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EXPERIMENTAL BASIS OF ENHANCING THE IMMUNOGENICITY OF INFLUENZA B VIRUS BY GENETIC RECOMBINATION

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Summary. — Redistribution of the immunogenicity marker in the course of genetic recombination of influenza B virus was studied in animal experiments on virus strains differing in their ability to induce antibody formation following a single peroral or intraperitoneal immunization. Immunogenicity of influenza virus could be enhanced by recombination of a strain possessing a low activity with a highly immunogenic homotypic strain. Efficiency of the transfer of the immunogenicity marker depended on the properties of viruses used for recombination. Strains at a low passage level proved to be more prospective "donors of immunogenicity" than hyperattenuated thermosensitive viruses which were unsuitable for this purpose. There was no complete correlation between haemagglutinating activities of influenza B viruses and of the recombinants.

Key words: influenza B virus; recombination; immunogenicity

Introduction

Recombination of homotypic influenza A viruses leads to redistribution of a variety of genetic markers (virulence, reproduction activity, properties of surface antigens, ability to plaque formation, etc.). Strains with a high reproductive activity for inactivated vaccine and attenuated ones for live vaccine can thus be rapidly obtained (Kilbourne, 1969; Polezhaev *et al.*, 1974, 1978; Aleksandrova, 1977; Polezhaev and Aleksandrova, 1978). In the first case, a newly appearing epidemic virus is crossed with strain A/PR/8, which results in an increased activity of the recombinants to reproduce in developing chick embryos. To obtain a live vaccine, wild virus is crossed with a virus of obsolete antigenic structure, innocuous for man.

In the present study we investigated the redistribution of the mouse immunogenicity marker in the course of recombination of influenza B virus strains differing in their ability to stimulate the production of antihaemagglutinating antibodies in the blood of immunized animals. We studied the relationship between the haemagglutinating activity and immunogenicity

of the viruses tested. One of the strains used for recombination (B/Leningrad/14/17/55) also had a ts marker and thus we also checked the relationship between this marker and immunogenicity of the recombinants for mice.

Materials and Methods

Viruses. The two wild strains B/Leningrad/2/3/67 (B/67) and B/Leningrad/14/3/76 (B/76) had undergone 3 chick embryo passages. The vaccine strain B/Leningrad/14/17/55 (B/14/17), which had undergone 33 chick embryo passages (16 passages at optimal temperature and 17 passages at a temperature lowered to 25—28 °C), was characterized by complete areactogenicity for children and thermosensitivity, i.e. lowered activity to reproduce at 37.5 °C. Two groups of recombinants of B/76 virus were tested: 1 — with strain B/67, and 2 — with the cold-adapted thermosensitive B/14/17 virus.

Determination of infectivity and haemagglutinating activity. Groups of 10 embryos each were inoculated with the viruses in a dose of 3—4 log EID_{50}/0.2 ml and incubated at 32 °C for 72 hr. The haemagglutinin titres were determined in allantoic fluid samples from individual embryos. Fluids with the highest haemagglutinin titres were used for immunization of mice.

Preparation of recombinants. Chick embryos were inoculated with a mixture of native parent viruses in a dose of 6 log EID_{50}/0.2 ml and incubated at 32 °C for 48 hr. Recombinants were selected after 2 passages in the presence of 16—32 neutralizing units of antiserum to the donor-virus (B/14/17 or B/67) by subsequent cloning by the limiting dilution method in chick embryos at 32 °C for 72 hr. Clones with an antigenic characteristic of B/67 virus were selected. The specificity of surface antigens (haemagglutinin and neuraminidase) was determined as described (Polezhaev *et al.*, 1978).

Immunization of mice. Groups of 10—15 white mice weighing 10—12 g were immunized with a single peroral or intraperitoneal (i.p.) dose of virus (titre 6—7 log EID_{50}/ml). In the former case, 0.1 ml of virus was administered per os to mice that had been previously kept for 36—48 hr without water. In i.p. immunization, 0.5 ml of virus-containing allantoic fluid was injected. Mice in the control groups were given either per os or i.p. placebo in the form of allantoic fluid from uninfected chick embryos.

Determination of immunogenicity of the viruses. Individual mouse sera taken 12—14 days after immunization were heated for 30 min at 58 °C and examined in haemagglutination inhibition (HI) tests for antihaemagglutinating antibody. The geometric mean titres (GMT) of antibody were then calculated for each mouse group.

Thermosensitivity of the viruses ($RCT_{37.5}$ marker) was determined by comparing the infectivities in chick embryos incubated at optimal (32 °C) and increased (37.5 °C) temperature.

Results

Thermosensitivity and haemagglutinating activity of parent strains and recombinants of influenza B virus

All original and recombinant strains of influenza B virus examined reproduced actively in chick embryos at the optimal temperature irrespective of their previous passage history. The infectious titres of the original viruses and the two groups of recombinants B/76-B/67 and B/76-B/14/17 did not differ from each other by more than 1.5 log EID_{50}/0.2 ml.

The strains studied differed in several biological properties — ability to reproduce at increased temperature and haemagglutinating activity (Table 1).

The haemagglutinin titre in chick embryos infected with the low-passage B/67 strain did not surpass 128—256. Both the second epidemic virus B/76 and the cold-adapted B/14/17 that had undergone numerous chick embryo passages showed a high haemagglutinating activity (titres 512—1024).

Table 1. Characteristics of recombinants of influenza virus B/76

Parent viruses and recombinants		Ability to reproduce in chick embryos (log EID_{50}) 0.2 ml) at			Haemagglutinin titre	Immunogenicity for mice* after immunization			
						per os		intraperitoneally	
		32 °C	37.5 °C	Diff.		Exp. 1	Exp. 2	Exp. 1	Exp. 2
B/76		7.24	3.0	4.24	512–1024	3.5	<2.0	6.5	3.2
B/67		7.25	4.5	2.75	128–256	26.0	18.4	37.0	17.1
Recomb.	1	7.5	4.75	2.75	128–256	16.0	13.9	34.0	19.7
	2a	7.25	4.75	2.5	128–256	26.0	19.7	37.0	21.1
	2b	7.75	5.5	2.25	128–256	9.8	8.0	16.0	12.1
	3	7.5	3.25	4.25	128–256	21.1	16.0	8.6	13.9
	4	7.0	2.0	5.0	128–256	9.8	8.6	13.0	16.0
	5	7.25	3.0	4.25	128–256	24.2	21.1	9.2	12.1
	6	7.5	3.75	3.75	128–256	13.0	12.1	10.6	13.9
	7	7.25	2.25	5.0	128–256	19.7	16.0	9.2	11.3
	8	7.0	4.25	2.75	128–256	17.1	14.9	21.1	28.0
B/76		7.7	3.5	4.2	512–1024	3.5	<2.0	6.5	2.7
B/14/17		8.25	2.25	6.0	512–1024	28.0	12.1	194.0	84.0
Recomb.	9	8.2	1.7	6.5	512–1024	<2.0	<2.0	<2.0	<2.0
	10	9.0	4.0	5.0	512–1024	2.1	<2.0	8.0	5.3
	11	7.0	2.7	4.3	512–1024	<2.0	<2.0	2.1	2.0
	12	8.24	1.24	7.0	512–1024	2.1	<2.0	6.5	6.5
	13	8.24	1.24	7.0	512–1024	7.5	5.3	7.5	6.5
	14	7.0	3.0	4.0	512–1024	2.6	2.3	7.5	3.5
	15	7.25	4.25	3.0	512–1024	3.5	3.2	7.5	6.1
	16	8.5	2.25	6.25	512–1024	2.6	2.1	6.5	4.6

* GMT of antihaemagglutinating antibody.

Recombinants of these viruses (B/76 and B/14/17) also produced high levels of haemagglutinin (titres 512—1024).

The haemagglutinating activity of the second group of recombinants B/76-B/67 was $\geq$ 4-fold lower and corresponded to the haemagglutinating activity of the original B/67 virus (titre 128—256). The low-passage wild strains B/76 and B/67 were characterized by moderate thermosensitivity. Their reproduction at 37.5 °C ($RCT_{37.5}$ marker) was lowered by 3—4 log EID_{50}/0.2 ml. The cold-adapted B/14/17 virus that had undergone 17 passages at 25 °C, showed the highest thermosensitivity ($RCT_{37.5}$ — 6 log). Recombinants of this virus with strain B/76 also had a ts genetic marker. $RCT_{37.5}$ of most recombinants reached thermosensitivity values characteristic of the cold-adapted parent B/14/17 virus, or differed from it by not more than 1.0—1.7 log.

Recombinants B/76-B/67, like the original parent strains, reproduced at increased temperature more actively, i.e. they were characterized by moderate thermosensitivity.

Immunogenicity of parent viruses and recombinants for white mice

To compare the immunogenicity of the parent strains and their recom-

binants, white mice were immunized in parallel with similar virus concentrations (6—7 log EID_{50}/0.2 ml).

As shown in Table 1, strain B/76 proved to be the least immunogenic, inducing the lowest levels of antihaemagglutinins after a single peroral or i.p. immunization (GMT 3.5 and 6.5, respectively). The second low-passage strain B/67 and the cold-adapted B/14/17 virus were more immunogenic and induced GMT of antibody 26.0—28.0 after peroral and 37.0—194.0 after i.p. immunization.

The main aim of the present study was to investigate the possibility of increasing the immunogenicity of the little active B/76 strain by genetic recombination with the more immunogenic influenza B viruses. The data presented in Table 1 showed that recombinants of B/76 virus with B/67 and B/14/17 viruses possessed a different ability to induce antibody formation. Recombinants B/76-B/67, retaining surface antigens of the little active B/76 virus, gained from the B/67 virus a higher immunogenicity. GMT of antibody after immunization with recombinants 2a, 3 and 5 reached values of the parent B/67 virus and two other recombinants (2b and 4) reached a somewhat lower GMT value (9.8). The remaining recombinants were characterized by moderate immunogenicity (GMT 13.0—19.7) which, however, was higher than that of the original B/76 virus.

These data indicate that, by the method of genetic recombination, increased ability to antibody formation can be transferred from the more active parent to a little active influenza virus with simultaneous preservation of the antigenic specificity of surface antigens of the less immunogenic virus.

Different results were obtained on recombination of B/76 virus with the other active virus B/14/17. In this case the higher immunogenicity could not be transferred to the recombinants. The immunogenicity of all B/76-B/14/17 recombinants tested corresponded to the very low one of B/76 virus and there was no correlation between the haemagglutinating activity and immunogenicity of the recombinants obtained. All B/76-B/14/17 recombinants were characterized by a high ability to haemagglutinin formation taken over from B/14/17 virus, but did not stimulate active formation of antihaemagglutinins under conditions of a single peroral or i.p. immunization.

Discussion

The possibility of rapid attenuation of newly appearing virulent influenza viruses by genetic recombination with obsolete vaccine strains offers a basis for contemporaneous methods of obtaining strains for the preparation of a live influenza vaccine. As the efficiency of the recombination method depends on the properties of the parent viruses, obtaining of guaranteed areactogenic recombinants presumes that virus quite innocuous for highly susceptible persons, in particular children, is used as a donor of attenuation.

Our investigations have shown that recombinants obtained by crossing a virulent virus with a good donor of attenuation frequently are characterized by low vaccine efficiency which to a considerable degree is determined by the properties of the virulent parent virus (Aleksandrova *et al.*, 1979). This phe-

nomenon is particularly typical of laboratory strains of influenza B virus which frequently are unable to stimulate an intense formation of anti-influenza immunity in vaccinees.

In the present study we demonstrated in experiments on mice the possibility of increasing the immunogenicity of influenza B virus by genetic recombination. By crossing the little active epidemic strain B/Leningrad/14/76 with the homotypic highly immunogenic B/Leningrad/2/67 virus we obtained recombinants possessing surface antigens of the little active virus, which had inherited from B/67 virus the ability to an active stimulation of antibody formation in the blood of immunized animals (the intensity of reproduction in mouse lungs was not investigated).

The results obtained stressed the necessity of selection of a suitable "donor of immunogenicity" for increasing the vaccinal properties of a little immunogenic virus. This necessity was demonstrated by the absence of a similar effect of an increased vaccine potency of the little active B/76 strain on crossing with B/14/17, another highly immunogenic virus.

The differences between strains B/14/17 and B/67 concerning their ability to confer the ability of stimulating antibody formation on the little active B/76 virus could be due to the peculiarities of B/14/17 virus. The latter include 1) long-term passaging in chick embryos and 2) high thermosensitivity and complete loss of reactogenicity for the main host — man, due to multiple ts mutations in the viral genome.

On crossing with B/76 virus, the recombinants probably had gained these defects from the B/14/17 strain and this had an unfavourable effect on the immunogenicity of the recombinants obtained.

The experimental data presented above showed that, in principle, it is possible to transfer the immunogenicity marker by the recombination method from a highly active virus to a virus with low immunogenicity. The experiments also showed that the regularity of this transfer depends on the properties of the "donor of immunization". Thermosensitive viruses are unsuitable for the latter purpose.

We found that the correlation between immunogenicity and haemmagglutinating activity may be disturbed in the recombinants obtained. The highest levels of immunogenicity were registered in strain B/67 and recombinants B/76-B/67, characterized by a lower haemagglutinating activity as compared with recombinants B/76-B/14/17 ,which possessed a higher haemagglutinatting activity. These findings indicate that immunogenicity is governed not only by genes coding for the surface proteins, but also by other so far not defined genes of influenza virus.

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