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PHENOTYPIC MIXING BETWEEN VESICULAR STOMATITIS AND UUKUNIEMI VIRUSES

J. DRAGÚŇOVÁ, O. KOŽUCH, M. GREŠÍKOVÁ

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

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Summary. — The population of vesicular stomatitis virus (VSV) which reproduced in cells preinfected with Uukuniemi virus (UUK) contained a proportion of VSV(UUK) pseudotypes. The virions containing the VSV genome were resistant to anti-VSV serum and neutralized with anti-UUK serum. In addition to previous reports on phenotypic mixing of different families of enveloped viruses, the combination of rhabdovirus surface antigen with bunyavirus genome is described.

Key words: *Rhabdoviridae*; *Bunyaviridae*; *phenotypic mixing*

Phenotypic mixing between representatives of different enveloped virus families has been described especially between VSV and avian or murine leukaemia viruses (Závada, 1972). Phenotypic mixing has been also demonstrated between RNA and DNA viruses, e. g. between VSV and herpes simplex virus (Huang *et al.*, 1974) or vaccinia virus (Lukashevich and Závada, 1982). As envelope glycoprotein donor may serve also viruses not producing complete virions, the synthesis of functional surface glycoprotein(s) being a sufficient condition. This is exemplified by pseudotype of VSV with the chick helper factor (Love and Weiss, 1974), or with defective measles virus isolated from a patient with subacute sclerotizing panencephalitis (Wild *et al.*, 1976). It has been suggested, that phenotypic mixing might be universal in any pairwise combination of enveloped viruses, although representatives of some viral families have not been tested yet (Závada, 1982). One of these is the family Bunyaviridae; therefore, we decided to test the mixing of Uukuniemi virus with VSV (type Indiana).

A confluent monolayer of mink CCl 64 cells (Petri dishes, diameter of 5 cm) was infected first with Uukuniemi virus, supplied with Basal Eagle's medium containing 5% of inactivated calf serum and incubated at 37 °C for 48 hr. Subsequently, the cultures were infected with VSV at multiplicity of infection (MOI) of 100 PFU/cell and the samples taken at various incubation intervals (at 37 °C) were plated in mink cells. A parallel titration of neutralized and non-neutralized samples was performed. Neutralization test was carried out at 37 °C for 90 min with sheep anti-VSV serum (final dilution in reaction mixture was 1 : 500). The anti-VSV serum had a 50% neutralization titre

Table 1. Time dependence of VSV (Uukuniemi) pseudotype production

Hours after infection with VSV	VSV only		VSV + UUK*	
	Treatment with anti-VSV serum			
	no	yes	no	yes
3	5.8	< 1	4.9	< 1
4	7.0	< 1	5.9	1.3
4.5	7.5	< 1	6.5	3.8
5	8.2	< 1	7.2	4.2
5.5	8.5	< 1	7.9	2.5
6	9.0	< 1	8.0	< 1
6.5	9.2	< 1	8.2	< 1
8	9.0	< 1	8.5	< 1
10	9.0	< 1	8.8	< 1

* grown in cells preinfected with Uukuniemi virus
Data indicate titres in log PFU/ml

of 2×10^6 for 100 PFU. Serial tenfold dilutions were plated for plaques. Under these conditions, the virions with Uukuniemi virus genome do not form any visible plaques. As control, VSV grown in mink cells was used. The plaques were counted within 24 hr incubation at 37 °C.

To identify the apparent VSV pseudotype, additional test sera were included in the neutralization experiment. For this purpose, the progeny of mixed UUK + VSV infection was harvested at the interval of 5 hr after superinfection with VSV, when according to orientation experiment the apparent pseudotype reached its maximum of infectivity. The infectious

Table 2. Neutralization of VSV phenotypically mixed with Uukuniemi virus

Serum No.	VNT	PsNT
1	10 000	40 000
2	< 10	< 10
3	< 10	< 10
4	< 10	< 10
5	< 10	< 10
6	< 10	< 10

1 = Mouse serum with neutralizing antibodies to Uukuniemi virus
2 = Mouse serum against mouse hepatitis virus (50% neutralization titre = 1000)
3 = Human serum with neutralizing antibodies to tick-borne encephalitis virus (50% neutralization titre = 64)
4. 5. 6 = Normal mouse sera
VNT = virus neutralization test (the test was carried out according to the method of Kožuch and Mayer (1975).
PsNT = pseudotype neutralization test

medium was diluted as above and incubated with anti-VSV serum for 30 min. Residual infectious virus was diluted to give 100 PFU/ml, then it was mixed with additional test sera and further incubated at 37 °C for 60 min. Finally, the neutralization mixtures were plated for plaques.

The infectious titre of control VSV and VSV grown in the presence of Uukuniemi virus increases as a function of time (Tab. 1). After neutralization with anti-VSV serum, the infectivity of control VSV drops below the level of detection. On the other hand, VSV grown with Uukuniemi virus show a fraction, resistant to anti-VSV serum, which reaches maximum at 5 hr after superinfection of the cells with VSV. This fraction indicates that phenotypic mixing of VSV with UUK has taken place.

The aim of neutralization experiment with phenotypical mixed VSV was to decide, whether this anti-VSV serum resistant fraction represents the VSV(UUK) pseudotype, or the pseudotype of VSV with some contaminating viruses (e. g. mouse hepatitis virus). These possibilities have been suspected, since UUK had been previously passaged in mouse brains. All control sera caused only insignificant neutralization of pseudotype VSV (UUK). Only the anti-Uukuniemi hyperimmune serum neutralized the pseudotype (Tab. 2). The 50% neutralization titre was 4-fold higher for VSV(UUK), than for UUK only (assay performed in PS cells). This test demonstrated that pseudotype particles VSV(UUK) could have been produced.

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DISTRIBUTION OF MOUSE CYTOMEGALOVIRUS IN ORGANS OF WHITE MICE EXPERIMENTALLY INFECTED BY NATURAL ROUTE

J. SVOBODOVÁ, *D. BLAŠKOVIČ, D. HUČKOVÁ

Department of Virology and Microbiology, Faculty of Natural Sciences, Comenius University and *Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

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Summary. — One, 10, 21-day-old and adult mice were inoculated by peroral and/or intranasal routes with mouse cytomegalovirus (MCMV). In animals surviving generalized infection, the virus could be demonstrated in salivary glands up to 123 days post-infection (p. i.). In mouse females which had eaten their infected and diseased offspring, the virus was detectable in salivary glands up to day 121, p. i. On day 16 p. i., the virus was present in salivary glands, lungs and kidneys of mice of different age groups, but no virus was recovered from their Gasserian ganglia. These results were compared with those obtained after infection with murine alphaherpesvirus.

Key words: *mouse cytomegalovirus*; *experimental infection*; *virus distribution*

Introduction

Pathogenetic studies with murine herpesvirus (MHV) (Blaškovič *et al.*, 1980, Čiampor *et al.*, 1981; Svobodová *et al.*, 1982*a*, *b*) in mice infected by natural route demonstrated the presence of this virus in various organs of infected animals and the dynamic nature of its persistence in lungs and latency in Gasserian ganglia (Blaškovič *et al.*, 1984; Rajčáni *et al.*, 1985). No virus was demonstrated in the salivary glands of MHV-infected laboratory mice. Because the population of free living small rodents *Apodemus flavicollis* and *Clethrionomys glareolus* in Czechoslovakia (Mistríková and Blaškovič, 1985) showed positivity for both MHV and MCMV, it appeared reasonable to compare the persistence of MCMV in salivary glands with the persistence of MHV in Gasserian ganglia. This study was performed also with the aim to find further biological criteria distinguishing between MHV (subfamily Alphaherpesvirinae) (Matthews, 1982) and MCMV.

Materials and Methods

Outbred 1, 10, 21 day old and adult white mice from Dobrá Voda breed were used throughout. In addition, females which have eaten their infected and diseased offspring were included into the experiments. The route of infection and the infectious dose of MCMV strains Smith (1954)

Table 1. One-day-old suckling mice infected by oral route with $10^{3.5}$ $TCID_{50}$ in 0.01 ml of MCMV (strain Smith)

Organs	Days p.i.							
	2	4	5	7	10	12	13	16
Salivary glands	+	+	+	+	+	+	+	+
Lungs	+	+	+	+	+	+	+	+
Liver	—	—	n.d.	n.d.	n.d.	n.d.	—	—
Spleen	+	—	+	+	tox	n.d.	+	+
Kidney	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	n.d.	—	—
Brain	—	—	n.d.	n.d.	n.d.	n.d.	—	—
Gasserian ganglia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	—	—

n.d. = not done; — = no virus recovered; tox = toxic suspension

used in a titre of $10^{4.5}$ $TCID_{50}$/0.1 ml imitated natural infection. Volumes of 0.01, 0.02, 0.05 and 0.1 ml respectively, were given perorally (p.o.) and/or intranasally (i.n.).

The presence of the virus distributed via blood to different organs of infected animals was proved by inoculation of the 10% homogenized organ suspension to the primary mouse embryo cell (MEC) monolayer cultures cultivated under standard conditions in basal Eagle's medium supplemented with 10 per cent inactivated bovine serum.

Results

The virus distribution at different time intervals p. i. in one-day-old mice is presented in Table 1. The infected suckling mice became ill and the severe diseased offspring were eaten by their mothers. When these were investigated on days 76, 89 and 121, MCMV was recovered from their salivary glands, but not from their lungs, spleen and kidneys. Ten days old mice have not developed any overt symptoms of disease. Animals killed by aether

Table 2. Ten day-old suckling mice infected by oral route with $2 \times 10^{3.5}$ $TCID_{50}$ in 0.02 ml of MCMV

Organs	Days p.i.				
	2	5	8	13	16
Salivary glands	+	+	n.d.	+	+
Lungs	+	+	+	+	+
Liver	—	—	n.d.	—	—
Spleen	+	+	n.d.	—	—
Kidney	+	+	n.d.	—	+
Brain	n.d.	—	n.d.	—	n.d.
Gasserian ganglia	n.d.	—	n.d.	—	n.d.
Tonsils, mouth mucosa	+	+	n.d.	n.d.	n.d.
Trachea	n.d.	+	n.d.	n.d.	n.d.

Explanation as in Table 1.

Table 3. 21-day-old mice infected by oral route with $5 \times 10^{3.5}$ $TCID_{50}$ in 0.05 ml of MCMV

Organs	Days p.i.				
	3	5	7	10	18
Salivary glands	+	+	+	+	+
Lungs	+	+	+	−	−
Liver	−	−	−	−	−
Spleen	+	+	+	−	−
Kidney	−	+	+	−	n.d.
Heart	+	−	−	−	−
Brain	−	−	−	−	−
Gasserian ganglia	n.d.	−	−	−	−

Explanation as in Table 1.

anaesthesia at different time intervals revealed the presence of infectious virus in organ suspensions (Table 2).

In 21-day-old mice inoculated similarly as the 10-day-old animals the virus could be detected up to day 18 as indicated in Table 3. Adult mice infected with 0.1 ml of virus with infectious titre of $10^{4.5}$ $TCID_{50}$/0.1 ml were followed for virus persistence. Salivary glands, lungs, spleen and kidneys were investigated on days 31, 42 and 123 p. i. At these intervals the virus was constantly present in salivary glands, in lungs up to the day 31 p. i., its titre in organs reaching $10^{5.5}$ $TCID_{50}$/ml. No virus was recovered from spleen and kidneys at any of these intervals.

Discussion

The aim of this study was to compare the pathogenesis of MHV (Alphaherspesvirinae) first isolated in our laboratory in 1980 with MCMV (Betaherpesvirinae). The pathogenesis of MHV after experimental infection resembling natural conditions of virus infection and its possible transmission (horizontal infection from mother to the offspring, excretion by nasal or oral discharge and by urine to the environment) were previously described (Blaškovič *et al.*, 1984; Rajčáni *et al.*, 1985). The pathogenesis of MCMV was described by Lussier (1973), Hudson (1979) and Tonari and Minamishima (1983). The main interest was paid to long-term virus persistence in different organs of experimental animals to investigate its significance in nature and possibly also in human infections with the viruses of two different genera. MHV persisted in lungs, kidneys and brain of 1-day-old mice for at least 9 days p. i. Ten-day-old animals harboured the virus for at least 11 days in Gasserian ganglia, 14 days in kidneys and 141 days in lungs. In 21 day old mice the MHV could be recovered from Gasserian gnaglia and from kidney up to day 28 and from the lungs up to day 141 p. i. The horizontal transmission from suckling mice to their mothers (oral infection) was effective at least 141—169 days after the presumed infection and the virus could be recovered from Gasserian ganglia and lungs. The virus spread by blood

stream; the persistence in the lungs was of productive type in contrast to the static, nonproductive persistence of herpes simplex virus in experimentally infected rabbits and mice (Rajčáni *et al.*, 1969; Baringer, 1975; Stevens, 1975; Klein, 1982; Rajčáni and Blaškovič, 1986).

In comparison to MHV, the MCMV also spreads by blood stream. The persistence of the virus in salivary gland was decisive for the pathogenesis and for virus excretion. It was accompanied with short-term virus persistence in kidneys and possible virus excretion in urine. No virus presence was detected in neural tissue. The site of virus persistence in lungs and sensory ganglia (MHV), or in salivary glands and lungs (MCMV) and their character may serve as biological marker for distinction of herpesviruses of subfamily Alpha- and Betaherpesvirinae.

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