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TO THE ANTIGENIC CLASSIFICATION OF SOME VIRUSES FROM THE TICK-BORNE ENCEPHALITIS COMPLEX BY MONOCLONAL ANTIBODIES

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Summary. — Two monoclonal antibodies (MoAb) to the Skalica virus from the tick-borne encephalitis (TBE) complex were used to compare Karshi and Royal Farm viruses with the Russian spring-summer encephalitis, Central European encephalitis (Hypr and Kumlinge strains) Skalica, Langat and Powassan viruses. The first MoAb was prepared by fusion of P_3NS_1 cells with BALB/c mouse spleen cells, immunized with the Skalica virus; it was of IgM class and reacted in haemagglutination-inhibition (HI) test (MoAb type 1). The second MoAb was of IgG class and reacted in complement-fixation (CF) test (MoAb type 2). MoAb type 1 reacted in the HI test with Russian spring-summer encephalitis (RSSE), Central European encephalitis (CEE) virus strains, Skalica and Langat viruses. No reaction was observed with Powassan, Karshi, and Royal Farm viruses. MoAb type 2 reacted in the CF test with all members of tick-borne encephalitis complex except the Powassan, Karshi, and Royal Farm viruses.

Key words: *antigenic classification*; *viruses of the tick-borne encephalitis complex*; *monoclonal antibodies*

The viruses belonging to the tick-borne encephalitis complex (Clarke, 1964) have been already compared by the use of MoAb (Grešíková and Sekeyová, 1984; Gaidamovich *et al.*, 1986). MoAb to the Skalica reacted with all members of TBE complex, except of the Powassan virus. MoAb to the Russian spring-summer encephalitis (RSSE) virus reacted with the members of TBE complex except of Powassan, Langat, and Skalica viruses.

Recently Karshi and Royal Farm viruses have been included in the TBE complex (Calisher, 1988). It was of interest to answer the question of antigenic relationships of these viruses by the use of MoAb.

Viruses and antigens. Following viruses of the TBE complex were used: Russian spring-summer encephalitis (Sofin strain), Central European Encephalitis (Hypr and Kumlinge strains) Skalica, Langat, Powassan, Karshi, and Royal Farm viruses. Antigens for HI and CF tests

Table 1. Tick-borne flaviviruses used for serological comparison by monoclonal antibodies

Virus	Isolated from	Year	Location
Russian spring summer encephalitis	human	1937	Primorskii Kray, U.S.S.R.
Hypr strain of CEE	human	1953	Brno, Czechoslovakia
Kumlinge strain of CEE	*Ixodes ricinus*	1959	Åland, Finland
Skalica	*Clethrionomys glareclus*	1974	Radimov forest, Czechoslovakia
Langat	*Ixodes granulatus*	1956	Malaysia
Powassan	human	1958	Ontario, Canada
Karshi	*Ornithodoros papillipes*	1972	Uzbekistan, U.S.S.R.
Royal Farm	*Argas hermanni*	1968	Kabul, Afghanistan

CEE = Central European encephalitis

were prepared from the brain of viruses infected suckling mice by the sucrose-acetone method (Clarke and Casals, 1958).

Monoclonal antibodies. The method of hybridoma production was described previously (Novák *et al.*, 1983; Kushch *et al.*, 1986).

Serological tests. For serological comparison the haemagglutination-inhibition (HI) and the complement-fixation (CF) tests were used. The HI and CF tests were carried out as microtests in plastic plates. The HI test was performed as described by Clarke and Casals (1958).

At 30 min, at 1, 2, and 18 hr after inoculation 0.4 ml of the red cells suspension was added to the mixtures in the wells. The system was incubated for 45 min until reading. To remove the non-specific inhibitors, the ascitic fluids were treated with acetone.

The complement-fixation test was performed with 2 units of complement in veronal buffered solution for 30 min, 2 hr, and 18 hr according to Casals (1967).

Seven viruses of the TBE complex have been compared: RSSE, CEE (Hypr and Kumlinge strains), Skalica virus, Langat, Powassan, Karshi, and

Table 2. The reactivity of monoclonal antibodies with some viruses of tick-borne encephalitis complex in the haemagglutination-inhibition (HI) test

Antigen	HI titres after		
	30 min	2 hr	18 hr
Russian spring-summer encephalitis	40	80	160
Hypr strain of CEE	320	640	1280
Kumlinge strain of CEE	640	1280	1280
Skalica	640	1280	2560
Langat	160	320	1280
Powassan	0	0	0
Karshi	0	0	0
Royal Farm	0	0	0

CEE = Central European encephalitis

Table 3. The titres of monoclonal antibodies in the complement-fixation (CF) test with some viruses of the TBE complex

Virus	CF titres after incubation of		
	30 min	2 hr	18 hr
Russian spring-summer encephalitis	0	0	128/8*
Hypr strain of CEE	0	0	256/256
Kumlinge strain of CEE	0	0	512/32
Skalica	0	0	256/256
Langat	0	0	256/8
Powassan	0	0	0
Karshi	0	0	0
Royal Farm	0	0	0

CEE = Central European encephalitis
* Titre of MoAb/titre of antigen

Royal Farm viruses (Table 1). For comparison, MoAb of IgM and/or IgG classes were used. MoAb of IgM class (MoAb type 1) reacted in HI test, MoAb of IgG class (MoAb type 2) reacted in CF test.

By HI tests the highest titres were obtained with the Skalica and Kumlinge antigens; 4-fold differences were obtained with Langat antigen and 16-fold differences with the RSSE antigen after 30 min incubation and/or after 2 hr incubation.

No antigenic relationships were detected with the Powassan, Karshi, and Royal Farm viruses (Table 2). When the incubation of MoAb with the antigens was prolonged to 18 hr, higher HI titres were observed with all antigens tested except of Powassan Karshi, and Royal Farm viruses.

Table 4. Identification of tick-borne encephalitis (TBE) virus strains by monoclonal antibodies using haemagglutination-inhibition (HI) test

Isolated strains	HI titres* with MoAb	HI titres* with reference TBE antiserum
Apodemus flavicollis No. 6	5 120	320
A. flavicollis No. 12	10 256	320
A. flavicollis No. 28	5 128	320
Ixodes ricinus No. 155	1 280	320
I. ricinus No. 156	1 280	160
I. ricinus No. 159	1 280	160
Patient's blood sample	2 560	640

* The antibodies and the antigen were incubated for 18 hr

Using MoAb in CF test, a close relationship was detected among RSSE, CEE (Hypr and Kumlinge strains), Langat, and Skalica viruses. No reactions were detected with the Powassan, Karshi, and Royal Farm viruses (Table 3). Finally, MoAbs (type 1) were used for the identification of CEE virus strains. As shown in Table 4, higher antibody titres were detected with the MoAb than with the reference TBE antiserum.

In our investigations, 2 types of MoAbs (IgM class and IgG class) o TBE virus have been used to specify the antigenic classification of Karshi and Royal Farm viruses. In the previous study, the close relationships between the RSSE, kEE, louping-ill, Negishi and Omsk haemorrhagic fever viruses have been detected. When the antigens were incubated with MoAb for 30 min, 4 to 8 times lower titres were obtained with Langat and Kyasanur forest disease viruses. The MoAb to Skalica virus did not react with Powassan virus (Grešíková and Sekeyová, 1984). MoAb to RSSE did not react with Powassan, Langat-TP-21 and Skalica viruses (Gaidamovich *et al.*, 1986).

A number of virus strains associated with encephalitis in Europe and in the U.S.S.R. have been registered in the International Catalogue of Arboviruses (Karabatsos, 1985). The homogeneity among TBE virus strains isolated in Europe was described by analysis of structural glycoproteins (Heinz and Kunz, 1981) and by the use of MoAb (Heinz *et al.*, 1982). In our study no significant differences in the reactivity between Hypr and Kumlinge strains were detected.

Recently, the Karshi and Royal Farm viruses were included (Calisher, 1988) into the tick-borne encephalitis complex. In order to clarify the classification of these viruses we compared them antigenically with MoAb. By the use of HI and CF tests no relationships of the Powassan, Karshi, and Royal Farm viruses to the tick-borne encephalitis complex was found.

It would be useful to compare antigenically all the registered tick-borne encephalitis virus strains.

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