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PREPARATION OF INFLUENZA B VIRUS RECOMBINANT STRAINS

R. YA. PODCHERNYAEVA, M. V. SHCHIPANOVA, V. S. ELKIN, E. I. MELNICHENKO

D. I. Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences, 123 098 Moscow, U.S.S.R.

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Summary. — The study of antigenic and biologic properties of influenza B epidemic viruses isolated in 1979 and 1983 and laboratory strain B/Lee/40 has revealed some differences in their biologic properties. The most marked changes have been found in the haemagglutinin (HA) and neuraminidase (NA) indicating that influenza B viruses underwent dramatic antigenic drifts during the period in question. The strains obtained by genetic recombination have inherited surface antigens of epidemic influenza B/Singapore/222/79 and B/USSR/100/83 viruses and preserved the HA thermolability inherent to these viruses. They have, however, acquired the marker of reproduction in chick embryos and the immunogenicity from the donor strain B/Lee/40. These recombinants can be, therefore, recommended as vaccine strain candidates.

Key words: influenza B virus; recombinants; biologic properties; haemagglutinin; neuraminidase; immunogenicity

Introduction

Over the last decade, 4 influenza B epidemics have been registered in the U.S.S.R. The latest of these were caused in 1980—1981 by viruses antigenically identical to the reference strain B/Singapore/222/79 (Yakhno *et al.*, 1982) and in 1983 by antigenically modified virus B/USSR/100/83 (Isachenko *et al.*, 1984).

The study of antigenic and biologic properties of epidemic influenza B viruses from 1980—1983 and of the prototype strain B/Lee/40 allowed us to select for recombination experiments the actively reproducing laboratory strain B/Lee/40 as donor and as recipients the reference strains recommended by WHO, namely B/Singapore/222/79 and B/USSR/100/83. We present the results of the investigation of antigenic and biologic properties of the parent and recombinant influenza B virus strains.

Materials and Methods

Viruses. Strains B/Lee/40, B/Singapore/222/79 and B/USSR/100/83 were from Virus Strains Collection of the D. I. Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences. Pathogenic strain B/Singapore/222/79 Pm^+ has been selected after 15 passages of the initial strain

through mouse lungs; the titre of the virus at which the death rate of animals reached 50% was 3.5 log.

Sera. For se ect on of recombinants high-titre immune rat serum against purified and concentrated B/Lee/40 virus has been used. For identification of recombinants immune rat sera against all three strains have been prepared.

Recombination experiments have been carried out in chick embryos using two live influenza viruses (either B/Lee/40 and B/Singapore/222/79 or B/Lee/40 and B/USSR/100/83) a t ε dose of 10^7 EID_{50}/0.2 ml per embryo. Antigenic properties have been identified by means of con ventional techniques, i.e. haemagglutination inhibition (HI) and neuraminidase inhibition (NI) test. For determination of the sensitivity to inhibitors normal guinea pig, rat and human sera have been used devoid of antibodies against influenza B viruses. Heat resistance of HA and infectivity were tested by heating at 56 °C and 61 °C for 30 min. The ability of viruses to reproduce in the allantoic cavity of chick embryos at different temperatures of incubation (28, 3 € and 40 °C) as well as pathogenicity for albino mice were assessed according to the previously described procedure (Podchernyaeva *et al.*, 1972). For toxicity studies 4 albino mice weighing 11—12 g and 2 guinea pigs weighing 250—300 g have been used. They were given 0.5 and 2.0 ml of virus-containing fluid, respectively, by intraperitoneal (i.p.) route. The observations were conducted for 7 days weighing the animals daily. Immunogenic properties of the recombinant and parent strains were tested in albino mice weighing 11—12 g after two i.p. immunizations with 0.3 ml of allantoic virus at 7-day intervals; on day 21 after second immunization, the antibody titres have been determined in HI using CO_2 treatment for inhibitor removal. To determine the degree of the protective effect, specially selected pathogenic variant of B/Singapore/222/79 Pm^+ strain has been used; the mice were intranasally infected with this virus at a dose of 100 LD_{50}/0.05 ml per mouse. The deaths were registered for 14 days and the survival rate was calculated.

Results

First series of recombination experiments was carried out between strains B/Lee/40 and the epidemic strain B/Singapore/222/79. After 5 passages with antiserum against B/Lee/40 followed by 3 passages at limiting dilutions the recombinations yielded 4 reassortant strains (R_1—R_4). Their HA and NA were those of the epidemic strain B/Singapore/222/79, the HA being thermolabile (Table 1). Alike to strain B/Singapore/222/79, the recombinants were sensitive to human serum inhibitors (at titres 1/10—1/40) and resistant to guinea pig and rat serum inhibitors. All recombinants reproduced in chick embryos to high titres (up to 7.0—8.0 log EID_{50}) at optimal incubation temperature (36 °C), but poorly (4.25—5.0 log EID_{50}) at low temperature (28 °C); they did not reproduce at 40 °C. The recombinants were non-toxic for guinea pigs and albino mice up on i.p. infection and weakly pathogenic (0.4—2.0 log LD_{50}) for mice after intranasal infection (Table 2). The study of immunogenic activity has shown that in sera of mice immunized with recombinants R_3 and R_4, antibodies are produced at the same titres (1/256) as after immunization with the donor strain B/Lee/40. Recombinant viruses had a higher protective activity (82—94%) as compared to parent strain B/Singapore/222/79 (72%). The highest immunogenicity was found in recombinant strain R_4 (Table 2).

The second recombination series was carried out between strain B/Lee/40 and reference epidemic virus B/USSR/100/83. Four passages with serum B/Lee/40 followed by 4 passages at limiting dilutions yielded 2 reassortants (R_5 and R_6) having the HA and NA of epidemic strain B/USSR/100/83 (Table 1). The recombinant HA was as thermolabile as that of parent strain

Table 1. Antigenic and biologic properties of parent and recombinant strains of influenza B virus

Strain	HI			NI			HA	Thermosensitivity 61 °C, 30 min		Sensitivity to inhibitors in sera		
	B/Lee/40	B/Sing/222/79	B/USSR/100/83	B/Lee/40	B/Sing/222/79	B/USSR/100/83		HA	EID	human	guinea pig	rat
B/Lee/40	1 280	20	20	160	40	20	256	128	+	—	—	—
B/Sing/222/79	20	1 280	640	80	320	80	128	—	—	20	—	—
R_1	20	1 280	640	80	320	80	128	—	—	40	—	—
R_2	20	1 280	640	40	320	80	128	—	—	10	—	—
R_3	20	1 280	640	80	320	80	64	—	—	40	—	—
R_4	20	1 280	640	40	320	80	128	—	—	20	—	—
B/USSR/100/83	10	2 560	5 120	20	80	320	32	—	—	—	—	—
R_5	80	2 560	5 120	20	80	320	64	—	—	—	—	—
R_6	80	2 560	5 120	20	80	320	64	—	—	—	—	—

Table 2. Properties of parent and recombinant influenza B virus strains

Strain	Infectivity (log EID_{50}) at temperatures (degrees centigrade):			Pathogenicity (log LD_{50})	Immunogenicity	
					Antibody titre	Survival rate (%)
	28	36	40			
B/Lee/40	4.0	8.0	—	0.71	256	12
B/Singapore/222/79	4.25	7.25	—	1.71	128	72
R_1	4.25	7.0	—	1.71	64	88
R_2	5.0	8.0	—	2.0	128	92
R_3	5.0	7.75	—	0.4	256	82
R_4	4.25	8.0	—	0.71	256	94
B/USSR/100/83	4.0	6.5	—	0.4	64	60
R_5	4.25	8.0	—	0.5	64	56
R_6	4.0	8.0	—	0.4	128	65

B/USSR/100/83, but their reproduction in chick embryos (log 8.0 EID_{50}) was alike to that of B/Lee/40 virus. Both recombinants were non-toxic and non-pathogenic for laboratory animals. One of them had a more marked immunogenicity (Table 2) than the another.

Discussion

Several investigators (Korchanova *et al.*, 1983; Molibog *et al.*, 1982; Chakraverty, 1972, 1973) comparing the antigenic properties of HA and NA of different strains of influenza B virus showed that they possessed both common and different antigenic components depending on the year of isolation. We have previously (Podchernyaeva *et al.*, 1986) studied the antigenic and biologic properties of 3 reference influenza B virus strains isolated in 1940, 1979 and 1983. These strains appeared to be biologically different in some respects, the differences in HA and NA being the most marked, indicating that influenza B virus underwent dramatic drift changes over these years. The study of some biologic properties indicate higher thermostability of HA and lower sensitivity to serum inhibitors of these viruses, as compared to influenza A viruses, especially of serotype H3N2. In addition, influenza B viruses reproduced poorly at low temperature (28 °C) and did not reproduce at 40 °C. The recombinant strains produced by genetic recombination have inherited surface antigens from the epidemic influenza viruses B/Singapore/ /222/79 and B/USSR/100/83 and preserved the HA thermostability characteristic of these epidemic strains. They have, however, acquired the marker of reproducibility in chick embryos from the donor strain B/Lee/40. The latter allows one to use these recombinants as candidates for production of vaccine preparations (Kilbourne, 1969; Polezhaev *et al.*, 1978; Andzhaparidze

and Bektimirov, 1978; Smorodintsev, 1983). We believe that for successful vaccination against influenza it is also important to investigate the immunogenic activity of recombinant viruses. Not only the titres of the recombinant-induced antibodies should be taken into account but also the degree of resistance to challenge with a pathogenic variant, in this case B/Singapore/222/79 Pm^+. Because of the dissimilarity of HA and NA in viruses B/Lee/40 and B/Singapore/222/79, immunization with strain B/Lee/40 provided only a slight (12%) protective effect against virus B/Singapore/222/79 Pm^+. The highest protective effect was observed after immunization with the homologous virus (72%) and virus B/USSR/100/83 (60%), i.e. after immunization with viruses with similar antigenic specificities of HA and NA. This can also explain the higher level of immunity of mice to pathogenic strain B/Singapore/222/79 Pm^+ in response to administration of recombinants antigenically similar to virus B/USSR/100/83. Unlike to recombinants R_1—R_4, the recombinants R_5 and R_6 differed in the degree of immunity induced. This can be accounted for the fact that protection has been tested only with respect to the pathogenic variant B/Singapore/222/79 Pm^+ which was antigenically related more to recombinants R_5 and R_6 than to R_1—R_4. We believe that alike to our experiments with influenza A viruses H1N1 and H3N2 (Shchipanova *et al.*, 1984) antigenically homologous pathogenic variants should be used to provide more reliable data of the level of immunity to influenza B viruses.

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