

## IN VITRO CULTIVATION STUDIES ON THE SKALICA STRAIN OF TICK-BORNE ENCEPHALITIS VIRUS

M. GREŠÍKOVÁ, M. SEKEYOVÁ

Institute of Virology, Slovak Academy of Sciences, 809 39 Bratislava, Czechoslovakia

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*Summary.* — The Skalica strain of tick-borne encephalitis virus was cultivated in mouse organ cultures. In the fluids from spleen, liver, brain and spinal cord cultures, maximum infectivity titres of 8.1—8.3 log LD<sub>50</sub>/0.01 ml were found 6 and 9 days after inoculation. In the fluids from lymph node and muscle cultures, maximum infectivity titres of 4.2 and 6.5 log LD<sub>50</sub>/0.01 ml, respectively, were detected 6 days after inoculation. Neither interferon nor haemagglutinin nor complement-fixing antigen was demonstrated in media from explant cultures from the 3rd to the 15th day of cultivation. Fifteen days after seeding, haemagglutination inhibiting antibodies were detected in the fluid phase of lymph node, spleen and liver explant cultures derived from mice infected 6 days previously, as were complement-fixing antibodies in the fluid phase of lymph node, spleen, liver, lung, brain and spinal cord explant cultures derived from mice infected 14 days previously.

*Key words:* tick-borne encephalitis virus; mouse organ cultures; antibodies

### *Introduction*

Organ cultures may be considered to resemble the organism of animals more than tissue cultures. Therefore we used mouse organ cultures to study the multiplication of the Skalica strain of tick-borne encephalitis TBE virus. This strain is a spontaneous variant with lowered virulence for juvenile white mice after subcutaneous (s.c.) inoculation (Grešíková and Sekeyová, 1980). We also used the explantation method to study antibody production in mice infected with the Skalica strain of TBE virus.

### *Materials and Methods*

*Organ cultures* were prepared from the spleens, livers, inguinal lymph node, brains, spinal cords and muscles of juvenile mice. The organs were dissected into pieces 1—2 mm<sup>3</sup> in size and washed with medium 199, 6—9 pieces each were then placed on plastic grids in Petri dishes containing medium 199 with 10 % foetal calf serum and antibiotics. The organ cultures were incubated at 37 °C in a CO<sub>2</sub> atmosphere for 18 hr before infection with TBE virus strain Skalica (Grešíková *et al.*, 1976). The organ cultures were inoculated with 1 ml of a virus suspension with a titre of 5.5 log LD<sub>50</sub>/0.01 ml and incubated further in a CO<sub>2</sub> incubator at 37 °C for 15 days. The fluid phase

**Table 1. Multiplication of the Skalica strain of TBE virus in mouse organ cultures**

Organ culture	Virus titres (log LD <sub>50</sub> /0.01 ml) in fluid phase on days						
	1	2	3	6	9	12	15
Spleen	2.5	3.0	4.3	8.1	4.8	2.8	2.5
Liver	2.5	2.8	4.3	8.1	8.3	3.6	3.1
Lymph nodes	2.5	3.5	4.1	4.2	3.4	2.5	2.5
Brain	2.5	4.1	4.8	8.1	8.3	3.5	3.0
Spinal cord	2.3	3.5	4.3	8.1	8.3	3.5	3.0
Muscle	2.5	3.0	3.6	6.5	5.8	5.1	4.1

of organ cultures was taken 1, 2, 3, 6, 9, 12 and 15 days after inoculation (p.i.) and assayed for virus by intracerebral (i.c.) inoculation of 2–3 days old suckling mice or by the interference method. In the latter, tube cultures of chick embryo cells (10<sub>5</sub> cells in 1 ml) were grown in basal Eagle's medium (BEM) supplemented with 10 % calf serum and antibiotics. After 24 hr, the cells were inoculated with organ culture media. Three days later, 100 TCD<sub>50</sub> of Sindbis virus were added and after another 3 days the cytopathic effect (CPE) of Sindbis virus was recorded. Experiments in which the challenging virus caused no CPE were considered positive (Grešíková and Nosek, 1967).

*Serology.* Samples of organ cultures media were treated by acetone or by 2-mercaptoethanol and acetone like serum samples (Grešíková and Sekeyová, 1978). The haemagglutination (HA) and haemagglutination inhibition (HI) tests were carried out according to Clarke and Casals (1958) and the complement-fixation (CF) tests by the micromethod of Casals (1967).

### Results

#### *Multiplication of the Skalica strain of TBE virus in organ cultures*

In the course of culturing, cell proliferation was seen at the edge of explants from lymph nodes, spleen, liver, brain, spinal and muscles. The virus multiplied in all these organ cultures (Table 1). The maximum titre of 8.1–8.3 log LD<sub>50</sub>/0.01 ml was found in the spleen, liver, brain, and spinal cord cultures from the 6th to the 9th day p.i.

**Table 2. HI and CF antibodies in the fluid phase of organ cultures derived from mice infected with the Skalica strain of TBE virus**

Organ culture	Antibody titres on days of cultivation										
	3, 6, 9			12			15			15*	
	HI-A	HI-ME	CF	HI-A	HI-ME	CF	HI-A	HI-ME	CF	HI-A	HI-ME
Lymph nodes	0	0	0	0	0	0	2	0	16/4**	2	0
Spleen	0	0	0	0	0	0	2	0	16/4	0	0
Liver	0	0	0	0	0	0	2	0	8/4	2	0
Lungs	0	0	0	2	0	0	0	0	8/4	2	0
Brain	0	0	0	0	0	0	0	0	32/4	0	0
Brain	0	0	0	0	0	0	0	0	32/4	0	0
Spinal	0	0	0	0	0	0	0	0	8/4	2	0

\* Suspension of organ cultures.

\*\* Titre of fluid phase/titre of antigen.

HI antibodies were detected in organ cultures derived from mice on the 6th day p.i.

HI-A = HI antibodies in fluid phase treated by acetone.

HI-ME = HI antibodies in fluid phase treated by 2-mercaptoethanol and then by acetone.

CF antibodies were detected in organ cultures derived from mice on the 14th day p.i.

Neither interferon nor haemagglutinin nor CF antigen was found in the fluid phase of the organ cultures from the 3rd up to the 15th day of culture.

*Production of antibodies in mice inoculated with the Skalica strain of TBE virus*

To study antibody production, white mice were inoculated s.c. with the Skalica strain of TBE virus ( $10^{6.5}$  i.c. mouse  $LD_{50}/0.1$  ml); organ cultures were prepared on the 3rd, 6th, 9th, 12th and 15th day p.i. of the mice.

HI antibodies were detected 15 days after seeding in the fluid phase of lymph node, spleen and liver organ cultures derived from mice 6 days p.i. The results of the 2-mercaptoethanol treatment indicated that the antibodies were of the IgM type (Table 2). In organ cultures of lymph nodes derived 9 days p.i. of the mice, IgM antibodies of low titre were detected.

In organ cultures prepared 3, 6, 9 and 12 days p.i. of the mice, CF antibodies were not detected. Fifteen days after seeding, the fluid phase of organ cultures from the lymph nodes, spleen, lungs, liver, brain and spinal cord derived from mice 14 days p.i., contained CF antibodies (Table 2).

*Discussion*

The replication of arboviruses in laboratory rodent organ cultures was studied with Middelburg virus (de Vleeschauwer and Pattyn, 1974) and Western equine encephalomyelitis (WEE) virus (Grešíková and Monath, 1975). Spinal cord and muscle organ cultures support the multiplication of WEE virus and muscle organ cultures were the most productive in supporting the multiplication of Middelburg virus.

It was of interest to study the behaviour of an aberrant strain of TBE virus (Skalica) in mouse organ cultures. The Skalica strain is non-pathogenic for white mice on s.c. inoculation and causes threshold viraemia of short duration. It is a temperature-sensitive variant of TBE virus (Grešíková and Sekeyová, 1980).

In the present work the Skalica strain of TBE virus showed a considerably wide affinity to mouse organ cultures. Nevertheless, interferon, haemagglutinin, and complement-fixing antigen were not detected in the fluid phase of these cultures.

In the fluid phase of organ cultures of the spleen, lymph nodes and liver derived from mice 6 days p.i. with the Skalica strain, low titres of HI antibodies sensitive to 2-mercaptoethanol were detected 15 days after seeding.

In the fluid phase of organ cultures of lymph nodes, spleen, liver, lungs, brain and spinal cord derived from mice, 14 days p.i. with the Skalica strain, CF antibodies were detected 15 days after seeding.

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