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DIFFERENTIATION IN THE COMPLEMENT FIXATION TEST OF THE VIRUSES OF TICK-BORNE ENCEPHALITIS COMPLEX BY MEANS OF A TYPE-SPECIFIC SOLUBLE ANTIGEN

S. YA. GAIDAMOVICH, V. A. DEMENEV, V. R. OBUKHOVA

The D. I. Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences, 123 098 Moscow

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Summary. — Highly type-specific soluble antigens (SA) of viruses of the tick-borne encephalitis (TBE) complex were obtained by treatment of the sucrose-acetone antigens with 8 mol/l urea. Each SA reacted with the homologous serum only. The serum to SA of strain Sofyin and of Omsk haemorrhagic fever virus distinguished the TBE complex representatives; the difference between homologous serum titres was in the range of 4—5 two-fold dilution steps.

Key words: viruses of the tick-borne encephalitis complex; soluble antigen; serum to soluble antigen; complement fixation test

Introduction

The differentiation of viruses of the TBE complex has remained a problem up to present. In the majority of serological tests the typing of individual representatives of the complex is rather difficult. The differentiating capacity of virus antigens and antisera varies in respect with intraseries antigenic relations (Rzhakhova, 1967), the differences being of quantitative nature. We have tried to approach the problem by fulfilling the principle "all or none", i.e. aiming at a qualitative differentiation using conventional antisera.

As demonstrated with the Langat (Smith and Holt, 1961) and TBE viruses (Vereta and Levkovich, 1968; Lyapustin *et al.*, 1983) the representatives of the TBE complex can induce the synthesis of a nonstructural soluble antigen (SA) in infected cells. Studying other flaviviruses, a number of investigators have shown the high type-specificity of SA allowing to differentiate between the viruses of dengue complex (Cloud *et al.*, 1971), Japanese encephalitis virus (Lavrova and Obukhova, 1978), and West Nile virus (Gaidamovich, Lavrova, 1973). A somewhat lower differentiating capacity was found with the sera to SA among the geographic variants of the West Nile virus (Lavrova and Gaidamovich, 1978).

In this paper we report on the typing of prototype strains of TBE complex in the complement fixation (CF) test by means of SA and anti-SA sera.

Materials and Methods

Viruses of TBE complex were obtained from the museum of viruses of the D. I. Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences: Omsk haemorrhagic fever (OHF), strain Kubrin — 5th passage; Langat, strain TR-21 — 10th passage; Powassan, strain LB —

Table 1. Typing of TBE complex viruses using SA and SAA in CF test with IAF to complete virion

IAF	Antigens (Virus strains) Sofyin		Absettarov		OHF		Powassan		Louping ill		Negishi		KFD		Langat	
	SAA	SA	SAA	SA	SAA	SA	SAA	SA	SAA	SA	SAA	SA	SAA	SA	SAA	SA
Sofyin	**640**	**160**	40	0	80	0	160	0	80	0	320	0	640	0	320	0
Absettarov	160	0	**160**	**40**	80	0	40	0	20	0	80	0	80	0	320	0
OHF	80	0	40	0	**80**	**20**	160	0	40	0	40	0	80	0	320	0
Powassan	20	0	40	0	40	0	**640**	**80**	20	0	40	0	20	0	20	0
Louping ill	80	0	40	0	40	0	0	0	**160**	**80**	80	0	40	0	20	0
Negishi	80	0	40	0	40	0	320	0	20	0	**320**	**80**	80	0	20	0
KFD	320	0	40	0	40	0	320	0	20	0	160	0	**640**	**160**	80	0
Langat	80	0	40	0	40	0	160	0	20	0	160	0	160	0	**320**	**80**

Notice: antigen titres expressed as dilution reciprocals

4th passage; Kyassanur forest disease (KDF), strain R-9605 — 7th passage; sheep encephalitis (louping ill), strain I-40 — 7th passage; prototype Negishi strain — 6th passage; tick-borne spring summer encephalitis (TBE), strain Sofyin — 11th passage, strain Absettarov — 6th passage. The viruses were maintained in the brain of 2 to 3-day-old laboratory albino mice, the infectivity of the strains at intracerebral (i.c.) inoculation was 8.5—9.0 log LD_{50}/0.03 ml.

Antigens. Sucrose-acetone antigen (SAA) was prepared according to Clarke and Casals (1958) from the brain of 2—3-day-old infected mice. SA was prepared as follows: dry urea was added to SAA up to a final concentration of 8 mol/l (4.8 g urea per 10 ml of antigen) and the flask with the treated antigen was placed into 37—40 °C water bath. The required amount of urea was poured out instantly to finish dissolution within 10—15 sec. Then the antigen was placed into incubator (at 37 °C) for 10—30 min, then the mixture was dialyzed for 20—24 hr at 4 °C against

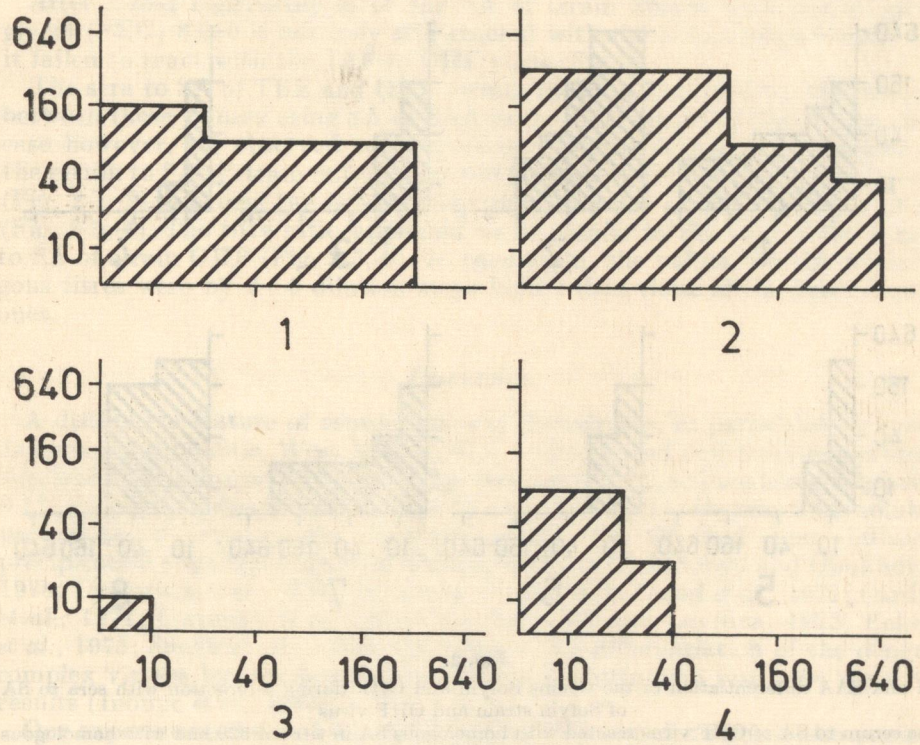

Fig. 1.

Antigenic interactions of the soluble (SA) and sucrose-acetone (SAA) antigens from strain Sofyin with homologous and heterologous IAF

After 5-fold concentration with PEG 6,000 SA reacts with the immune serum of homologous strain only.

1 — IAF to the complete virion of Sofyin strain and SA;
2 — IAF to the complete virion of Sofyin strain and SAA;
3 — IAF to the complete virion of OHF virus and SA;
4 — IAF to the complete virion of OHF virus and SAA.

Abscissae: IAF dilution reciprocals; ordinates: antigen dilution reciprocals.

100 volumes of borate buffer (pH 9.0). The resulting soluble antigen remains active for 2 months.

Sera. Immune ascites fluids (IAF) against whole virus were prepared using the method proposed by Gaidamovich *et al.* (1969). Rabbit immune sera to the SA of the strains Sofyin and OHF were prepared according to Backhausz (1967) in 7-month-old animals.

Complement fixation (CF) test was performed by micromethod with 2 units of complement.

Results

As shown in Table 1, 8 mol/l urea treatment of SAAs diminished the group-specific antigenic reactions to unnoticeable levels in the CF test. However, the incubation time required for a complete extinguishion of the group-speci-

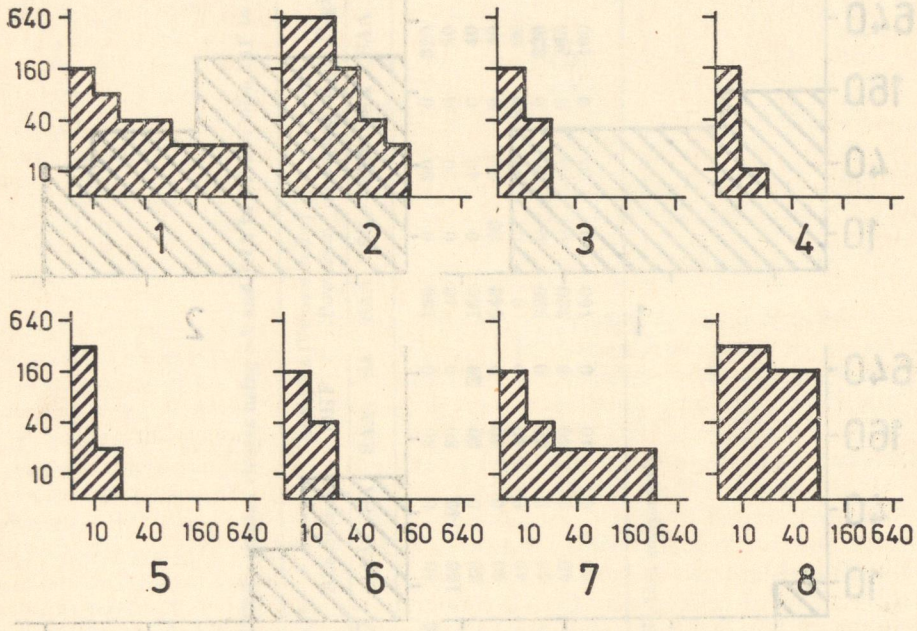

Fig. 2.

SA and SAA differentiation of the strains Sofyin and OHF during interaction with sera to SA of Sofyin strain and OHF virus

The serum to SA of OHF virus reacted with homologous SA in titre of 320 and with homologous SAA in titre of 80. The anti-SA serum of the Sofyin strain reacted in titres 640 and 160 with homologous SA and SAA, respectively. Heterologous titres were not higher than 20 in either case.

1 — Serum to SA Sofyin reacted with SA Sofyin
2 — Serum to SA Sofyin reacted with SAA Sofyin
3 — Serum to SA Sofyin reacted with SA OHF
4 — Serum to SA Sofyin reacted with SAA OHF
5 — Serum to SA OHF reacted with SA Sofyin
6 — Serum to SA OHF reacted with SAA Sofyin
7 — Serum to SA OHF reacted with SA OHF
8 — Serum to SA OHF reacted with SAA OHF

Abscissae: serum dilution reciprocals; ordinates: antigen dilution reciprocals.

ficity and the emergence of the species-specificity ranged from 10 to 30 min. The incubation time varied from experiment to experiment and was not a distinctive feature of the virus strain. CF activity of SA was 2 to 8 times lower than the baseline activity of SAA before urea treatment.

The differentiating capacity of the SA obtained in our experiments allowed a distinct typing of all the viruses of TBE complex; SA reacted only with the homologous sera. We observed no cross-reactions neither between antigenically related TBE and OHF viruses, nor between the geographic variants of TBE strains Sofyin and Absettarov. In some cases the difference between homologous and heterologous titres to SAA allowed to identify the individual representatives of the TBE complex.

After 5-fold concentration of the SA of strain Sofyin with polyethylene glycol (PEG) 6,000 it not only still reacted with the homologous serum, but it failed to react with the IAF to OHF virus (Fig. 1).

The sera to SA of TBE and OHF viruses could be also used to differentiate between these viruses using SA or SAA in the cross-titration (Fig. 2). In such case however, we observed some cross-interactions as homologous titres of the serum to SA of strain Sofyin were 640 for SA and 160 for SAA, respectively (Fig. 2-1,-2), whereas the heterologous titres did not exceed the value of 20 (Fig. 2-3,-4). The titre ratio appeared to be similar in the case of the serum to SA of strain OHF (Fig. 2-5,-8). In conclusion, the values of the homologous titres were by 3—5 dilution steps higher than those of the heterologous ones.

Discussion

A distinctive feature of reproduction of flaviviruses, in particular, dengue, Japanese encephalitis, West Nile, TBE is a virus-coded nonstructural protein deprived of haemagglutination activity resistant to 0.1% sodium laurylsulphate, 0.1% 2-mercaptoethanol and 6—8 mol/l urea. This highly thermostable soluble protein has a type-specific antigenic activity in CF and agar diffusion precipitation tests (Lavrova and Gaidamovich, 1975; Lavrova and Obukhova, 1978; Lyapustin *et al.*, 1983; Brandt *et al.*, 1970*a*, *b*; Cloud *et al.*, 1971; Cardiff *et al.*, 1971; Cornesky *et al.*, 1972; Gaidamovich and Lavrova, 1973; Eckels *et al.*, 1975; Smith *et al.*, 1970). In contrast, SA-differentiation of the dengue complex viruses by immune adherence haemagglutination test gave negative results (Inouye *et al.*, 1980).

Our experiments have shown that SAs of TBE complex viruses also possess a marked antigen specificity — this is likely related to the fact that SA protein contains only a type-specific determinant. We believe that in each particular case the choice of duration of the contact with 8 mol/l urea required for the degradation of the virus-specific proteins and the resulting disappearance of group-reactive determinant is determined by different multiplicity of the infection of the brain cells. This causes different accumulations of both virus-specific structural proteins, and of the virus-induced SA protein. We consider the decrease of CF activity of SA to the level of CF activity of SAA, for a decrease of the amount of the substrate in the antigen-antibody reaction. The

2 to 8-fold variations of CF activity can be also accounted for the different multiplicity of infection in each particular case.

The research undertaken by Lavrova and Gaidamovich (1978) demonstrated the dissimilar differentiating capacity of the serum to SA of the Indian and African variants of the West Nile virus, since, according to the authors, SA of the Indian strain contains both antigenic determinants — a group- and a species-specific. We have shown that the SA of the TBE complex viruses contains only a species-specific determinant. Nevertheless, the serum to SA of the strains Sofyin and OHF reacted also with the heterologous representatives. Our explanation of this is that in the course of the 8 mol/l urea treatment, the virus-specific proteins were deteriorated to a level negligible to react in CF, but sufficient to induce antibody response.

Thus, our investigations have shown that SA-differentiation of prototype strains of TBE complex viruses can be approached by the principle of "all or none", which significantly simplifies the identification. However, we cannot rule out that the antigenic SA-interactions with other geographic variants of the TBE complex, especially of the TBE virus, the results would be different. Recently monoclonal antibodies were obtained that could detect group and type specific determinants of TBE complex viruses (Grešíková *et al.*, 1984; Stephenson *et al.*, 1984) but the SA differentiation is much cheaper than the hybridoma techniques.

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