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PERORAL IMMUNIZATION OF ADULT WHITE MICE WITH THE SKALICA STRAIN FROM THE TICK-BORNE ENCEPHALITIS VIRUS COMPLEX

E. ELEČKOVÁ, M. GREŠÍKOVÁ

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czechoslovakia

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Summary. – Adult white mice immunized perorally with the infectious Skalica strain from the tick-borne encephalitis (TBE) virus complex did not show any clinical symptoms of illness. 56 % of experimental animals immunized with two doses of the Skalica virus (the titer of virus was $6x10^{10}$ LD_{50}) were protected against the challenge with the Hypr strain of TBE virus. All mice immunized with the Skalica virus and having haemagglutination-inhibiting antibodies higher than 1:80 survived the challenge with the given dose of virulent TBE virus. No differences in the immunogenicity and protectivity were observed in experimental animals infected with infectious Skalica virus by oesophageal probe, or by drinking virus-containing medium. A higher protective activity against the virulent Hypr virus was observed in adult white mice immunized subcutaneously with the Skalica virus.

Key words: tick-borne encephalitis virus complex; Skalica strain; peroral immunization; haemagglutination-inhibiting antibodies

Introduction

The Skalica strain isolated from bank vole *(Clethrionomys glareolus)* (Grešíková *et al.*, 1976) is related to the Langat virus (Guirakhoo *et al.*, 1991). Close relationships between the Skalica, Langat and Hypr strain of tick-borne encephalitis viruses were demonstrated by kinetic haemagglutination-inhibition tests (Elečková *et al.*, 1990).

Adult white mice infected subcutaneously (s. c.) with the Skalica virus showed no manifest clinical symptoms of illness (Grešíková and Sekeyová, 1980). This strain was immunogenic after peripheral application to adult white mice (Grešíková and Sekeyová, 1981; Grešíková *et al.*, 1986).

The aim of the present study was to analyse the immune response of adult white mice after peroral infection with the Skalica virus.

Materials and Methods

Viruses. Skalica virus has undergone passages (i. c.) in suckling white mice. For immunization purposes, the virus was cultivated in chick embryo or Vero E6 cells, in Earle's minimal essential medium (MEM) with or without 5 % foetal bovine serum. The virus was harvested after 72 hr incubation at 37 °C.

Virus titration. Serial 10-fold dilutions of the culture fluid were injected intracerebrally (i. c.) into suckling mice and the LD_{50} titers were determined routinely.

Immunization of mice. For s. c. immunization groups of 4 adult white mice were inoculated with two 100 LD_{50} doses of the Skalica virus. The second dose was given 14 days after the first dose. The mice were challenged with the virulent Hypr strain s. c. 14 days after the second dose of Skalica virus. For peroral immunization the probe with 0.2 ml of infectious Skalica virus was applied directly into the oesophagus of mice. The second dose of the virus was applied after 14–28 days. The blood samples were taken from immunized animals before the application of the second virus dose and 14 days later. The mice were challenged with 100 LD_{50} of virulent TBE virus (Hypr strain) s. c. 14 days after the second dose of the Skalica virus. In addition, 16 adult white mice were infected by drinking ad libitum of medium containing infectious Skalica virus.

Serological examination. The sera of mice were extracted by acetone and adsorbed with goose erythrocytes prior testing for the presence of haemagglutination-inhibiting (HI) antibodies. The antigens for the HI tests were prepared by sucrose-acetone extraction of suckling mice brain according to the method described by Clarke and Casals (1958). Four to eight haemagglutinating units of antigen were used in the tests.

Results

Adult white mice immunized s. c. with the Skalica virus were protected against the challenge with 1000 LD_{50} of the Hypr virus strain. After immunization with two doses of the Skalica virus the protection effect was higher (Table 1).

Adult white mice immunized with two doses of the Skalica virus ($2x10^4$ LD_{50}) by oesophageal probe produced HI antibodies with the titers ranging from 1:10 up to 1:320 (the mean titer was 1:46.7). From 34 immunized mice only 4 (12 %)

Table 1. Protective activity of Skalica virus applied s. c. against challenge with Hypr virus

Mice immunized with 1 dose of Skalica virus		Mice immunized with 2 doses of Skalica virus		Non-immunized mice	
Challenge with resp. dose of Hypr (LD_{50})	Death/ Survival	Challenge with resp. dose of Hypr (LD_{50})	Death/ Survival	Challenge with resp. dose of Hypr (LD_{50})	Death/ Survival
10^6	1/3	10^6	0/4	10^6	4/0
10^5	1/3	10^5	0/4	10^5	4/0
10^4	1/3	10^4	0/4	10^4	4/0
10^3	0/4	10^3	0/4	10^3	4/0
10^2	0/4	10^2	0/4	10^2	4/0
10^1	0/4	10^1	0/4	10^1	4/0

were protected against the challenge with TBE virus (Table 2).

When experimental animals were immunized with higher dose of virus ($6x10^{10}$ LD_{50}), HI antibodies were detected with mean titer 1:173.7. From 27

Table 2. HI antibodies in the sera of mice immunized perorally with the Skalica virus

Serum No.	the 1st dose ($2x10^4$ LD_{50})	HI titer* after the 2nd dose ($2x10^4$ LD_{50})	Challenge (10^2 LD_{50})
1	20	20	-
2	80	20	-
3	10	20	-
4	40	320	320
5	40	80	-
6	20	20	-
7	320	20	-
8	80	20	-
9	20	20	-
10	10	20	-
11	160	20	-
12	20	320	640
13	20	20	-
14	20	10	-
15	0	10	-
16	10	20	-
17	40	10	-
18	80	20	-
19	20	20	-
20	40	20	-
21	20	10	-
22	40	160	640
23	0	20	-
24	20	40	-
25	80	40	-
26	80	10	-
27	80	20	-
28	80	10	-
29	40	20	-
30	40	10	-
31	20	160	1280
32	40	10	-
33	10	10	-
34	20	40	-
Mean	47.6	46.7	720

* Reciprocal values of dilutions
- Mice dead after challenge

immunized mice 15 (56 %) were protected against the challenge with TBE virus (Table 3).

We compared also two types of the peroral route of the application of the Skalica virus to adult white mice. Twenty one mice were immunized with the Skalica virus ($6x10^4$ LD_{50}) by the oesophageal probe and 16 mice were allowed to drink medium containing infectious virus ($10^{5.5}$ LD_{50}) ad libitum. No significant differences in HI titers were observed in both groups (Table 4).

Table 3. HI antibodies in the sera of mice immunized perorally with the Skalica virus

Serum No.	the 1st dose ($6x10^{10}$ LD_{50})	HI titer* after the 2nd dose ($6x10^{10}$ LD_{50})	Challenge (10^2 LD_{50})
1	10	10	-
2	160	320	640
3	40	10	160
4	80	160	640
5	10	40	-
6	10	20	-
7	20	160	160
8	40	320	320
9	0	0	-
10	10	10	-
11	40	320	320
12	10	320	320
13	10	20	-
14	10	160	-
15	20	10	-
16	40	320	320
17	40	80	320
18	80	640	160
19	0	80	-
20	0	320	640
21	40	40	160
22	10	20	-
23	0	10	-
24	20	320	640
25	20	320	320
26	40	10	-
27	20	640	640
Mean	28.8	173.7	384

* Reciprocal values of dilutions
- Mice dead after challenge

Table 4. HI antibodies in the sera of immunized with the Skalica virus administered by the peroral probe or by drinking virus – containing medium

Mouse No.	HI titer* after immunization by peroral probe			Mouse No.	HI titer* after drinking virus – containing medium		
	The first dose ($6x10^4$ LD_{50})	The second dose ($6x10^4$ LD_{50})	Challenge (10^2 LD_{50})		The first dose ($6x10^4$ LD_{50})	The second dose ($6x10^4$ LD_{50})	Challenge (10^2 LD_{50})
1	0	80	320	1	40	160	320
2	10	80	160	2	10	10	–
3	20	20	–	3	10	20	–
4	10	10	–	4	10	320	320
5	0	160	320	5	0	20	–
6	0	10	–	6	40	80	640
7	10	80	320	7	20	80	640
8	0	80	160	8	0	40	–
9	10	80	640	9	20	80	640
10	10	40	–	10	10	40	–
11	20	20	–	11	20	80	–
12	20	160	640	12	10	10	–
13	0	10	–	13	10	160	640
14	10	320	640	14	10	0	–
15	10	10	–	15	10	20	–
16	10	160	640	16	10	10	–
17	20	20	–				
18	0	10	–				
19	80	320	640				
20	40	40	640				
21	10	20	–				
Mean	13.8	82.3	465.4		14.3	70.6	533.3

* Reciprocal values of dilutions
– Mice dead after challenge

Discussion

The isolation of the Skalica virus was described in 1976 (Grešíková *et al.*, 1976). After peripheral inoculation of adult white mice with this virus no clinical symptoms of illness were observed; however, antibody production could be demonstrated (Grešíková *et al.*, 1986). It was therefore of interest to examine antibody production in adult white mice after peroral immunization. Mice inoculated perorally with the Skalica virus did not show any symptoms of illness. These results contrast with the experiments carried out with a virulent TBE virus, the Hypr strain. Adult white mice inoculated perorally with infected milk died after an incubation period of 9–11 days with typical symptoms of encephalitis (Grešíková, 1957). The questions arise as to whether the Skalica virus when administered perorally was blocked by inhibitors present in the gastrointestinal tract and/or the Skalica virus is more sensitive to the acid pH than the Hypr virus. Mice inoculated with virus-contaminated sour milk (the Hypr strain of TBE virus was used) died after an incubation of 8–9 days with the symptoms of encephalitis (Grešíková-Kohútová, 1959). It was also demonstrated that the Skalica virus, in comparison to virulent Hypr virus strain, is more sensitive to the action of non-specific inhibitors (Grešíková and Sekeyová, 1981), similarly to TBE viral strains with reduced virulence (Mayer *et al.*, 1967).

Using the Skalica strain, an experimental vaccine was prepared. Immunized animals produced antibodies and were protected against a virulent TBE virus (Grešíková *et al.*, 1986).

There is also a theoretical possibility that live attenuated Skalica vaccine administered perorally might protect the vaccinated subject against virulent TBE virus strains.

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