

ANTIGENIC RELATIONSHIPS AMONG VIRUSES OF THE TICK-BORNE ENCEPHALITIS COMPLEX AS STUDIED BY MONOCLONAL ANTIBODIES

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Summary. — Tick-borne encephalitis (TBE) monoclonal antibodies showed haemagglutination-inhibiting (HI) activity against viruses belonging to the TBE complex except of Powassan virus. The HI titre with Kyasanur forest disease virus was lower than with tick-borne encephalitis virus, when monoclonal antibodies were incubated with the antigen at +4 °C for 30 min. Langat virus could be distinguished from other viruses of the TBE complex when the antigen was incubated with monoclonal antibodies at +4 °C for 18 hr. A close antigenic relationship was demonstrated between tick-borne encephalitis, louping-ill, Negishi and Omsk haemorrhagic fever viruses.

Key words: tick-borne encephalitis complex; monoclonal antibodies; antigenic relationships

Introduction

The tick-borne encephalitis complex consists of seven viruses: Powassan, louping-ill, tick-borne encephalitis (Central European and Far Eastern subtypes), Negishi, Omsk haemorrhagic fever, Kyasanur forest disease and Langat (Clarke, 1964). Using the kinetics of HI tests, it was possible to differentiate tick-borne encephalitis virus strains isolated in closely adjacent areas (Grešíková, Sekeyová, 1981). Therefore, the antigenic relationship of viruses belonging to the tick-borne encephalitis complex was studied by means of monoclonal antibodies (Novák *et al.*, 1983).

Materials and Methods

Antigens. All antigens used, namely Powassan, louping-ill, tick-borne encephalitis (Central European, the Hypr strain and the Skalica strain, Far Eastern tick-borne encephalitis), Negishi, Kyasanur forest disease (KFD) and Langat were prepared by the sucrose-acetone method (Clarke and Casals, 1958).

Monoclonal antibodies. Monoclonal antibodies to tick-borne encephalitis virus were prepared from hybrid P₃-NS₁/1-Ag₄-1 and BALB/c mouse spleen cells. Mice were immunized with the Skalica strain of TBE virus (Grešíková *et al.*, 1976) as described (Novák *et al.*, 1983). After 3 weeks of cultivation, 14 hybridomas with HI activities were obtained. The highest titres of monoclonal antibodies were obtained from hybridomas Nos 9, 15 and 16. These cell lines were cloned, recloned and inoculated i.p. into BALB/c mice. For the production of ascitic fluid, Freund's adjuvant,

Table 1. The results of haemagglutination-inhibition (HI) test with monoclonal antibodies to tick-borne encephalitis virus

Antibody No.	HI titre with the Skalica antigen		NI
	Ascitic fluid treated with acetone	Ascitic fluid treated with ME and acetone	
9	162,240	0	2.4
16	80	10	2.1

ME = 2-mercaptoethanol

Pristane, inactivated *Staphylococcus aureus* cells (90 million cells), respectively, were applied to BALB/c mice prior immunization. In the present studies, monoclonal antibodies No. 9 and No. 16 were used.

Haemagglutination-inhibition (HI) tests. HI tests were carried out according to the methods described by Clarke and Casals (1953). Complement-fixation (CF) tests were performed as described by Casals (1967).

Results

Nine strains of TBE complex have been compared: Powassan, louping-ill, Central European TBE (the prototype Hypr strain and the Skalica strain), Far Eastern TBE, Omsk haemorrhagic fever (OHF), Negishi, Kyasanur forest disease (KFD) and Langat, using monoclonal antibodies to the Skalica strain of TBE. The hybridomas were found to produce antibodies of IgM class (Table 1) since the neutralization activity of the monoclonal antibodies was detected only when anti-IgM antibodies were added; the neutralization

Table 2. The haemagglutination-inhibiting (HI) titres of monoclonal antibodies to some arboviruses of group B (family Togaviridae)

Arbovirus antigen	HI titre with the clone 9		HI titre with TBE antiserum
	1st sample	2nd sample	
Tick-borne encephalitis	10,240	10,240	320
Omsk haemorrhagic fever	1,280	1,280	160
Kyasanur forest disease	0	0	0
West Nile	0	0	80
Dengue type 1	0	0	10
Dengue type 2	0	0	0
Dengue type 3	0	0	40
Dengue type 4	0	0	80
Yellow fever	0	0	20
Sindbis	0	0	0

Table 3. The titres of monoclonal antibodies in haemagglutination-inhibition (HI) tests with viruses belonging to the tick-borne encephalitis complex (30 min incubation)

Antigen	HI titres with the monoclonal antibody No. 9		HI titre with reference TBE antiserum
	Ascitic fluid prepared with Freund's adjuvant	Ascitic fluid prepared by inoculation of inactivated <i>Staphylococcus aureus</i>	
TBE Hypr	640	320	160
TBE Skalica	640	320	80
Louping-ill	640	320	40
Far Eastern TBE	320	160	160
Negishi	640	320	80
Powassan	0	0	10
Omsk haemorrhagic fever	640	320	160
Kyasanur forest disease	40	40	80
Langat	320	160	80

index (NI) of the clone 9 was 2.4 and that of the clone 16 was 2.1. The monoclonal antibodies showed no complement-fixing activity, but they had a HI activity reacting exclusively with the haemagglutinins of the Skalica and OHF viruses (Table 2). A close antigenic relationship was demonstrated between Central European TBE, louping-ill, Negishi and Omsk haemorrhagic fever viruses by 30 min incubation with the antigens listed above (Table 3). The HI titres with these antigens were 640, whereas with the Powassan virus the titre was 64 times and with the KFD virus 16 times lower.

When incubation of monoclonal antibodies with the antigens of the TBE complex was prolonged to 18 hr, HI titres of 640—1280 were obtained with TBE virus, Hypr and Skalica strains, with louping-ill, Far Eastern TBE virus, Negishi and OHF viruses. No reaction was obtained with the Powassan virus (Table 4). The HI titres with KFD virus were 4 times lower and with Langat virus the HI titre was 8 times lower.

By the use of monoclonal antibodies to the Skalica strain of TBE virus, it was possible to differentiate by the haemagglutination-inhibition tests the Powassan virus, isolated in Nearctic geographical regions from the viruses of TBE complex, isolated from Palearctic regions.

Discussion

Louping-ill, Negishi, Central European and Far Eastern TBE viruses form a compact complex in respect to their antigenic composition (Clarke, 1964). However, by the kinetics of the HI and complement-fixation tests it was possible to differentiate Central European TBE strains (Grešiková and Sekeyová, 1981). The differences observed were regarded as significant

Table 4. The titres of monoclonal antibodies in haemagglutination-inhibition (HI) tests with viruses belonging to the tick-borne encephalitis complex (18 hr incubation)

Antigen	HI titres with monoclonal antibody No. 9		HI titre with reference TBE antiserum
	Ascitic fluid prepared with Freund's adjuvant	Ascitic fluid prepared by inoculation of inactivated <i>Staphylococcus aureus</i>	
TBE Hypr	1 280	640	160
TBE Skalica	1 280	640	80
Louping-ill	640	320	40
Far Eastern TBE	640	640	320
Negishi	1 280	640	160
Powassan	0	0	20
Omsk haemorrhagic fever	1 280	640	160
Kyasanur forest disease	320	160	80
Langat	160	160	80

characteristics of the intratypic groups of TBE. However, quantitative evaluation of enzyme immunoassay results with monoclonal antibodies to the structural glycoprotein of TBE virus did not reveal any differences in reactivity among TBE virus strains isolated in different European natural foci (Heinz *et al.*, 1982).

In this study on antigenic relationships among the viruses of TBE complex, monoclonal antibodies (Novák *et al.*, 1983) were used. The virus used for immunization was the Skalica strain (Grešíková *et al.*, 1976), which is non-pathogenic for adult white mice after subcutaneous inoculation (Grešíková and Sekeyová, 1981; Rajčáni and Grešíková, 1982) so that it does not require any inactivation before immunization. The supernatants from hybridomas and ascitic fluids were found to produce highly specific HI antibodies of the IgM class. The specificity of ascitic fluids was proved by HI tests, in which 10 togaviruses were tested. HI activity was observed only with the viruses of the TBE complex. Out of nine strains of the TBE complex studied in HI test with monoclonal antibodies, 4 antigenically closely related strains were found: Central European TBE, louping-ill, Negishi and Omsk haemorrhagic fever viruses. These findings may be a useful tool in studies on the evolution of TBE viruses as well as for a potential use of the Skalica strain of TBE virus as a candidate for a vaccine not only against Central European TBE but also against louping-ill, OHF and Negishi.

References

- Casals, J. (1967): Immunological techniques for viruses, pp. 163—181. In: K. Maramorosch and H. Koprowski (Eds): *Methods in Virology*, vol. 3, Academic Press, New York—London.
- Clarke, D. H., and Casals, J. (1958): Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. *Am. J. trop. Med. Hyg.* **7**, 561—573.

- Clarke, D. H. (1964): Further studies on antigenic relationships among the viruses of the group B tick-borne encephalitis complex. *Bull. Wld Hlth Org.* **31**, 45—56.
- Grešíková, M., Mrciak, M., Brtek, V., and Sekeyová, M. (1976): Isolation and identification of tick-borne encephalitis virus from a bank vole (*Clethrionomys glareolus*) trapped in the vicinity of Radimovsky forest (Western Slovakia), pp. 105—110. Proceedings of 2. *Internat. Arbeitskolloquium über Naturherde von Infektionskrankheiten in Zentraleuropa*, Graz, 25. 2.—28. 2. 1976.
- Grešíková, M., and Sekeyová, M. (1981): Some aspects of biological and antigenic properties of freshly isolated tick-borne encephalitis virus strains, pp. 150—161. In Ch. Kunz (Ed.): *Tick-borne encephalitis*. Proc. Intern. Symp. Baden/Vienna. Facultas-Verlag, Wien.
- Heinz, F. X., Berger, R., Majdic, O., Knapp, W., and Kunz, Ch. (1982): Monoclonal antibodies to the structural glycoprotein of tick-borne encephalitis virus. *Infect. Immun.* **37**, 869—874.
- Novák, M., Grešíková, M., Sekeyová, M., Russ, G., Zikán, J., Pospíšil, M., and Čiampor, F. (1983): Production and characterization of hybridomas cell line secreting monoclonal antibodies with haemagglutination-inhibiting activity to tick-borne encephalitis virus. *Acta virol.* **27**, 34—42.
- Rajčáni, J., and Grešíková, M. (1982): Pathogenicity of the Skalica strain (from the tick-borne encephalitis complex) for white mice. *Acta virol.* **26**, 264—269.