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VESICULAR STOMATITIS VIRUS PSEUDOTYPE WITH NEUTRALIZATION ANTIGEN OF TICK-BORNE ENCEPHALITIS VIRUS

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Summary. — Vesicular stomatitis virus (VSV), when grown in dual infection with tick-borne encephalitis (TBE) virus, produced pseudotype VSV (TBE) with surface proteins provided by TBE virus. This phenomenon can be employed in a rapid, accurate and sensitive test for detection and assay of neutralizing antibodies specific for TBE virus.

Key words: vesicular stomatitis virus; tick-borne encephalitis virus; phenotypic mixing

VSV grown in mixed infection with other enveloped viruses often forms pseudotype particles. These contain the genome (as well as internal proteins) of VSV, but a majority of surface proteins is provided by the other virus. Therefore they are resistant to VSV specific neutralizing antibodies, but they are sensitive to antibodies specific for the donor of surface antigens (Závada, 1982). Such VSV pseudotypes afford a sensitive, rapid and accurate assay system for neutralizing antibodies directed to enveloped viruses which do not produce plaques at all, or only after a prolonged incubation period. So far, it has been used mostly with various oncoviruses.

Since TBE virus does not plaque easily, we investigated the possibility of producing VSV (TBE) pseudotypes which could serve for detection and titration of TBE virus-specific neutralizing antibodies.

The Indiana type of VSV and the Skalica strain of TBE virus were used. Skalica strain, when compared to the standard TBE virus strains, is characterized by a decreased virulence, while serologically it is indistinguishable from them (Grešíková and Sekeyová, 1979).

In an experiment aimed at demonstrating the VSV (TBE) pseudotypes, chick embryo cell (CEC) cultures were infected first with TBE virus at multiplicity of infection (MOI) of 0.1 PFU/cell. After 48 hr of incubation at 37 °C, the cultures were superinfected with VSV (MOI= 100 PFU/cell). A parallel CEC culture was infected with VSV only at MOI of 10 PFU/cell (TBE-preinfected cultures required a higher MOI of VSV to overcome the virus interference). Progeny virus was harvested after additional incubation at 37 °C for intervals indicated in Table 1, and then plaque-assayed either untreated with antisera (= total VSV) or after treatment with anti-VSV serum [= VSV(TBE) pseudotype]. The neutralization mixtures contained infectious tissue culture fluid diluted 1 : 10 and anti-VSV serum diluted 1 : 500. This serum had a 50 % neutralization end-point titre of 2×10^6 for 100 PFU of VSV. Plaque assay for VSV as well as for the VSV (TBE) pseudotype was performed in mink CCL 64 cell line; this one represents a selective plaquing system for virions with VSV genome. Both VSV and TBE virus plaque in CEC, although TBE plaques appear only after 5 to 7 days. In the pseudotype neutralization test for TBE virus-

Table 1. Production of VSV and of VSV (TBE) pseudotypes in dependence on time after VSV infection

Treatment with anti-VSV serum	VSV and TBE virus titres (log PFU/ml) in CEC cultures infected with			
	pure VSV		VSV + TBE virus*	
	no	yes	no	yes
Hours after VSV infection:				
4	7.5	<1	6.9	<1
4.5	7.9	<1	7.5	1.3
5	8.2	<1	7.9	1.7
5.5	8.8	<1	8.3	3.9
6	9.0	<1	8.9	2.7
8	9.2	<1	9.0	<1
10	9.2	<1	8.9	<1

* VSV grown in the cells pre-infected with TBE virus.

specific antibodies, the reacting mixtures contained appropriate dilution of VSV produced in a culture mixedly infected with TBE virus, anti-VSV serum so as to give 100 PFU/ml of VSV (TBE) pseudotype per ml and dilution of test human sera as indicated in Table 2. These mixtures were incubated for 60 min at 37 °C and subsequently plaqued in mink CCL 64 cells.

The haemagglutination inhibition test (HIT) was carried out according to Clarke and Casals (1958); the complement-fixation test (CFT) by the micromethod of Casals (1967). For virus neutralization test (VNT) was used TBE virus in dilution of 100 PFU per ml. The virus was mixed with diluted test sera, incubated at 37 °C for 90 min, and plated for plaques in CEC. The plaques were counted after 7—9 days. The results of this VNT modification are in agreement with those of tube-neutralization test (Libíková and Vilček, 1961).

The purpose of the first experiment was to find whether VSV can produce pseudotype virions with TBE virus specific surface antigen, and if so, what

Table 2. Comparison of HIT. CFT. VNT and PsNT

No. of serum	Antibody titres with the used test			
	HIT	CFT	VNT	PsNT
1	40	0	20	40
2	640	32	16	32
3	20	0	0	0
4	2 560	NT	32	64
5	640	NT	16	64
6	20	NT	0	0
7	0	0	0	0

NT = not tested

is the optimum interval for harvesting the phenotypically mixed virus. The results (Table 1) showed, that indeed, in mixed infection with TBE virus, VSV produced a fraction of virions resistant to an excess of anti-VSV serum — presumably the VSV (TBE) pseudotype. This pseudotype reached its highest titre 5.5 hr after VSV infection, dropping rapidly at later intervals. Apparently, the pseudotype is rather labile and is rapidly heat-inactivated. In the parallel culture, infected with VSV only, no such fraction of infectivity resistant to anti-VSV serum was detected.

Identity of this VSV infectivity fraction, resistant to anti-VSV serum, as the VSV (TBE) pseudotype is presented in Table 2. In this table, a set of human sera was parallely tested for neutralization of VSV (TBE) pseudotype in pseudotype neutralization test (PsNT) of TBE plaque forming capacity in VNT and in HI and CF tests. It can be seen that highest antibody titres in PsNT were two-fold higher than titres in simple TBE virus neutralization. Although both the simple VNT and the PsNT detect the same type of antibodies (neutralizing antibodies), the HIT and CFT each detects antibodies directed to different viral antigens. A similarly superior sensitivity of PsNT compared to simple VNT has been observed also before in a different system (Clapham *et al.*, 1984).

Since the VSV (TBE) neutralization test turns out to be specific and easy to perform, it appears to be suitable for assay of neutralizing antibodies directed to TBE virus in the sera of patients with the clinical diagnosis of TBE. Of advantage is the use of TBE virus strain Skalica, characterized by a decreased virulence, which lowers the risk of laboratory TBE infection. The PsNT has two advantages over the simple VNT: it gives two-fold higher antibody titres, and its results can be scored as early as after 24 hr, while the simple TBE VNT requires 5—7 days.

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