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ANTIGENIC VARIATION OF THE VIRUSES BELONGING TO THE TICK-BORNE ENCEPHALITIS COMPLEX AS REVEALED BY HUMAN CONVALESCENT SERUM AND MONOCLONAL ANTIBODIES

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Summary. — Solid-phase enzyme-linked immunoassay (ELISA) was used for the detection of antigenic relationships and/or differences among the viruses belonging to the tick-borne encephalitis (TBE) complex. Monoclonal antibodies of IgM class with haemagglutination-inhibiting activity to the Skalica strain of TBE virus were used to compare the TBE complex viruses. Antigenic analysis of 9 viruses of the TBE complex, isolated from Eurasia and America showed close relationships among them. Nevertheless, it was possible to differentiate the Skalica strain from Langat, louping-ill and Omsk haemorrhagic fever (OHF) viruses by ELISA when monoclonal antibodies and antigens were diluted 1 : 10,000. Monoclonal antibodies to the Russian spring-summer encephalitis virus did not react with the Skalica strain in immunofluorescence test. By the use of convalescent serum no reaction was found with louping-ill, Russian spring-summer encephalitis, Powassan and OHF viruses in haemagglutination-inhibition (HI) test.

Key words: viruses of the tick-borne encephalitis complex; antigenic variants; differentiation; monoclonal antibodies; convalescent serum

Introduction

The viruses belonging to the tick-borne encephalitis (TBE) complex are distributed in three very vast zoogeographical regions: Palearctic, Oriental and Nearctic. The viruses comprising the complex are closer related to each other than to other members of the family Togaviridae (Clarke, 1962). By the use of monoclonal antibodies to the Skalica strain of TBE virus in HI test it was possible to differentiate the Powassan virus isolated in Nearctic geographical regions from the viruses isolated in Palearctic regions (Grešíková, Sekeyová, 1984). Monoclonal antibodies to Russian spring-summer encephalitis showed the close relationship among viruses belonging to the

TBE complex except of Powassan, Langat and Skalica virus (Gaidamovich *et al.*, 1986).

In the present study we applied human convalescent serum to compare the viruses belonging to the TBE complex.

Materials and Methods

Monoclonal antibodies (MA). Hybridomas were obtained by the fusion of P_3-NS 1-Ag 4-1 myeloma cell lines with the spleen cells of BALB/c mice immunized with the Skalica strain of TBE virus (Novák *et al.*, 1983). Second type of monoclonal antibodies was prepared by the fusion of NSO myeloma cells with the spleen cells of BALB/c mice immunized with the Russian spring-summer encephalitis, 4072 strain (Gaidamovich *et al.*, 1986).

For comparison, the serum of a patient with diagnosis of tick-borne encephalitis was used.

Viruses and antigens. All viruses of the TBE complex were used: tick-borne encephalitis, the prototype strain Hypr, the Skalica strain (Grešíková *et al.*, 1976), Russian spring-summer encephalitis (RSSE), the prototype Sofyin strain, Omsk haemorrhagic fever (OHF) virus, louping-ill, Kyasanur forest disease (KFD) virus, Langat TP-21, Negishi and Powassan viruses. Antigens for haemagglutination, haemagglutination-inhibition (HI) and complement-fixation (CF) tests were prepared from the brain of virus-infected suckling mice by sucrose-acetone extraction (Clarke and Casals, 1958).

Serological tests. HI tests were performed as described by Clarke and Casals (1958). Complement-fixation tests were performed according to Casals (1967). The immunofluorescence (IF) test was made in virus-infected Vero, PS or SPEV cells grown on slides placed into Petri dishes. After 24—48 hr, the infected cells were fixed with acetone, stored at —20 °C until examined by the indirect IF method as described (Coons and Kaplan, 1950).

The solid phase ELISA was carried out in 96-well plastic panels. The antigens were prepared in CV_1 monkey or Vero cells. The cells were maintained in Earle's minimal essential medium (MEM) with 10 % inactivated calf serum and antibiotics. After 24 hr incubation at 37 °C, the cultures were inoculated with the viruses of the TBE complex. The antigens were allowed to adsorb for 2 hr at 37 °C. Non-adsorbed virus was removed and fresh MEM was added. The infected cells were cultured for 24—48 hr at 37 °C; the cultures fluids were discarded, the cells were covered with PBS, frozen and thawed and then centrifuged at $60 \times g$ for 20 minutes; the supernatant was used as antigen.

Polystyren microtiter plates were coated with antigens and incubated overnight at 22 °C. The plates were washed 3 times with phosphate buffered saline containing 0.5 % Tween 20. To each well were added 100 µl of monoclonal antibodies diluted from 1 : 320 up to 1 : 10.280. After incubation for 18 hr at +4 °C, the wells were washed 3 times with PBS — Tween 20, then 100 µl of anti-mouse immunoglobulin labelled with horseradish peroxidase was added. After 3 washes with phosphate-buffered saline, the substrate (o-phenylendiamine) was added; the enzyme reaction was stopped after 30 min. at room temperature by addition of $2N\ H_2SO_4$. The absorbance was measured by Microelisa minireader MR 590 (Dynatech Laboratories, Inc.). The test was considered positive when absorbance of the test sample with virus antigen was 2.1 higher than with control antigen.

Results

The relationship among viruses of TBE complex in solid phase ELISA was first tested using selected viruses and monoclonal antibodies to the Skalica strain of TBE virus. Dilution of monoclonal antibodies (1 : 320—1 : 20 000) were screened in box titration with the antigens of the TBE complex, diluted from 1 : 320—1 : 10,280. The members of the TBE complex were cross reactive up to the dilution 1 : 2,560; the optimal dilution of antigens and monoclonal antibodies for the differentiation was 1 : 10,280. The differences among the extinction values could indicate antigenic diffe-

Table 1. Comparison of the viruses from TBE complex by ELISA

Antigens	Antigen dilution to monoclonal antibody dilution		
	2560/2560*	5120/5120*	10.000/10.000*
Skalica	0.42**	0.38	0.36
Hypr	0.50	0.55	0.24
Louping-ill	0.36	0.35	0
RSSE	0.57	0.57	0.1
OHF	0.46	0.29	0
KFD	0.29	0.19	0.1
Langat	0.46	0.46	0
Negishi	0.53	0.48	0.2
Powassan	0.49	0.21	0.2
Control			
West Nile	0.0	0.0	0.0

* dilution reciprocals
** absorbance at 490 nm

rences between the Skalica and Hypr strains on one hand, and the louping-ill, Langat and OHF viruses on the other (Table 1).

The second type of monoclonal antibodies was prepared from the fusion of NSO myeloma cell line with the spleen cells of BALB/c mice immunized with the RSSE virus, strain 4072 and with the Skalica strain. 98 hybrid cells producing fluorescent antibodies against TBE were primary cloned. Supernatants of the cloned and grown hybrid cells were repeatedly examined for the presence of IF antibodies; (Gaidamovich *et al.*, 1986) 56 hybridomas producing IF antibodies have been detected, the highest titre being 1 : 80 (Grešíková *et al.*, 1984). Selected hybridomas were tested in ELISA (Table 2) which revealed higher titres than the IF test. The specificity in IF test of monoclonal antibodies was examined against the Skalica and Hypr antigens (Central European subtypes of TBE). Monoclonal antibodies to RSSE virus reacted exclusively with the Hypr strain.

Table 2. The titre of monoclonal antibodies in IF test and ELISA

Hybridoma	IF titre	ELISA titre
K 31-3	40	2 560
KEN 12-1	10	1 280
K 11-1	20	2 560
KEN 9-1	5	2 560
N 58-3	20	2 560
NEK 6-3	10	640
N 66-4	80	1 280
K 8 C 2	5	320
NEK 9-4	40	1 280

Table 3. Titres of human convalescent serum in HI test with viruses belonging to the TBE complex

Antigen	HI titres with convalescent serum		HI titre with the reference TBE antiserum
	2 hr of incubation	24 hr of incubation	
TBE Hypr strain	160	320	160
TBE Skalica strain	40	40	80
Louping-ill	0	0	40
Russian spring-summer encephalitis	0	20	160
Negishi	40	80	80
Powassan	0	0	10
Omsk haemorrhagic fever	0	0	160
Kyasanur forest disease	40	80	80
Langat	80	80	80

Finally, we used human convalescent serum of a patient hospitalized at the Infectious Disease Department of the district hospital in Topoľčany with diagnosis TBE virus infection. As shown in Table 3, the patient's serum when incubated for 2 hr at +4 °C reacted in HI tests in highest titre with strain Hypr, and in lower titres with the Skalica strain, Negishi, KFD and Langat antigens. No reaction was observed with louping-ill, RSSE, Powassan and OHF viruses, respectively. Similar results were obtained when incubating the serum with antigens for 24 hr; in addition a cross reaction with RSSE antigen was observed.

Table 4. The titre of human convalescent serum in CF test with viruses belonging to the TBE complex

Antigen	CF titres with human serum*	
	2 hr incubation	24 hr incubation
TBE (strain Hypr)	8/4	16/4
TBE (strain Skalica)	0	0
Louping-ill	16/8	16/4
RSSE	16/8	16/8
Negishi	16/64	32/32
Powassan	0	0
Omsk haemorrhagic fever	8/8	16/8
Kyasanur forest disease	0	0
Langat	0	8/4

* Serum titre/antigen titre

The antigenic relationships among the viruses belonging to the TBE complex were studied by the use of convalescent human serum in CF tests. As shown in Table 4, the serum incubated for 2 hr at +4 °C with the antigen reacted with TBE, louping-ill, Negishi and OHF viruses. No reaction was observed with the Skalica strain of TBE, Powassan, KFD and Langat antigens. Similar results were obtained when incubating the serum with the antigens for 24 hr.

Discussion

Separation of viruses belonging to the TBE complex according to their vectors and geographical distribution is of biological significance probably representing a selection by the vector. It was supposed that monoclonal antibodies are extremely sensitive for detection of antigen changes. By the use of monoclonal antibodies a close relationship was demonstrated in the HI-test among the viruses isolated from Palearctic zoogeographical region but no relationship was found to the Powassan virus isolated in the Neoarctic zoogeographical region (Grešíková and Sekeyová, 1984).

The results obtained by the use of monoclonal antibodies to TBE virus isolated in Europe showed cross reactions with all TBE virus strains and with West Nile virus. This can be explained by homogeneity of the structural glycoproteins of TBE and West Nile viruses (Heinz *et al.*, 1982). Stephenson *et al.* (1984) using a panel of monoclonal antibodies to TBE virus investigated their ability to react with the intracellular TBE virus polypeptides 58 K and 51 K. It has been shown that there are conserved epitopes on TBE virus-specific proteins, the highest level of conservation was found on the 51 K polypeptide. The 58 K polypeptide also contained some conserved epitopes, but, in addition, it contained several highly variable epitopes.

Radioimmunoassay revealed two epitopes on the 51 K polypeptide: one was common for RSSE and Western strains of TBE, another specific for the Western strains only, including louping-ill virus (Stephenson *et al.*, 1984).

It is of interest that by using monoclonal antibodies we were able to detect differences in ELISA between louping-ill, OHF viruses (isolated in Palearctic region), Langat virus (isolated in Oriental region) and the Skalica strain (isolated in Palearctic region). By the use of human convalescent serum, it was possible to differentiate the viruses isolated in the Palearctic region from the virus isolated in the Nearctic region. Differences were found also between the viruses isolated in Palearctic region, namely between the viruses isolated in Central Europe (Hypr and Skalica) on one hand, and louping-ill virus isolated in Scotland and N. Ireland or RSSE and OHF viruses isolated in Siberia, on the other hand.

Thus, by convalescent serum the distinction was made between two viruses which appeared identical by monoclonal antibodies between the Western strains of TBE and louping-ill viruses.

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