.

Of the B/Victoria/2/87-like viruses, B/USSR/2/87 differs from B/Victoria/2/87 by 4 amino acids and 14 nucleotides, whereas B/Novgorod/21/91 differs from B/Victoria/2/87 by 8 amino acids and 19 nucleotides. A comparison at the amino acid level failed to identify any unique amino acid changes in B/USSR/2/87 that are shared with the HA1 of previously sequenced viruses of the B/Victoria/2/87 lineage (Fig. 2). The HA of the B/Novgorod/21/91 isolate shared amino acid changes that were observed in B/Victoria/2/87-like viruses that circulated during 1989-90 at positions 73 (methionine to threonine) and 137 (valine to isoleucine). In addition, amino acid changes at positions 129 (threonine to lysine) and 172 (proline to serine) (Fig. 2) had also been observed in an isolate from 1989, B/Victoria/19/89 [15]. Isolates with these amino acid changes were also observed in Finland during the end of the 1989-90 season [16], in Australia and Czechoslovakia during the 1990-1 influenza season, and in China during the 1991-2 and 1992-3 influenza seasons (data not shown), suggesting that similar viruses have circulated widely.

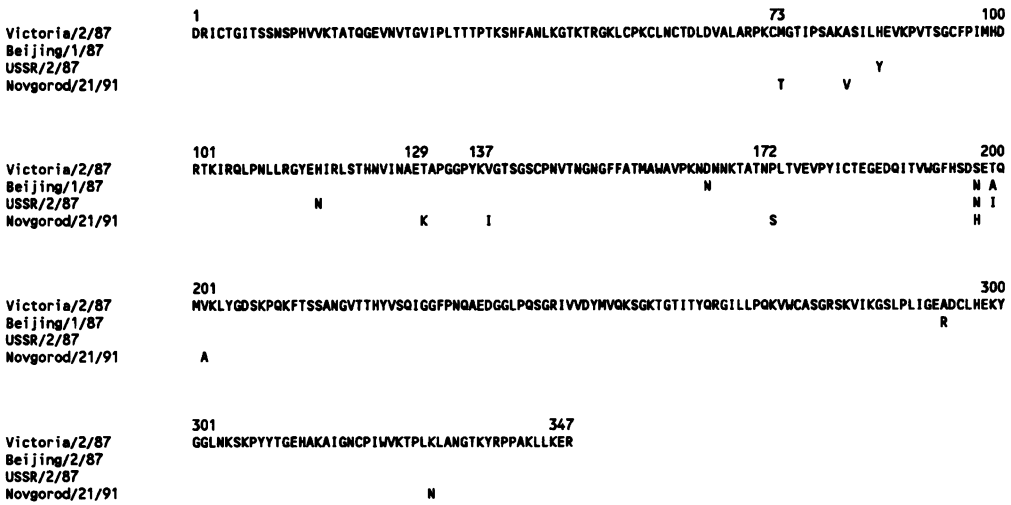
B/Moscow/2/90 differs from B/Yamagata/16/88 by 8 amino acids and 16 nucleotides, and is most closely related at the amino acid level to B/Victoria/103/89, which was isolated during the 1989-90 influenza season [15]. B/Moscow/2/90 shared a unique amino acid change with this virus at position 129 (arginine to glycine) and differed from it by only three amino acids (Fig. 2).

1ATGAGGCAATAATTGACTACTCATG57TGTAGCTAATGCTCACTTGGGATAGACTGCTCAAACTGCCCCACTGCGCATCGGATCTGACTTCCAACTGCTTCAAGG66GAGTGC				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
151	AMTGTGACTG57GATACCACCTGCACACACCAACCMAATCTCAATTTTCCAAATCTCAAGGCAACAAAACAGGGAAACTATGCCCAAGTGTTCACAAGTGTCAACTGCGACAGATCTGGAAGTGGCTTGGCGAGAACCAAGTSCATG565				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
301	ACCATACCTTGGCCAAAAGCTCAATACCTCATGAGTGCACCACTGTTACACTCTGG67GTTCTTCTATATGSCAGGAGACAATAATAGACAGCTACCCCAATCTTCTCACAGAGATACGAACATAATCAG57TATACACCCATACG7T				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
451	ATCAACGCGAAGAACGCACCCAGGAGACCTTACAAAAGTTGGAACTTGCCTTAGCTTGCCTTGCCTTACCATTTG9AAACGGATCTTGGGCTGTGCCAACAATGGCTTGG66GTTCCGCAACAATGGCTTGGGCTGTCCGCAACAAAACAGGCAACAAATCCATTACCA				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
601	GTAGAGTACCATTCATTTGTACAGAGGAGAGAGAGACCAATTTACTGTTTTGG66GTTCCACTCTGTATAGCAGAACCCAAATG57AAAACCTCTATG56AGACTCAAGGCTCAGAGAGTTCACCTCATCTGCGCAATG56AGTGG56GAGTGC				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
751	GTTTCACAGATTG57GCTTCCCAATCCAGCAGAGAGGAGG66CTACCACAGAGG66GTAGAAATGTTTTGTGATTCAGTTG57TTCAGAAATCTGGAAACAGAGAGAGTACTTACCAGAGG66GTTATTTATGGCTCAAAAAGT5756				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
901	TECCCAAGTGGCAGAGCAAGTAAATAAAG56TCTTGCCTTTAATG57TGAAGCAGATTTG57TCCACAGAAATAG57G57TAACAAAGCAGGCTTACTTACACAGGAGGAGCAATG56CAAAGCCATAG56AAATTC56CAATAT56				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					
1051	GTGAAACACCTTGA6CTG56CAATG56AACAAATATAGACTCTGCAACCTATTAAG56AAG56				
Victoria/2/87					
Beijing/1/87					
USSR/2/87					
Kovgorod/21/91					
Yamagata/16/88					
Moscow/2/90					

Fig. 1. Nucleotide sequence comparison of the HA1 coding region of influenza B isolates from Russia. Sequences begin with the HA1 initiation codon at base 34 and end at the HA1–HA2 cleavage site at base 1119. B/Victoria/2/87, B/Beijing/1/87 and B/Yamagata/16/88 (5) are used as reference sequences. Dashes (-) indicate nucleotide deletions.

The heterogeneity that is observed in the deduced amino acid sequence at positions 197 and 199 (Fig. 2) in all three of the isolates examined has been reported to be associated with influenza B virus propagation in embryonated hens' eggs. Influenza B viruses passaged in mammalian cells or sequenced directly from

A



B

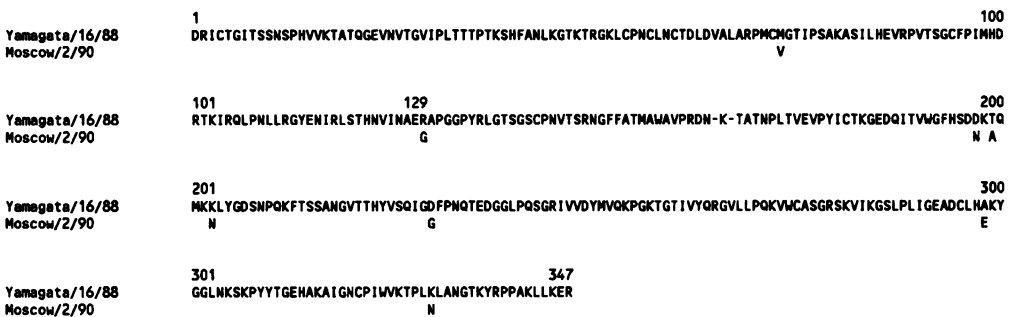


Fig. 2. Deduced amino acid sequence comparison of the HA1 coding region of (A) B/Victoria/2/87-like and (B) B/Yamagata/16/88-like influenza B isolates from Russia. Sequences begin with the first amino acid after the signal peptide cleavage and end at the HA1-HA2 cleavage site at residue 347. B/Victoria/2/87, B/Beijing/1/87 and B/Yamagata/16/88 [5] are used as the reference sequences. A dash (-) indicates an amino acid deletion.

clinical specimens are reported to possess a potential glycosylation site at amino acids 197-199 whereas viruses passaged in eggs often lose this site [15, 17-19]. These host cell-associated changes may result in minor antigenic variation when influenza B viruses are tested with polyclonal antiserum [15, 20].

Evolutionary relationships of the HA genes of influenza B viruses from Russia and of several previously characterized strains are shown in Figure 3. The co-circulation of multiple sublineages of B/Yamagata/16/88-like viruses has recently been described [15]. In this study, these sublineages are represented by B/Yamagata/16/88, B/Hong Kong/22/89 and B/Victoria/103/89. The isolate B/Moscow/2/90 is most closely related to the sublineage represented by B/Victoria/103/89.

Among the B/Victoria/2/87-like viruses, B/USSR/2/87 is most closely related to B/Texas/37/88 at the nucleotide level. Similar viruses circulated in the United

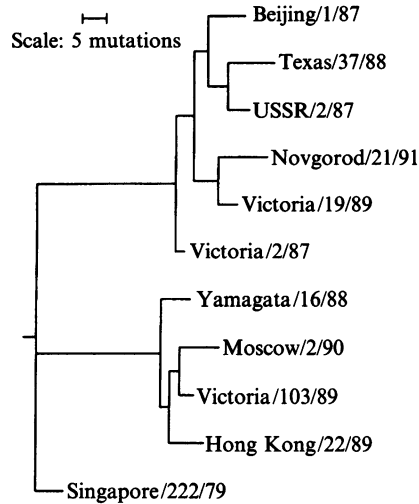


Fig. 3. Evolutionary relationships of the HA1 domains of the hemagglutinin genes of influenza B viruses, 1979–91. Sequence data were analysed by using version 7.0 of the sequence analysis software package of the University of Wisconsin Genetics Computer Group [21]. Version 3.4 of the Phylogeny Inference Package (PHYLIP [22]) with the DNAML (maximum likelihood algorithm) program was used to estimate phylogenies from the nucleotide sequences; the phenogram was plotted using the Drawgram program. Both software packages were run on a VAX computer (Digital Equipment Corporation). Sequences from previous reports are B/Victoria/2/87, B/Beijing/1/87, B/Yamagata/16/88, B/Texas/37/88 [5], B/Hong Kong/22/89, B/Victoria/19/89, B/Victoria/103/89 [15] and the root virus B/Singapore/222/79 [9].

States during the 1988–9 epidemic [5] and in Finland at the beginning of the 1989–90 influenza season [16]. The isolate B/Novgorod/21/91 is most closely related at the nucleotide level to B/Victoria/19/89, a relationship that was also suggested by comparison of the amino acid sequences.

This work will aid in evaluation of the results of vaccine trials conducted in Novgorod, Russia, where a mass vaccination campaign using both live and inactivated vaccines was conducted during the 1990–1 influenza season. Outbreaks of type B influenza occurred in Russia, including the city of Novgorod, during the study. Co-circulation of both B/Victoria/2/87-like and B/Yamagata/16/88-like viruses in Russia during the 1990–1 influenza season has previously been reported [23]. In this study we have characterized viruses isolated in Russia from each lineage during the 1990–1 influenza season and shown them to be genetically related to previously described viruses isolated during the 1989–90 season. In addition, a reported antigenic variant (B/USSR/2/87) from the 1987–8 season was examined and found to be genetically related to viruses that circulated in the United States during the epidemic season of 1988–9. Analysis of nucleotide sequences has proved to be much more sensitive than antigenic analysis for describing the evolutionary patterns of influenza B viruses [5, 15]. The results presented here confirm the wide distribution of genetic variants of influenza B that were initially identified in the United States [5] and Australia [15], and subsequently in Finland [16], Czechoslovakia and China (unpublished results).

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