Genetic characterization of the hemagglutinin gene of influenza B virus which predominated in the 1985/86 Canadian influenza season

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OBJECTIVE: To characterize the hemagglutinin (HA) gene of B/Canada/3/85, a prototype strain of influenza B virus variants that emerged in the 1984/85 influenza season and predominated in the 1985/86 season in Canada.
DESIGN: Sequencing and comparison of the HA genes of B/Canada/3/85 and the vaccine strains for the 1985/86 season, B/USSR/100/83, and for the 1986/87 season, B/Ann Arbor/1/86.
RESULTS: B/Canada/3/85 was similar to B/Ann Arbor/1/86 and significantly different from B/USSR/100/83. Phylogenetic analysis of the HA1-coding sequences indicated that B/Canada/3/85 and several other 1985 strains isolated in distant parts of the world were very closely related and were early variants representing the emergence of a new lineage, the B/Victoria/2/87 lineage. B/Canada/3/85 differed from B/USSR/100/83 in nucleotide sequence by 3.44% and in amino acid sequence by 3.33%. There was also an insertion of two amino acids in the HA1 region of B/Canada/3/85.
CONCLUSIONS: B/Canada/3/85 was one of the herald strains for the 1985/1986 influenza B epidemic. The amino acid mutations and the two-codon insertion together may account for the observed antigenic changes in the HA of the influenza B variants.

Key Words: *Antigenic drift, Evolution, Hemagglutinin, Influenza B virus*

Caractérisation génétique du gène de hémaglutinine du virus grippal de type B ayant predominé de la saison 1985/86 au Canada

OBJECTIF : Caractériser le gène de l'hémmaglutinine d'une souche prototype pour des variants du virus grippal de type B, B/Canada/3/85 ayant émergé de la saison de grippe 1984/85 et prédominé lors de la saison 1985/86 au Canada.
DESIGN : Séquençage et comparaison du gène HA de B/Canada/3/85 B à ceux des souches vaccinales de la saison

voir page suivante

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1985/86, B/URSS/100/83 et de la saison 1986/87, B/Ann/Arbor/1/86.
RÉSULTATS : B/Canada/3/85 est semblable à l'B/Ann Arbor/1/86 mais diffère de façon significative de B/URSS/100 /83. L'analyse phylogénique des séquences codant HA1 indique que B/Canada/3/85 et quelques autres souches apparues et isolées dans diverses régions du monde en 1985, sont trés apparentées et représentent les premiers variants d'une nouvelle lignée, la lignée B/Victoria/2/87. La séquence du gène HA de B/Canada/3/85 diverge de celle B/URSS/100/83 à 3.44% et celle de ces acides aminés B 3.33%. Une insertion de deux acides aminés est aussi présente dans la région HA1 de B/Canada/3/85.
CONCLUSIONS : B/Canada/3/85 fût une des souches précurseurs de l'épidémie de grippe de type B en 1985/86. Les mutations d'acides aminés et l'insertion de deux codons pourraient être la cause des changements antigéniques observés chez la protéine HA des variants du virus grippal de type B.

Influenza B virus is a member of the *Orthomyxoviridae*, which also includes influenza A and C viruses. Influenza A , B or C viruses have eight or seven negative sense RNA genome segments (1). The RNA polymerases encoded by these viruses lack proofreading activity, which gives rise to a high mutation rate and thus, at least in part, their characteristic antigenic drift. Because of the segmented nature of the genome, influenza A viruses also exhibit genome segment reassortment. This reassortment results in dramatic changes in antigenicity (antigenic shift), which may lead to influenza pandemics (2). However, a pandemic caused by antigenic shift has not been reported for influenza B and C viruses, probably because of few virus subtypes and animal reservoirs, although reassortment has been documented for both types (3,4). Research on the origin of reassorting genome segments for influenza A virus has indicated that novel genes probably came from avian influenza strains (5,6). By comparison, antigenic drift and the features of such drift, especially for influenza B and C viruses, are less clearly understood. Different lineages of influenza B viruses, B/Victoria/2/87-like viruses and B/Yamagata/16/88-like viruses have been cocirculating worldwide (7), although the latter has accounted for the majority of isolates in most countries since the 1990/91 season (8,9).

Although no influenza B virus pandemic has been reported, the disease burden caused by this virus is significant. In Canada, influenza B accounted for 33% of laboratory-confirmed influenza infections between 1980/81 and 1992/93 (unpublished). Scrutiny of the data showed that in the 1985/86 season there were 759 laboratory-confirmed influenza B infections in Canada, close to the number of laboratory-confirmed influenza A infections in the same period. None of the 759 influenza B isolates characterized was antigenically close to the vaccine strain for that season, B/USSR/100/83 (10).

To characterize further the hemagglutinin (HA) gene of the influenza B virus circulating during the 1985/86 season and to investigate the evolutionary biology of the virus, the HA gene of the prototype strain, B/Canada/3/85, was sequenced and compared with B/USSR/100/83 and another vaccine strain, B/Ann Arbor/1/86.

MATERIALS AND METHODS

The influenza virus B/Canada/3/85 was isolated in Winnipeg, Manitoba and submitted to Laboratory Centre for Disease Control (LCDC), Ottawa, Ontario in February 1985 and then passaged three times in embryonated hen eggs (10). The vaccine strains, B/USSR/100/83 and B/Ann Arbor/1/86, were received from the World Health Organization Collaborating Center for Influenza at Centers for Disease Control and Prevention, Atlanta, Georgia. Strains were antigenically characterized by the hemagglutination inhibition (HI) assay (11).

Viral RNA was extracted using the Trizol reagent and protocol from Gibco BRL, a method using guanidine isothiocyanate and phenol to dissociate RNA from proteins and to inhibit RNase activity (12). The RNA pellet was resuspended in RNase-free water and converted into cDNA in standard reverse transcription reactions (13) containing M-MLV reverse trancriptase (Gibco BRL) and primers specific for the conserved termini of the hemagglutinin gene of influenza B viruses. The cDNA was amplified by the polymerase chain reaction (PCR) in a Perkin Elmer 9600 thermocycler using Taq DNA polymerase (Boerhinger Mannheim) and the specific primers.

PCR amplicons were separated on 1% low melting point agarose gel (BRL, Maryland) and purified using the wizard PCR prep kit (Promega, Wisconsin). The amplicon was sequenced by using successive primers (about 200 nucleotides apart) and the ABI prism kit (Perkins-Elmer) according to the manufacturer's specifications. Twenty-five cycles of PCR at 96°C for 10 s, 50°C for 5 s and 60°C for 4 mins were performed. Sequencing reaction products were purified using Centrisep gel permeation columns (Princeton Separation, New Jersey) and dried under vacuum. Samples were processed using an

TABLE 1
Cross reactivity between the hemagglutinins of three influenza B viruses

	Hemagglutination inhibition titre of fowl antisera raised to antigens		
Antigens	**B/USSR/100/83**	**B/Canada/3/85**	**B/Ann Arbor/1/86**
B/USSR/100/83	1280	80	40
B/Canada/3/85	160	320	160
B/Ann Arbor/1/86	160	320	320

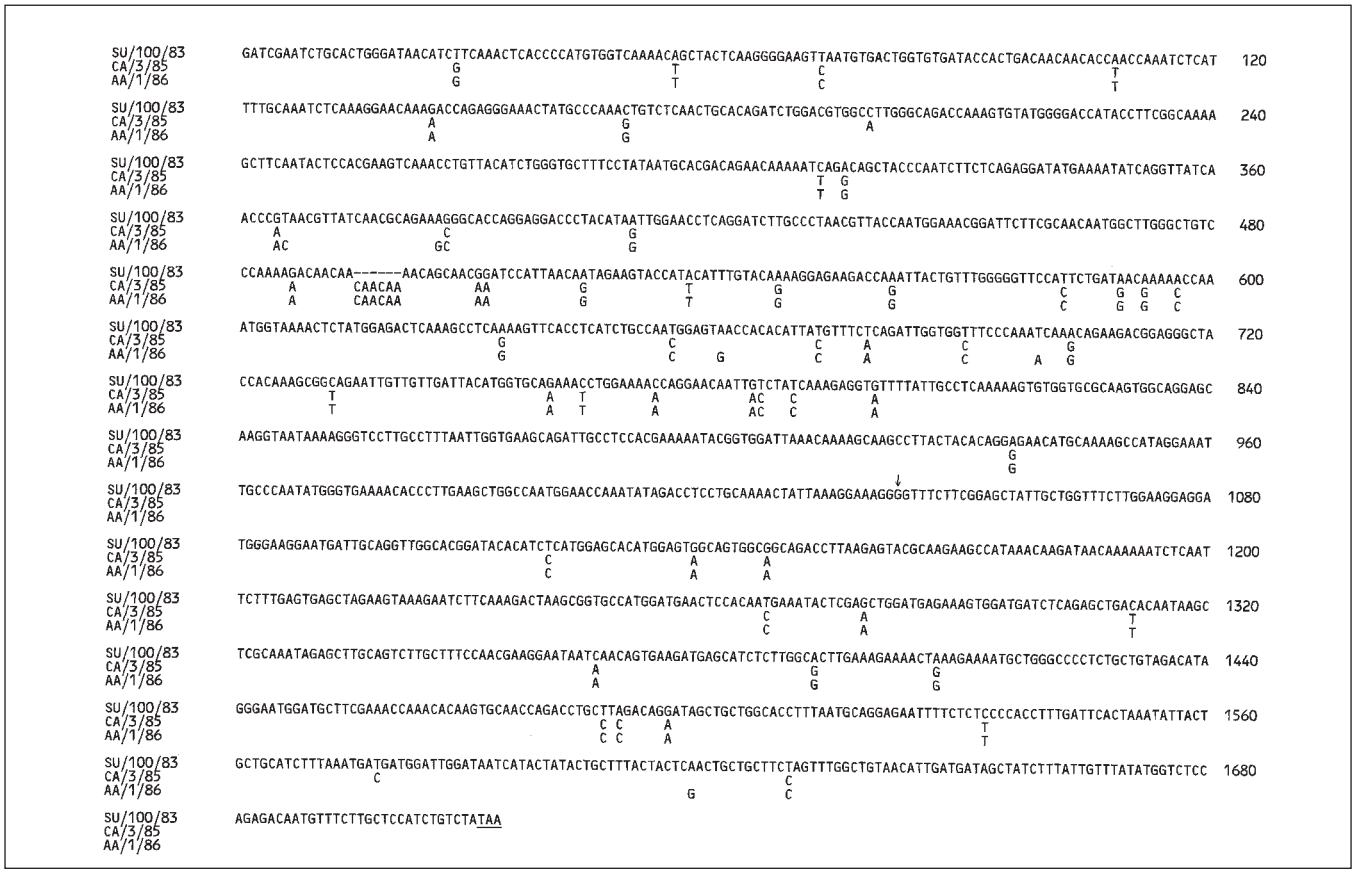

Figure 1) *Comparison of nucleotide sequences encoding the HA1 and HA2 domains. Sequence alignment was performed by using the CLUSTAL W program (J Felsenstein, Department of Genetics, University of Washington, Seattle, Washington). The start of HA2 domain is marked with an arrow and the translation termination codon is underlined. Hyphens indicate lack or absence of nucleotides and positions are created for the purpose of sequence alignment. AA/1/86 B/Ann Arbor/1/86; CA/3/85 B/Canada/3/85; SU/100/83 B/USSR/100/83*

automatic sequencer (Perkins-Elmer) in the core facility of LCDC. All sequence data are results of at least two independent determinations.

Sequences were assembled and analyzed using the program PCGene (Intelligenetics, California) and CLUSTAL W (14). Phylogenetic trees were drawn with programs DRAWTREE and DRAWGRAM in the PHYLIP package (J Felsenstein, Department of Genetics, University of Washington, Seattle, Washington).

RESULTS

Comparison of B/Canada/3/85 with B/USSR/100/83 and B/Ann Arbor/1/86: The HI assay (11) was used to analyze the antigenicity of the three virus strains (Table 1). Antiserum to B/USSR/100/83 was eightfold less reactive with B/Canada/3/85 and B/Ann Arbor/1/86 than with the homologous strain. Reactivities of all antisera to B/Canada/3/85 and B/Ann Arbor/1/86 were essentially the same. Nucleotide sequences of the HA gene and the deduced amino acid sequences were obtained for the three influenza B strains. The nucleotide sequence of the HA gene of B/USSR/100/83 (SU/100/83) and that of the HA1 region of B/Ann Arbor/1/86 (AA/1/86) matched those available from Genbank (Maryland) (15), except for two synonymous substitutions in B/USSR/100/83. The last three nucleotides of the HA1 and the whole region of the HA2 of AA/1/86, which were not available from GenBank, were derived from the work reported in this paper. Comparison of nucleotide sequences is presented in Figure 1. The nucleotide and amino acid sequences of B/Canada/3/85 and B/Ann Arbor/1/86 differ only by 0.41% (seven of 1713) and 0.18% (one of 570), respectively; whereas those of B/Canada/3/85 and B/USSR/100/83 differ by 3.44% (59 of 1713) and 3.33% (19 of 570), respectively. The genetic differences are consistent with, but not necessarily directly related to, the identified antigenic diversity. The fact that B/Canada/3/85 was passaged three times in eggs should not have had a major impact on sequence differences. It is well established that in most instances the egg-adapted virus has a single base substitution when compared with either the corresponding cell culture isolate or virus in the original clinical specimens (16). The majority of the nucleotide and amino acid substitutions among the three strains cluster within the HA1 region. Between B/Canada/3/85 and B/USSR/100/83, 44 of 59 nucleotide substitutions and all 19 amino acid substitutions occurred in the HA1 region, including a two-codon insertion.

B/Canada/3/85 HA sublineage: To determine the position of B/Canada/3/85 in the evolutionary lineage of influenza B viruses, its HA1-coding region was aligned with those of 16 other influenza B viruses obtained from GenBank and from this work. A phylogenic tree was constructed with the CLUSTAL W program, which uses the neighbour-joining method

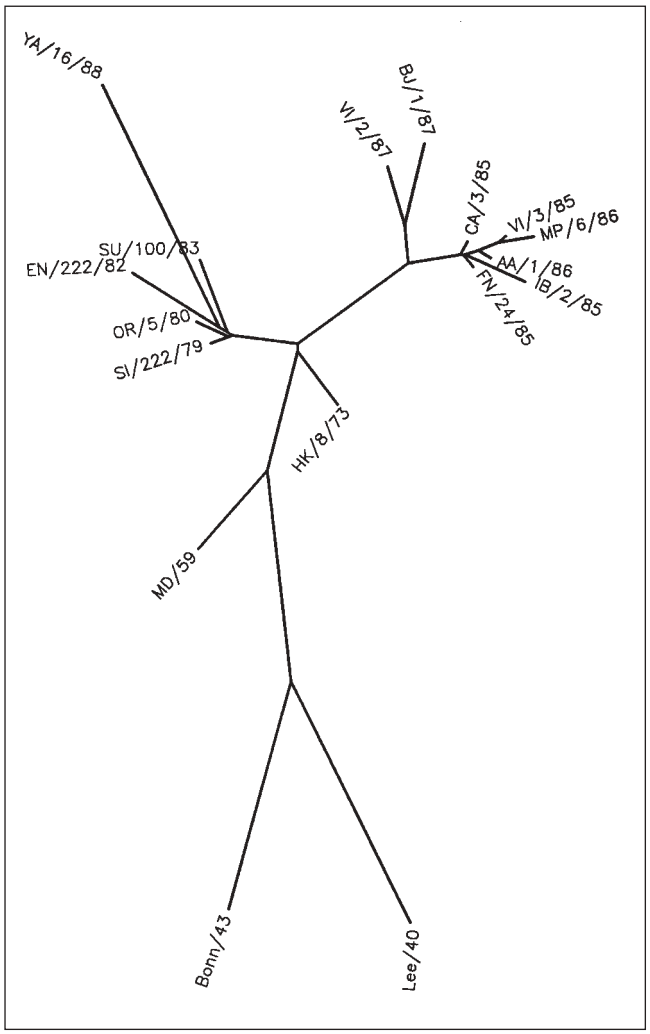

Figure 2) *A phylogenetic tree of the HA1-coding region of 17 influenza B strains isolated between 1940 and 1988. Sequence data were extracted from GenBank and analyzed using CLUSTAL W (14). The tree shown here was drawn using the program DRAWTREE in the Phylip package (J Felsenstein, Department of Genetics, University of Washington, Seattle, Washington). AA/1/86 B/Ann Arbor/1/86 (GenBank accession number U70385); BJ/1/87 B/Beijing/1/87 (X53098); Bonn/43 B/Bonn/43 (X13550); CA/3/85 B/Canada/3/85 (U70384); EN/222/82 B/England/222/82 (M18384); FN/24/85 B/Finland/24/85 (L19646); HK/8/73 B/Hong Kong/8/73 (M10298); IB/2/85 B/Ibaraki/2/85 (M36107); Lee/40 B/Lee/40 (K00423); MD/59 B/Maryland/59 (K00424); MP/6/86 B/Memphis/6/86 (X13551); OR/5/80 B/Oregon/5/80 (K02713); SI/222/79 B/Singapore/222/79 (K00038); SU/100/83 B/USSR/100/83 (X13552); VI/3/85 B/Victoria/3/85 (X13553); VI/2/87 B/Victoria/2/87 (M58428); YA/16/88 B/Yamagata/16/88 (M58419)*

(Figure 2) (14). B/Canada/3/85 together with other 1985 and 1986 isolates form a sublineage that was close to the B/Victoria/2/87 lineage but distant from the B/Yamagata/16/88 lineage. However, B/USSR/100/83 was closer to B/Yamagata/16/88. Therefore, B/Canada/3/85 and other 1985 strains actually represented the emergence of a new lineage which was prominent between 1985 and 1989 (7, unpublished data). One difference between B/Canada/3/85 and B/USSR/100/83 was a six-nucleotide insertion after nucleotide 494 (measuring from the start of the HA1-coding sequence) (Figure 1); B/Yamagata/16/88 does not have this insertion (7).

DISCUSSION

A unique challenge in the control of influenza is continually emerging new virus variants. The surveillance strategy has been to screen isolates worldwide and monitor emerging virus strains with antigenically different HA in order to predict the epidemic strains for the next season; these presumed epidemic strains are then included in the new influenza vaccines. This strategy has provided essential information for effective vaccine formulation changes. However, the predicted epidemic strains and vaccine component strains may not always match the predominating strains for the new season because of the failure to identify herald strains or identify the herald strains early enough. There is also the problem of determining which competing variant strains identified in different regions are most likely to become the epidemic strain continent-wide or across the globe. This was true for influenza B virus as illustrated by the Canadian 1985/86 influenza season when 759 laboratory confirmations of influenza B/Canada/3/85-like strains were documented, none of which were antigenically similar to that season's vaccine strain, B/USSR/100/83. The 1985/86 season had one of the highest influenza B activities in recent Canadian history (10). It was followed by a season in which the vaccine contained a close relative, B/Ann Arbor/1/86, but there was little influenza B activity – only 10 laboratory confirmations of influenza B in Canada (unpublished data). Several influenza B variants that belong to the same sublineage were isolated in distant parts of the world in 1985: Canada (B/Canada/3/85), Finland (B/Finland/24/85), Japan (B/Ibaraki/2/85) and Australia (B/Victoria/3/85). Either the variants emerged in different places, or more likely, variants that had previously deviated from the original dominant lineage developed and spread before becoming the dominant influenza B strain in Canada in 1985/86. Nevertheless, the vaccine component for the 1985/86 season was still B/USSR/100/83, and both Canada and United States experienced very strong influenza B epidemic activity (10,17). This experience stressed the importance of effective surveillance for antigenic drift of influenza B infections and illustrated that when strains emerge to produce early activity within a country or continent, an effective vaccine may not be available to protect against the first wave of major activity.

In retrospect, B/Canada/3/85, the herald strain for the 1985/1986 season in Canada, was serologically distinct from B/USSR/100/83 as seen by an eightfold difference in their HI titres and a 3.33% difference at the amino acid level. There was also an insertion mutation of two amino acids in B/Canada/3/85, compared with B/USSR/100/83. These facts should be considered important indicators for future influenza B surveillance, although other factors such as immunity level of the population and strain transmissibility are also important considerations. With the availability of facilities for genetic characterization of influenza variants at LCDC, future isolates that

have significantly different antigenicity or are hard to cultivate will be subject to genetic analysis. Accumulation of information about genotypic sequence variations and comparison with corresponding antigenic heterogeneity and other epidemic data may allow the assessment of the effect of specific sequence changes on antigenic expression and possibly on epidemic pattern. Such information may help in the identification of herald strains for vaccine strain selection.

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