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Generation of the influenza B viruses with improved growth phenotype by substitution of specific amino acids of hemagglutinin

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Abstract

Variability in growth characteristics of influenza B viruses remains a serious limitation in the manufacture of inactivated influenza vaccines. Currently, serial passage in eggs is the strategy used in most instances for selection of high growth virus variants. In previous studies we found that adaptation of the strain B/Victoria/504/2000 to high growth in eggs was associated with changes only in hemagglutinin (HA). The high growth phenotype was associated with acquisition of either two (R162M and D196Y) or three (G141E, R162M and D196Y) amino acid (AA) substitutions, predicted to be near the receptor-binding domain of HA. In the present study we analyzed, using reverse genetics, the contribution to virus growth of each of these AA substitutions and determined their effect on antigenic properties. We found that G141E and R162M were most favorable for virus growth; however, only R162M could improve virus growth without antigenic alteration. Substitution D196Y had least effect on virus growth but substantially altered antigenic properties. Additional virus variants with AA substitutions at positions 126, 129, 137 and 141 were generated and characterized. The AA changes advantageous for growth of B/Victoria/504/2000 were also tested in the context of the HA of the B/Beijing/184/93, a virus with stable low-growth phenotype. All of the tested AA substitutions improved the replicative capabilities of the corresponding viruses, but only N126D and K129E had no effect on antigenicity. The results of our studies demonstrate that introduction of specific AA substitutions into viral HA can improve viral replicative efficiency while preserving the original antigenic properties.
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Keywords: Influenza B virus; Growth characteristics; Antigenic properties; Reverse genetics

Introduction

Influenza A and B viruses cause seasonal epidemics associated with morbidity, mortality and economic loss (Cox and Subbarao, 1999; Simonsen et al., 2000; Thompson et al., 2004). Vaccination remains the primary means of influenza prophylaxis and control. Currently licensed inactivated influenza vaccines are trivalent formulations that contain antigenic components of two subtypes of influenza A virus (H1N1 and H3N2) and one influenza B virus. The high rate of mutation and antigenic drift of influenza viruses require continuous epidemiological surveillance and annual reconsideration of influenza vaccine composition in order to assure that the vaccine virus strains are antigenically representative of natural field isolates. In case of selection of new vaccine virus strains, they have to be adapted to provide high yield in embryonated chicken eggs (eggs), the primary substrate for vaccine production. Influenza virus strains vary in their capability to replicate in eggs and low virus yield in eggs remains a serious problem in the manufacture of influenza vaccines. Conventional genetic reassortment technology, which has been successfully used for influenza A viruses for decades, has not been as successful for influenza B viruses (Goodeve et al., 1985; Kilbourne, 1969; Robertson et al., 1992; Vodeiko et al., 2003). Even when all six of the genome segments encoding for viral proteins other than the

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hemagglutinin (HA) and neuraminidase (NA) are inherited from high growth strains, the resulting reassortant viruses rarely exhibit improved growth characteristics. This fact is complemented by the observation that adaptation of influenza B viruses to high growth in eggs may be associated with amino acid substitutions in viral HA (Gambaryan et al., 1999; Govorkova et al., 1999a, 1999b; Lugovtsev et al., 2005; Oxford et al., 1990; Robertson et al., 1985, 1990, 1993; Saito et al., 2004; Wagner et al., 2002; Williams and Robertson, 1993).

In previous studies of the strain B/Victoria/504/2000, we found that adaptation to high growth in eggs was associated only with AA substitutions in HA (Lugovtsev et al., 2005). Two independently isolated virus variants adapted by consecutive passage in eggs acquired either two AA substitutions (R162M and D196Y) or three AA substitutions (G141E, R162M and D196Y), respectively, predicted to be near the receptor-binding domain of HA. Both variants exhibited significantly increased virus yields in eggs compared to the B/Victoria/504/2000 reference virus from which they were derived, with the virus variant containing all three substitutions demonstrating the best growth characteristics. At the same time both variants showed the noticeable divergence from the original virus by antigenic properties. In the present study, we expand current knowledge of the contribution of those AA substitutions in HA to virus growth characteristics and other phenotypic properties. We used reverse genetics to generate a series of B/Victoria/504/2000 virus variants with the genome identical to the consensus sequence of the reference strain B/Victoria/504/2000, but differing between each other only by the presence or absence of the targeted AA substitutions in their HAs. In addition to the previously identified AA changes, a range of other AA substitutions, introduced in proximity to the receptor-binding domain of the HA (AA positions 126, 129, 137 and 141), were tested for their effect on viral phenotype. Finally, we demonstrated that the AA substitutions beneficial for growth capability of the strain B/Victoria/504/2000 had similar effect when introduced into the HA of the B/Beijing/184/93, a strain with stable low-growth phenotype.

Results

Effect of substitutions G141E, R162M and D196Y on viral growth characteristics

Eight B/Victoria/504/2000-like virus variants, distinguished only by AA substitutions at positions 141, 162 and 196 in their HAs, were generated and characterized (Table 1). As we found in our previous study (Lugovtsev et al., 2005), virus with all three AA substitutions (variant #2) demonstrated the best growth characteristics and the highest titer of hemagglutination (1:2048), and also produced large plaques in MDCK. However, viruses even with a single substitution G141E (variant #3) or R162M (variant #5) also exhibited a significant growth improvement in eggs and MDCK, and increased titers of hemagglutination (1:1024 and 1:512, respectively), forming medium-size plaques in MDCK. Substitution D196Y (variant #4) contributed least to replication, failed to increase the hemagglutination titer (1:128) and resulted in the formation of small plaques in MDCK. Although all double-mutant viruses (variants #6, #7 and #10) showed high growth phenotype, none of them had a yield in eggs significantly higher than single-mutant viruses having either G141E or R162M. Variant #1 (containing unmodified HA), like its natural counterpart, reference virus B/Victoria/504/2000 (data not shown), exhibited low-growth phenotype in eggs and MDCK.

Table 1
Phenotypic characteristics of the B/Victoria/504/2000 variants differing at AA positions 141, 162 and 196 of HA

Virus [a]	AA at position			Infectious titers, $\log_{10}$/0.1 ml ± SD [b]		HA titer [c]	Mean plaque size (mm) ± SD [b]
	141	162	196	EID_{50}	$TCID_{50}$		
Variant-1	G	R	D	7.4±0.1	6.7±0.2	128	0.9±0.4
Variant-2	E	M	Y	8.9±0.3	8.8±0.1	2048	2.2±0.3
Variant-3	E			8.4±0.3	8.7±0.1	1024	1.7±0.5
Variant-4			Y	7.8±0.1	7.2±0.2	128	0.5±0.2
Variant-5		M		8.3±0.1	8.2±0.2	512	1.5±0.4
Variant-6	E		Y	8.6±0.3	8.8±0.1	1024	1.8±0.6
Variant-7	E	M		8.6±0.3	8.6±0.4	1024	1.8±0.4
Variant-10		M	Y	8.6±0.3	8.4±0.4	1024	1.4±0.4

[a] All viruses were generated using reverse genetics. The nucleotide sequences of all eight genome segments of Variant-1 are identical to the corresponding consensus sequences of the natural reference strain B/Victoria/504/2000; other variants differ by AA substitutions introduced into their HAs.
[b] Data represent the mean (±standard deviations) of three independent experiments. $P<0.02$ for all comparisons versus Variant-1.
[c] Titers are expressed as the reciprocal of the highest virus dilution producing hemagglutination (see Materials and methods for details).

Effect of substitutions G141E, R162M and D196Y on viral antigenic properties

The antigenic properties of the generated viruses were tested by hemagglutination inhibition assay (HI) (Table 2). Ferret sera against natural reference strain B/Victoria/504/2000 and seven plasmid-derived virus variants were used. All viruses with modified HAs were evaluated in comparison with variant #1, which had the HA of the reference virus. Among the viruses with a single AA substitution, variant #4 (D196Y) showed strongest antigenic divergence from non-mutated virus (variant #1). On the other hand, the substitution R162M had minimal effect on antigenic properties, and the resultant virus (variant #5) was the only one antigenically indistinguishable from the reference variant #1 (≤2-fold difference of the corresponding HI titers). Variant #3 (G141E) demonstrated moderate antigenic divergence.

All virus variants having more than one AA substitution were antigenically distinguishable from the reference variant #1 containing unmodified HA (≥4-fold difference of the corresponding HI titers). The most divergent antigenic patterns were observed for viruses containing AA substitution D196Y, especially in combination with G141E (variants #6 and #2). Variant #7 (G141E and R162M) showed moderate antigenic deviation, similar to that of variant #3 containing single substitution