Acta virol. **37**: 101-105, 1993

FURTHER ISOLATION OF MURINE HERPESVIRUSES FROM SMALL MAMMALS IN SOUTHWESTERN SLOVAKIA

O. KOŽUCH[1], M. REICHEL[1], J. LEŠŠO[2], A. REMEŇOVÁ[2], M. LABUDA[1], J. LYSÝ[3], J. MISTRÍKOVÁ[2]

[1]Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava; [2]Department of Microbiology and Virology, Faculty of Natural Sciences of Comenius University; and [3]Institute of Zoology and Ecosozoology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received January 17, 1992; revised March 5, 1992

Summary. - A total of 69 small mammals of 6 species were collected in localities Marcelová and Kopáč (southwestern Slovakia) and investigated. Two strains of murine herpesvirus (MHV) have been isolated and reisolated from *Apodemus flavicollis*. Both virus strains killed suckling mice after i.c. and i.p. inoculation. Adult mice were killed 4–7 days after i.c. inoculation. Cross antigenic reactions among ^{14}C aminoacid hydrolysate labelled MHV-72 infected Vero cells and mouse immune sera against the two new isolates and rabbit immune sera raised against purified MHV strains No. 72 and No. 76 were done. Profiles of immunoprecipitated proteins are almost identical. All used immune sera cross-reacted with major proteins of MHV-72 strain. We conclude that the two virus isolates from *Apodemus flavicollis* are new strains of MHV.

Key words: murine herpesvirus; small mammals; virus isolation; identification; southernwestern Slovakia

Six strains of a novel MHV were isolated from two species of wild murine rodents *Clethrionomys glareolus* (MHV-60, MHV-68 and MHV-72) and *Apodemus flavicollis* (MHV-76, MHV-78 and MHV Šumava A.f.) from two distant regions of Czech and Slovak Federative Republic. The first isolation of five strains of MHV was done in the region near Bratislava (western Slovakia) and the isolation of the sixth strain of MHV in Šumava, southwestern Bohemia (Blaškovič *et al.*, 1980; Mistríková and Blaškovič, 1985). Previously published data (partial biological, morphological and pathogenetic studies) suggested that MHV belongs to alphaherpesvirus subgroup (Čiampor *et al.*, 1981; Svobodová *et al.*, 1982*a, b*; Blaškovič *et al.*, 1984; Rajčáni *et al.*, 1985). Efstathiou *et al.* (1990*a,b*) investigated the genome of MHV-68 and identified nine genes which encode amino acid sequence with greater similarity to proteins of the gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri than to the homologous products of the alphaherpesviruses varicella-zoster virus and herpes simplex-virus type 1 or the betaherpesvirus human cytomegalovirus.

The aim of this presentation was to identify two isolates of herpes virus from *Apodemus flavicollis* from the region of southwestern Slovakia (Marcelová, Kopáč).

Small mammals were live-trapped in Swedish metal traps, using oat flakes as bait. After transportation to the laboratory in Bratislava the animals were sacrified, necropsied and their organs (brain, lungs, liver and spleen) were collected aseptically. Ten percent suspensions of brains and pools of the other organs were made in 2 ml of minimal essential medium (MEM) containing 10 % heat inactivated newborn bovine serum. Suspensions were centrifuged at low speed 3000 rpm for 15 min and supernatants were inoculated intracerebrally (i.c.) into five 1 to 3 days old mice, 0.01 ml per mouse.

African green monkey kidney cells (Vero), rabbit embryo fibroblast cells (REF), pig kidney epithelial cells (PS) and chick embryo cells were cultured in Eagle's basal medium (BEM) supplemented with 5–10 % inactivated bovine serum (IBS). These cells were used throughout all experiments.

The MHV-72 strain isolated from *Clethrionomys glareolus* and the MHV-76 isolated from *Apodemus flavicollis* (Blaškovič *et al.*, 1980) were compared with two earlier isolates No. 4556 and No. 5682 from *Apodemus flavicollis* collected in southwestern Slovakia. Immunoprecipitation and preparation of immunoprecipitated polypeptides for SDS-gel electrophoresis were carried out according to Raučina *et al.* (1984). The samples were analysed in 10 % polyacrylamide gel cross linked with methylenbisacrylamide as previously described (Matis and Rajčáni, 1980).

Table 1. Murine herpesviruses isolated from organs of mammals collected in two localities of southwestern Slovakia

Locality	Marcelová		Kopáč	
Year of collection	1982		1984	
Species	No. of collected animals	No. of isolates (from organs)	No. of collected animals	No. of isolate (from brains)
Apodemus flavicollis	7	1	26	1
Apodemus sylvaticus	22	0	3	0
Clethrionomys glareolus	N.D.	N.D.	6	0
Microtus arvalis	3	0	N.D.	N.D.
Sorex araneus	1	0	N.D.	N.D.
Sorex minutus	1	0	N.D.	N.D.
In total	34	1	35	1

N.D.: not done

Mouse immune sera were prepared by intraperitoneal (i.p.) inoculations of 16–20 g outbred mice with 0.1 ml of the 10 % brain suspension containing the isolates in six weeks intervals. The rabbit immune sera against purified MHV-72 and MHV-76 were kindly provided by Dr. Stančeková. Virus-neutralization tests (VNT) were performed according to Svobodová *et al.* (1982*b*). The titer of sera in VNT were for MHV-72 – 1:128, MHV-76 – 1:128 MHV-4556 – 1:128 and MHV-5682 – 1:512.

A total of 69 small mammals of 6 species were collected in 1982 and 1984 years in localities Marcelová and Kopáč. The most numerous rodent species were: *Apodemus flavicollis, Apodemus sylvaticus* and *Clethrionomys glareolus* (Table 1). Two strains of murine herpesviruses have been isolated and reisolated from *Apodemus flavicollis:* one strain (No. 4556) from a brain (locality Marcelová, October, 1982) and one strain (No. 5682) from mixture of organs (locality Kopáč, July, 1984).

Both virus strains killed suckling mice after i.c. and i.p. inoculation. The incubation period was 5–7 days in the first mouse passage and was shortened to 3–4 days in subsequent passages. These virus strains killed adult (8–10 g) mice on the day after i.c. inoculation. No symptoms of disease were observed in adults mice after s.c. inoculation.

The virus isolates produced a cytopathic effect in 24 hr growth old chick embryo cell cultures, PS, Vero (clones E_6) and REF cells on 3–5 days after inoculation.

Experiments were done to prove that the two new isolates from *Apodemus flavicollis* are new strains of MHV. Rabbit immune sera raised against purified MHV-72 and MHV-76 strains, respectively, and mouse immune sera raised against virus isolates No. 4556 and No. 5682, respectively, were tested in VNT with the MHV-72 strain. The VNT clearly showed that MHV-72 reacted with both homologous and heterologous immune sera. Svobodová *et al.* (1982*b*) also found that five MHV isolates (No. 60, 72, 76 and 78) reacted in VNT with homologous and heterologous immune sera raised against these isolates. No antigenic relatedness were observed in VNT between MHV strains and HSV-1, HSV-2, pseudorabies virus, bovine herpesvirus type 1 and/or murine cytomegalovirus (Svobodová *et al.*, 1982*b*).

Cross-antigenic reactions were done among ^{14}C aminoacid hydrolysate labelled MHV-72 infected Vero cells and mouse immune sera raised against two isolates No. 4556 and No. 5682 and rabbit immune sera raised against purified MHV strains 72 and 76.

The electrophoretic profiles of immunoprecipitated proteins are almost identical. All used immune sera cross-reacted with major proteins of MHV-72, for example with 161, 138, 84; 74, 50, 36, 28.5 K (Fig. 1). The mouse immune sera against isolates No. 4556 and No. 5682 were prepared against 10 % brain suspension of infected mice. The rabbit immune sera were prepared against purified MHV-72 and MHV-76 (Svobodová *et al.*, 1982*b*). This is a possible explanation why mouse immune sera precipitated more MHV-72 – specific polypeptides than rabbit immune sera.

Two control experiments were done. Infected Vero cells with the MHV-72 strain were immunoprecipitated with nonimmune rabbit and nonimmune

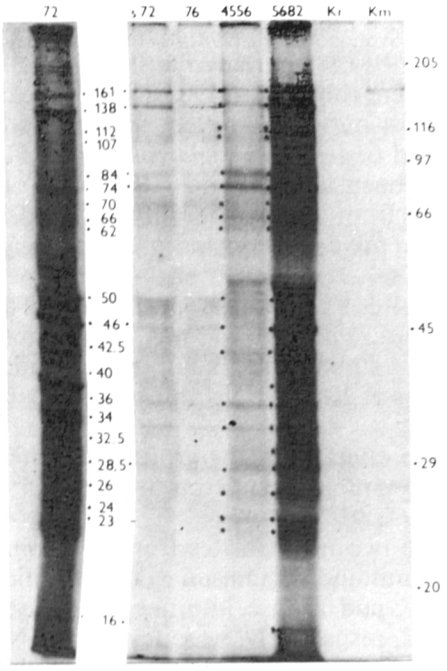

Fig. 1
Electrophoretic profiles of polypeptides of MHV-72 infected Vero cells precipitated with immune rabbit and mouse sera
72: ^{14}C-aminoacid hydrolysated labelled MHV-72 infected Vero cells used for immunoprecipitation.
72, 76: MHV-72 infected Vero cells immunoprecipitated with rabbit immune sera raised against purified MHV-72 and MHV-76.
4556, 5682: MHV-72 infected Vero cells immunoprecipitated with mouse immune sera raised against 10 % brain mouse suspensions infected with MHV-4556 and MHV-5682.
Kr, Km: MHV-72 infected Vero cells immunoprecipitated with nonimmune rabbit and nonimmune mouse sera. Individual bands of polypeptides are characterized by their relative molecular weight x 10^{-3}.

mouse sera (Fig. 1) while ^{14}C aminoacid hydrolysate labelled proteins on noninfected Vero cells were immunoprecipitated with immune rabbit and immune mouse sera raised against MHV strains (not shown). Only weak reaction between nonimmune mouse serum and 161 K protein of MHV-72 strain was found. No other nonspecific reactions were observed. On the basis of these results we considered the two virus isolates from *Apodemus flavicollis* immunologically identical with previously isolated strain of MHV-72 and belonging to the group of murine herpesviruses.

In our presentation, six species of small mammals were investigated and only two virus isolates were obtained from *Apodemus flavicollis*. Our results have confirmed that the new isolates are immunologically identical with MHV-72. It is in conformity with previous experiments that all six previously described strains of MHV are immunologically identical (Svobodová *et al.*, 1982*b*, and our unpublished data), even when they have been from two species of small rodents.

Until the present time, all murine herpesviruses have been isolated from *Apodemus flavicollis* or *Clethrionomys glareolus*, respectively. These species are probably the main maintenance hosts of MHV in nature. The circulation and distribution of MHV in nature is not completely understood and therefore ecological studies will continue.

References

Blaškovič, D., Stančeková, M., Svobodová, J., and Mistríková, J. (1980): Isolation of five strains of herpesvirus from two species of free living small rodents. *Acta virol.* **24**, 468.

Blaškovič, D., Stančeková, M., and Rajčáni, J. (1984): Experimental pathogenesis of murine herpesvirus in newborn mice. *Acta virol.* **28**, 225–231.

Čiampor, F., Stančeková, M., and Blaškovič, D. (1981): Electron microscopy of rabbit embryo fibroblast infected with herpesvirus isolates from *Clethrionomys glareolus* and *Apodemus flavocollis*. *Acta virol.* **25**, 101–107.

Efstathiou, S., Ho, Y. M., and Minson, A. C. (1990*a*): Cloning and molecular characterization of murine herpesvirus genome. *J. gen. Virol.* **71**, 1355–1364.

Efstathiou, S., Ho, Y. M., Hall, S., Styles, C. J., Scott, S. D., and Gompels, U. A. (1990*b*): Murine herpesvirus 68 is genetically related to the gamma herpesviruses Epstein-Barr virus and herpesvirus saimiri. *J. gen. Virol.* **71**, 1365–1372.

Matis, J., and Rajčáni, J. (1980): Preparation of immune sera to immediate early and early polypeptides specified by herpes simplex virus type 1. *Acta virol.* **24**, 105–113.

Mistríková, J., and Blaškovič, D. (1985): Ecology of the murine alphaherpesvirus and its isolation from lungs of rodents in cell culture. *Acta virol.* **29**, 312–317.

Rajčáni, J., Blaškovič, D., Svobodová, J., Čiampor, F., Hučková, D., and Staneková, D. (1985): Pathogenesis of acute and persistent murine herpesvirus infection in mice. *Acta virol.* **29**, 51–60.

Raučina, J., Matis, J., and Leššo, J. (1984): Defective glycoprotein C of the syncytial strain of herpes simplex virus type 1. *Acta virol.* **28**, 457–463.

Svobodová, J., Blaškovič, D., and Mistríková, J. (1982a): Growth characteristics of herpesviruses isolates from free living small rodents. *Acta virol.* **26**, 256–263.

Svobodová, J., Stančeková, M., Blaškovič, D., Mistríková, J., Leššo, J., Russ, G., and Masárová, P. (1982*b*): Antigenic relatedness of alphaherpesvirus isolated from free-living rodents. *Acta virol.* **26**, 438–443.