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CHARACTERISTICS OF SOME TICK—BORNE ENCEPHALITIS VIRUS STRAINS ISOLATED IN SLOVAKIA

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Summary. — Several tick-borne encephalitis (TBE) virus strains isolated in Slovakia from 1974—1978 were characterized by their cytophatic abilities, sensitivity to heating at 50 °C and to 2 M urea, and pathogenicity for white mice. Strains isolated from *Ixodes ricinus* ticks differed in pathogenic properties from those isolated from *Clethrionomys glareolus*. The latter strains (Skalica) may be considered a spontaneous variant of TBE virus with decreased virulence for mice.

Key words: *tick-borne encephalitis virus*; *biological properties*

In the period from 1974—1978 we attempted to disclose new natural foci of tick-borne encephalitis (TBE) virus in Slovakia. Four virus strains designated "Skalica" were isolated from the blood and organs of a bank vole (*Clethrionomys glareolus*) in 1974 (Grešíková *et al.*, 1976). In the present work strain 53 was used. Another 4 strains of TBE virus were isolated from *Ixodes ricinus* ticks in 1978.

The purpose of this study was to compare some biological properties of the newly isolated virus strains and to determine their laboratory characteristics.

Virus isolation. The isolation experiments from different tick instars were carried out in suckling white mice and chick embryo cells (Grešíková and Nosek, 1967), those from organs of a bank vole (*Clethrionomys glareolus*) in suckling white mice.

Laboratory characterization of the isolated virus strains. The strains were cultivated in chick embryo cells (CEC) as described (Grešíková and Kožuch, 1965). The effects of temperature and urea were investigated according to the method described by Grešíková (1959) and Mayer and Sabó (1966). Plaque titration was carried out in PS cells (de Madrid and Porterfield, 1969).

Serological tests. The isolated virus strains were identified by haemagglutination-inhibition (HI) and complement-fixation (CF) tests. In the haemagglutination and HI tests antigens prepared by acetone extraction were used. The micromethod of HI tests was performed in plastic plates with 4—8 haemagglutinating units of antigen and reference sera extracted by acetone (Clarke and Casals, 1958). The CF test was performed according to Casals (1967) in plastic plastes. Mixtures consisting of one drop serum, two drops of complement (2 units) and one drop antigen were incubated for 18 hr at 4 °C. Afterwards 2 units of haemolytic system were added and the results were read after an additional 30 min incubation at 37 °C.

In suckling white mice we succeeded in the isolation of 4 strains of TBE virus from *I. ricinus* ticks (Ir 13 from the locality Nemečky and Ir 32, Ir 33 and Ir 48 from the locality Devín) and 4 strains from organs of a bank vole

Table 1. Multiplication of TBE virus strains in PS cells

Virus strain	Passage history (No. of mouse passages)	CPE	Virus titre log CPD_{50}/ /0.1 ml
Ir 13	1	0	<2
Ir 32	2	+	5.5
Ir 33	1	0	<2
Ir 33	2	+	5.5
Skalica	4	0	<2
Hypr	58	+++	6.5

(*C. glareolus*) in the locality Skalica. The strains were identified by HI and CF tests.

Of the Ir strains, only strain Ir 13 exhibited an interference with Sindbis virus in CEC. Neither this nor the strain Skalica exerted a cytophatic effect in PS cells (Table 1). We decided, therefore, to investigate some biological properties of strains Skalica and Ir 13 and to compare them with those of the prototype Hypr strain of TBE virus.

Table 2. Thermosensitivity of TBE virus strains cultivated in CEC cultures ar elevated temperature

Virus strain*	Extracellular virus titre (log mouse LD_{50}/0.01 ml) in CEC cultures after 18 hr of incubation at °C			
	40	41	42	43
Ir 13	9.5	9.5	9.5	7.0
Skalica	4.2	2.3	2.1	1.8
Hypr	9.5	9.5	9.5	6.3

*The titre of the inocula of all 3 strains was 8.5 log LD_{50}/0.01 ml.

The growth of these virus strains was investigated in CEC cultivated at 37 °C. Since no significant differences in the virus growth were observed, we studied the thermosensitivity of strains Ir 13, Skalica and Hypr in CEC during cultivation for 18 hr at the temperatures of 40, 41, 42 and 43 °C. Whereas

Table 3. Effect of 2 M urea on the haemagglutinating (HA) activity of TBE virus strains

Virus strain	HA titre before heating	HA titre after heating at 37 °C for 30 min	HA titre after treatment with 2M urea at 37°C for 30 min
Ir 13	640	640	160
Skalica	1280	1280	320
Hypr	1280	640	640

Table 4. Pathogenicity of TBE virus strains for susceptible (Černý Vůl) and insusceptible (Dobrá Voda) mice

Virus strain	log LD_{50} in susceptible mice		log LD_{50} in insusceptible mice	
	i. c. inoculation	s. c. inoculation	i. c. inoculation	s. c. inoculation
Ir 13	8.5	8.5	3.7	<2
Skalica	8.5	<2	3.5	<2
Hypr	8.5	8.5	6.2	2

strains Hypr and Ir 13 multiplied also at 40—42 °C, the titre of strain Skalica decreased at 40 °C by 4.3 log units. At 43 °C, the titre of strain Skalica decreased by 6.7 log units (Table 2).

Heating at 50 °C for 20 min of the strains Ir 13 (2nd mouse pass.), Skalica (4th mouse pass.) and Hypr (59th mouse pass.) resulted in a titre (log. i. c. mouse LD_{50}/0.01 ml) decrease from 9.5 to 7.5, 4.0 and 5.5, respectively. Thus the strains Skalica and Hypr were thermolabile, while the strain Ir 13 was thermostable.

Treatment with 2 M urea (Table 3) had no effect on the haemagglutination activity of the Hypr strain, but the haemagglutinin titre of Skalica and Ir 13 strains decreased 4-fold after urea treatment.

The pathogenicity for juvenile white mice of the newly isolated strains of TBE virus was compared with that of the prototype Hypr strain. In the less susceptible juvenile mice of the Dobrá Voda breed the newly isolated strains showed lower pathogenicity than the prototype virus strain after intracerebral inoculation. In the susceptible juvenile white mice of the Černý Vůl breed only the strain Skalica was found non-pathogenic after subcutaneous inoculation of 10^6 LD_{50} of virus (Table 4).

Subcutaneus inoculation of juvenile white mice with strain Ir 13 was followed by intense and long-lasting viraemia (sufficient for vector infection), whereas strain Skalica caused only threshold viraemia of short duration (Table 5).

Both newly isolated strains (Ir 13 and Skalica) were immunogenic. In the less susceptible white mice inoculated subcutaneously HI antibodies were found in titres from 20—40 as early as one week post infection, reaching values from 20 to 160 one week later.

Table 5. Viraemia in white mice after s. c. administration of TBE virus strains

Virus strain*	Viraemia (log'LD_{50}/0.01 ml) on days					
	1	2	3	4	5	7
Ir 13	3.5	3.5	3.5	3.1	<1	0.7
Skalica	2.0	2.0	1.0	0	0	0
Hypr	2.8	2.5	3.5	<1	<1	0.4

*The titre of the inocula of all 3 strains was 7.5 log LD_{50}/0.01 ml.
0 means no virus detected.

Table 6. Antibody production in adult white mice (Dobrá Voda) inoculated with 1 dose of TBE virus strains

Virus	Passage history (No. of mouse passages)	HI antibody titres* 7 days p. i.	14 days p. i.
Ir 13	2	20/0	40/10
Ir 32	2	40/20	160/40
Ir 33	2	40/20	80/20
Skalica	4	40/20	20/20
Hypr	58	40/10	20/10

*HI titres before/after mercaptoethanol treatment.

Treatment with 2-mercaptoethanol of sera harvested one week after subcutaneous inoculation led to a decrease in antibody titres in all sera tested. Mouse sera harvested two weeks after subcutaneous inoculation with strains Ir 13, Ir 32, Ir 33 and Hypr contained IgM antibodies, whereas sera of mice inoculated with strain Skalica and harvested at the same interval contained IgG antibodies (Table 6).

We consider the strain Skalica as a spontaneous variant of TBE virus with lowered virulence for juvenile white mice after subcutaneous inoculation. The strain forms small plaques (0.75 mm diameter) under agar, does not produce a cytophatic effect in PS cells, is thermolabile, is non-pathogenic for white mice inoculated subcutaneously and causes threshold viraemia of short duration. In these markers it differs from the prototype Hypr strain which forms larger plaques (1.5 mm diameter) under agar, produces a cytopathic effect in PS cells, is thermostable, is pathogenic for mice after subcutaneous inoculation and causes long-lasting intensive viraemia.

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