PATHOGENETICAL CHARACTERIZATION OF ISOLATE MHV-60 O MOUSE HERPESVIRUS STRAIN 68

M. PAPPOVÁ[1], M. STANČEKOVÁ[1], I. SPIŠŠÁKOVÁ[2], V. ĎURMANOVÁ[2], J. MISTRÍKOVÁ[1,2*]

[1]Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B2, 842 15 Bratislava, Slovak Republic; [2]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 05 Bratislava, Slovak Republic

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Summary. – Infection of mice with mouse herpesvirus strain 68 (MHV-68) is an excellent small animal model of gammaherpesvirus pathogenesis in a natural host. We carried out comparative studies on MHV-60, another isolate of MHV-68. The acute infection of BALB/c mice inoculated intranasally (i.n.) with MHV-60 as well as its impact on tumor development were investigated. During the acute phase of infection the lungs were the main tissues infected. Our results show that MHV-60 has similar pathological features like other 4 isolates so far examined, namely MHV-72, MHV-78, MHV-Šumava inclusive of MHV-68. Nevertheless, MHV-60 differed from other isolates in following features: (i) the acute phase of infection was established very soon and lasted 10 days post infection (p.i.) in contrast to 14–28 days p.i. in the abovementioned isolates with a peak on days 3–5 p.i. The virus could also be recovered from the spleen, thymus and kidneys but not in other investigated organs. A lymphoproliferative response was associated with splenomegaly. At this time an increase in the number of leukocytes and appearance of atypical leukocytes in peripheral blood were observed. (ii) the infection was localized in the lungs and spleen, while in other isolates it was detected in a much broader scale of organs, and (iii) the acute phase of infection was accompanied by a massive splenomegaly, which was characteristic for the chronic phase of infection. Despite the fact that after clearance of the acute infection the virus was hardly detected, the tumor formation was later observed in 22% of infected mice as compared to 5% in control non-infected mice.

Key words: MHV-60; MHV-68; pathogenesis; tumors; lymphoma

Introduction

Murine gammaherpesviruses are natural pathogens of wild murine rodents (Blaškovič *et al*., 1980), are associated with lymphomas (oncogenicity), can establish a latent infection in B lymphocytes (Nash and Sunil-Chandra, 1994; Nash *et al*., 1996), and share common genetic features with human gammaherpesviruses including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). These viruses are capable to infect both outbred and inbred mice (Blaškovič *et al*., 1984; Efstathiou *et al*., 1990; Mistríková and Blaškovič, 1985). As for these human gammaherpesviruses no animal model is available, murine gammaherpesviruses have been widely used in *in vivo* studies. Until now eight MHV-68 isolates have been described: MHV-60, MHV-68, MHV-72, MHV-76, MHV-78, (Blaškovič *et al*., 1980), MHV-Šumava (Mistríková and Blaškovič, 1985), MHV-4556, and MHV-5682 (Kožuch *et al*., 1993).

Although all these isolates seem to be very similar if not identical in antigenic properties (Svobodová *et al*., 1982), they may greatly differ in other biological properties. To date we regard MHV-60, MHV-72, MHV-76, MHV-78, MHV-Šumava, MHV-4556 and MHV-5682 as different

*Corresponding author. E-mail: virumis@savba.sk; fax: + 4212-60296436.

Abbreviations: EBV = Epstein-Barr virus; i.n. = intranasal(ly); IF = immunofluorescence; KSHV = Kaposi's sarcoma-associated herpesvirus; MAb = monoclonal antibody; MHV-68 = mouse herpesvirus strain; p.i. = post infection

isolates of MHV-68. MHV-68, namely its pathogenetical properties and host-virus interaction are most often studied. MHV-68 isolates can acutely infect multiple organs of mice, including the spleen, liver, lungs, kidneys, adrenal glands, heart and thymus (Nash *et al*., 1996; Sunil-Chandra *et al*., 1992; Rajčáni *et al*., 1985).

During experimental infection of mice, lymphoproliferative responses typified by splenomegaly can be seen (Ehtisham *et al*., 1993). This is reminiscent of infectious mononucleosis associated with EBV infection. Thus, it is now possible to study in detail events in the virus life cycle *in vivo,* using various MHV-68 isolates. MHV-60 together with MHV-72 and MHV-68 has been the three isolates obtained from bank voles. MHV-68 and MHV-72 share similar properties *in vitro* and *in vivo* (Mistríková *et al*., 1994).

In this study we focused on pathological features of BALB/c mice infected with MHV-60 and compared this with other isolates of MHV-68. Despite the fact that MHV-60 was isolated from the same species, *Clethrionomys glareolus* as MHV-68 and another isolate of this virus, namely MHV-72, it differed from them in virulence and pathogenicity.

Materials and Methods

Cells. Vero cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemeted with 3–10% of heat-inactivated bovine serum (BS) or fetal calf serum (FCS), glutamine (30 mg per 100 ml) and gentamicin (8 mg per 100 ml).

Virus growth and titration. A virus stock was prepared by infection of Vero cells with MHV-60 at a multiplicity of infection (MOI) of 0.5 PFU/cell. The medium harvested on days 2–6 p.i. was clarified by low-speed centrifugation and the supernatant was dispensed in 1.5 ml aliquots and stored at -80°C until used. The virus titer was assayed by plaque titration using Vero cells grown in DMEM at 37°C in 5% CO_2 for 4–6 days.

Mice. Female inbred SPF BALB/c mice were obtained initially from the VELAZ farm, Prague, Czech Republic, later from the Institute of Virology, Slovak Academy of Sciences, Bratislava.

Infection of mice. Mice were infected at the age 4–5 weeks i.n. with 3 x 10^5 PFU of MHV-60 under light ether anesthesia.

Titration of virus in mice. At various time intervals after infection mice were killed by cervical dislocation and the lungs, spleen, thymus, kidney, milk glands, bone marrow, peritoneal macrophages, liver and brain were carefully removed at various times after infection and stored at -80°C until titrated. Specimens were homogenized in 1.5–2 ml of DMEM supplemented with 10% of heat-inactivated BS to produce 10% suspensions. The presence of infectious virus was determined by plaque titration in Vero cells. A suspension of organs was adsorbed onto cell monolayers in 24-well plastic plates for 1.5 hrs and plaques were counted after 4–6 days. The titers were expressed as the numbers of PFU per ml of suspension.

Blood samples were taken from sinus orbitalis at different times p.i. for examination of leukocytes and were mixed with heparin (final concentration of 2–4 U/ml) to prevent blood clotting.

Staining of leukocytes. Blood smears, made immediately after blood collection, were stained as described previously (Mistríková *et al*., 2002).

Differential leukocyte count. Blood picture consisted of percentage calculation of each kind of leukocytes in a blood smear. In fact, numbers of each kind of leukocytes in 100 leukocytes were counted in blood smears.

Cytological examination. Infected cell cultures grown on glass cover slips were fixed in Carnoy solution for 15 mins and then were transferred to 96% ethanol. The fixed specimens were stained with hematoxylin-eosine.

Histology. For histological analysis tumor tissues were fixed in acetic-alcoholic formalin (73.5% ethanol, 24.5% glacial acetic acid, and 2% formalin). After standard paraffin embedding (Kutlík, 1950), 3–5 μm sections were prepared using a Reichert microtome (Austria). The sections were deparaffinized in xylol and graded alcohol, stained in 0.1% hematoxylin and 0.2% eosin (Kutlík, 1950), dehydrated, mounted into Canada balsam and examined in a Nikon E400 light microscope.

Immunofluorescence (IF) test. The presence of the virus antigen in tumor cells was determined by an indirect IF test and the percentage of positive cells per 1 x 10^6 cells was calculated. Suspensions obtained from tumor tissues were stained with a monoclonal antibody (MAb) prepared against the isolate MHV-Šumava diluted 1:100–500 and with a goat anti-mouse IgG (H+L) conjugated with rhodamine (Immunotech, Slovak Republic).

Results

Detection of MHV-60 in various organs of acutely infected mice

A group of 41 mice were infected i.n. with 2 x 10^5 PFU of MHV-60. Mice did not develop infection symptoms except for three of them, which showed first mild symptoms on 4–6 days p.i. and recovered after 9–13 days p.i. The symptoms were characteristic by a ruffled fur, physical inactivity and emaciation. None of these mice died. They were sacrificed on days 2, 3, 4, 5, 7, 10, 13, 18, 24, and 60 p.i. and samples of the lungs, spleen, thymus, kidneys, peritoneal macrophages, heart, brain, bone marrow, mammary glands and liver were taken and infectious virus was titrated (Table 1). Days for sacrification were chosen on the basis of previous experience with MHV-72 and MHV-Šumava (Mistríková *et al*., 1994, 2002). Peritoneal macrophages were obtained by perfusion. The highest virus titer (10^3 PFU/ml) was detected in the lungs on days 3–5 p.i. (Fig. 1). Between days 5 and 7 p.i. the virus began to decline and on day 7 p.i. it reached the titer of 10^1 PFU/ml. After day 7 p.i. the virus became undetectable. Lower levels of the virus than those in the lungs were found in the spleen between days 3 and 10 p.i. After this period the virus remained undetectable

These results indicate replication of MHV-60 in these two organs with some delay in the spleen. Low virus titers

Table 1. Detection of the virus in various organs of mice infected with MHV-60

Organ	Day p.i.									
	2	3	4	5	7	10	13	18	24	60
	Virus titer (PFU/ml)									
Lungs	10^2	10^3	10^3	10^3	10^1	–	–	–	–	–
Spleen		10^1	10^1	10^2	–	10^1	–	–	–	–
Thymus	–	–	–	10^2	–	–	–	–	–	–
Kidney	–	–	–	10^2	–	–	–	10^1	10^1	–
Peritoneal marcophage	–	–	–	–	–	–	10^1	–	–	–
Heart	–	–	–	–	–	–	–	10^1	–	–
Brain	–	–	–	–	–	–	–	–	–	–
Bone marrow	–	–	–	–	–	–	–	–	10^1	–
Mammary glands	–	–	–	–	–	–	–	–	–	–
Liver	–	–	–	–	–	–	–	–	–	–

Four to six weeks-old Balb/c mice were infected i.n. with 2 x 10^5 PFU of MHV-60 per mouse. Virus was titrated in Vero cells.
(–) = the virus was not detected either in the 1st or in the 2nd cell culture subpassage.

(10^1 PFU/ml) were sporadically found in other lymphoid organs like thymus, peritoneal macrophages and bone marrow as well as in the heart and kidneys. The mammary glands and liver showed absence of the virus during the whole period of observation. The absence of MHV-60 in the brain suggests that this isolate has no affinity to cells of CNS following i.n. inoculation. These findings are in agreement with those obtained earlier with other isolates of MHV-68, namely MHV-72 (Mistríková *et al*., 1994), MHV-Šumava (Mistríková *et al*., 2002) and MHV-78 (Mrmusová-Šupolíková *et al*., 2003) in which the virus was not found in the brain.

Table 2 shows that the acute infection with MHV-60 had effect on the number of leukocytes as well as on the whole leukocyte count of infected mice. The infection caused an increase in the leukocyte count to a peak of 30,000/μl on day 24 p.i.

This increase correlated with the maximum number of atypical blastic cells (26 %) accompanied by eosinofilia (3%) and massive splenomegaly (a 3-fold increase in size compared to controls). Pathological changes in the spleen of infected mice appeared as a result of infiltration of atypical cells from peripheral blood (Fig. 2). All these features described above are analogical to those caused by EBV during infectious mononucleosis (Flano *et al*., 2002).

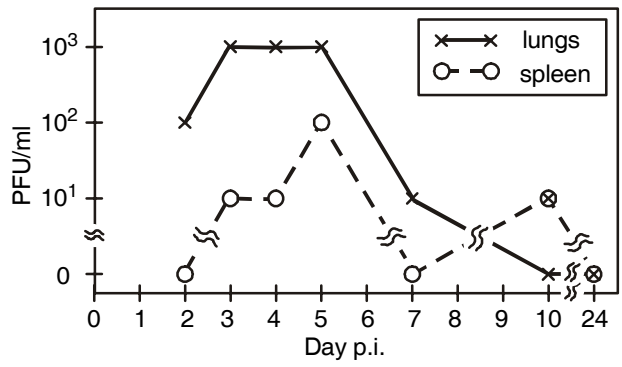

Fig. 1

Kinetics of MHV-60 in the mouse lungs and spleen

Oncogenicity of MHV-60

Long lasting infection of mice with MHV-60 resulted in lymphoproliferative disorders or tumor development. During a 21 months period of observation 9 (22%) of 41 infected mice developed tumors between days 277 and 633 p.i. During the same time period only 2 of 40 (5%) control mice formed spontaneous tumors ($p = 0.0260$, Chi-square test). A histological examination showed that a control mouse (aged 300 days) developed an undifferentiated skin carcinoma while another control mouse (aged 645 days) developed a lymphoma of cervical lymph nodes. The tumors in infected mice had various histology and location. The histology showed that only 2 (22%) of 9 tumors were lymphomas while the remaining tumors were carcinomas. The prevailing location was neck, axial and rectal regions, abdominal cavity and intestine (Table 3). These results are in contrast to those obtained with MHV-68 (Sunil-Chandra *et al*., 1994) and MHV-72 (Mistríková *et al*., 1996); over 50% of the tumors in the latter cases had lymphoma character. We were not able to isolate the virus from these tumors either by direct inoculation of homogenized tumor tissue to Vero cells or by co-cultivation of tumor fragments with Vero cells. However, using the method of indirect IF we detected 2% of MHV-60-positive cells in two samples of tumor tissue.

Table 2. Differential leukocyte count in mice infected with MHV-60

Day p.i.	Number of leu/μl	Type, number and percentage of leukocytes and spleen reactivity							
		Mo	Ly	Neu		Eoz	Baz	Atypical blast forms	Splenomegaly
				Sg	NSg				
C	7600	0	60%	40%	0	0	0	0	N
2	4500	0	80%	20%	0	0	0	0	N
3	5200	0	50%	50%	0	0	0	0	N
4	5300	1%	51%	47%	0	0	0	1%	N
5	5900	0	80%	20%	0	0	0	0	N
7	6700	0	40%	40%	20%	0	0	0	N
10	6500	0	63%	35%	0	0	0	2%	+
13	6000	2%	85%	5%	0	0	0	8%	+
18	6300	0	50%	44%	0	0	0	6%	++
24	30000	0	58%	13%	0	3%	0	26%	+++
40	8700	0	62%	25%	0	1%	0	12%	+
60	6300	2%	40%	48%	0	0%	0	10%	N

For the legend see Table 1.
C = control, non-infected mice; Leu = leukocytes; Mo = monocytes; Ly = lymphocytes; Neu = neutrophils; Sg, NSg = segmented, non-segmented granulocytes; Eoz = eosinophils; Baz = basophils. N = normal spleen; (+) = 2x normal spleen; (++) = 3x normal spleen; (+++) = 6x normal spleen.

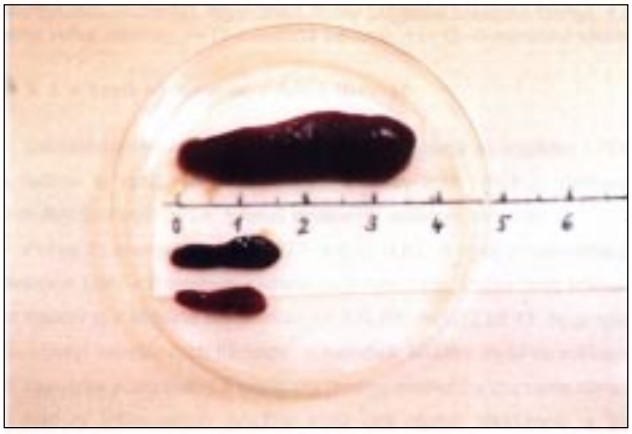

Fig. 2

Spleens of mice infected and non-infected with MHV-60

Discussion

MHV-60 is an isolate of MHV-68 from a wild mouse (*Clethrionomys glareolus*). MHV-68 is closely related to the KSHV and EBV. Because MHV-68, in general, is currently the only known gammaherpesvirus naturally infecting mice, it provides (i) an important opportunity to study the properties of the virus in inbred mice and (ii) a comparison of MHV-68 with primate gammaherpesviruses. Initially, all the studies have been concentrated on MHV-68 (Efstathiou *et al*., 1990a; 1990b; Ehtisham *et al*., 1993; Sunil-Chandra *et al*., 1992; Nash and Sunil-Chandra, 1994); later on, other MHV-68 isolates have been studied as well (MHV-72, MHV-76, MHV-78, and MHV-Šumava). The obtained results revealed some differences among the isolates concerning namely their pathological features. As the isolate MHV-60 has not been studied so much we decided to compare its pathological characteristics with those of other isolates of MHV-68.

We found that the lungs were the main target of MHV-60 after i.n. infection. The peak virus titer was reached on days 3–5 p.i. and the virus could not be detected after day 7 p.i. These results are in partial agreement with those obtained with MHV-68 and MHV-76 (Sunil-Chandra *et al*., 1992a; 1992b; Macre *et al*., 2001). The peak titers of MHV-Šumava appeared one week later with prolonged detection of the virus up to day 28 p.i. (Mistríková *et al*., 2002). In the spleen, MHV-60 was detected between days 3 and 10 p.i. with a peak on day 5 p.i. In the studies on MHV-68, the virus was detected only on day 3 p.i. (Sunil-Chandra *et al*., 1992a). In the spleen, Macre and coworkers (Macre *et al*., 2001) have detected MHV-76 between days 6 and 10 p.i. and MHV-68 between days 7 and 14 p.i. These authors have found the titer of MHV-76 1000 times lower than that of MHV-68. They have explained this difference as a result of 9538-bp deletion at the left end of the unique region the MHV-76 genome. The region is thought to play an important role in pathogenesis of acute MHV-68 infection. Rather different results have been described by Mistríková and coworkers (Mistríková *et al*., 2002) about MHV-Šumava and MHV-72. The appearance of MHV-Šumava in the spleen was significantly delayed, showing up between days 28 and 90 p.i. In the case of MHV-72 the virus was detected between days 7 and 28 p.i. (Mistríková *et al*., 1994).

Table 3. Localization and characteristics of tumors developed in mice infected with MHV-60

Tumor	Day p.i.	Tumorigenicity of MHV-60	
		Tumor localization and characteristics	Histology
T1	277	Neck; solid, inside liquid and tissue debris	Non-differentiated epithelial carcinoma
T2	308	Rectal region (2.4 g); solid with hemorrhagies attached to subcutaneous tissue	Non-differentiated carcinoma
T3	312	Axial, solid (3.9 g, 3 x 2 x 2 cm)	Non-differentiated adenocarcinoma
T4	385	Axial, subcutaneous, solid	n.t.
T5	394	A/neck (1.35 g), solid without or with hemorrhagies B/attached to intestine (0.1 g)	Partially diferentiated adenocarcinoma Lymphoepithelioma
T6	405	Rectal region (1.0 g)	n.t.
T7	566	Abdominal cavity (0.3 g), inguinal region; solid, with or without hemorrhagies	Non-differentiated spinocellular carcinoma
T8	630	Neck, solid	n.t.
T9	633	Neck (1.8 g), solid	Lymphoepithelioma

For the legend see Table 1.
n.t. = not tested

Infection of mice with MHV-60 frequently led to splenomegaly. Sunil-Chandra with coworkers (Sunil-Chandra *et al*., 1992a) has found a splenomegaly with MHV--68. In contrast, Mistríková with coworkers (Mistríková *et al*., 1994, 2002) has found a splenomegaly only during the chronic phase of infection with MHV-Šumava or MHV-72. During the acute phase of infection with MHV-76 Macre and coworkers did not observe a splenomegaly (Macre *et al*., 2001).

MHV-68 has been shown to be a B lymphotropic virus (Efstathiou *et al*., 1990b) In this study, we found that MHV-60, like other isolates of MHV-68, can also infect the thymus, kidneys, peritoneal macrophages and bone marrow (Sunil-Chandra *et al*., 1992a; 1992b; Mistríková *et al*., 1994, 2002; Macre *et al*., 2001). MHV-60, unlike MHV-72 and MHV-Šumava, does not replicate in the mammary glands (Rašlová *et al*., 2001; Mistríková *et al*., 2002). We were not able to detect MHV-60 in the brain; this result corresponds to our earlier results obtained with MHV-72 (Mistríková *et al*., 1994), MHV-Šumava (Mistríková *et al*., 2002), and MHV-78 (Mrmusová-Šupolíková *et al*., 2003).

The leukocyte count, differential leukocyte count and presence of atypical leukocytes following the MHV-60 infection are similar to those of other MHV-68 isolates and remind the situation seen following human EBV infection (Mistríková and Mrmusová, 1998; Mistríková *et al*., 2000, 2003).

Another pathological consequence of MHV-68 infection of mice, similar to EBV infection of man, is the ability of these viruses to induce a lymphoproliferative disease accompanied by splenomegaly and tumor development. We observed formation of lymphomas in 22% of BALB/c mice infected with MHV-60 in comparison to 5% of spontaneous tumors developed in the group of 40 non-infected mice. This result is similar (23%) to that obtained by Mistríková and coworkers with MHV-68 (Mistríková *et al*., 1996) but significantly less (50%) than that reported by Sunil-Chandra and coworkers (Sunil-Chandra *et al*., 1994) with MHV-68. After subtraction of the number of spontaneous tumors (5%), MHV-60 appears to be the most oncogenic isolate (appearance of virus-induced tumors in 17% of individuals) compared to MHV-Šumava (15%), MHV-72 (13%) and MHV-68 (11%) (Mistríková *et al*., 2000). The results of our pathogenetic study on MHV-60 presented here are similar to those reported on MHV-68 and its various isolates by other authors but differ in the following aspects: (i) the infection was localized in the lungs and spleen, while with other isolates it was detected in a much broader scale of organs, (ii) the acute phase of infection was established very soon and lasted 10 days only in contrast to 14–28 days with other isolates, and (iii) it caused massive splenomegaly in acute phase of infection in spite of the fact that it was characteristic for the chronic phase of infection. Despite the fact that after clearance of the acute infection the virus was hardly detected, the tumor formation was later observed in as much as 22% of mice which indicates a changeover of acute into chronical phase of infection. Even though only 2% of tumor cells carried a detectable amount of the virus, we can assume that even undetectable amount of the virus is able to maintain the tumor phenotype of cells.

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Note. The authors claim that all procedures using animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes from 1986.

References

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

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

Efstathiou S, Ho YM, Minson AC (1990a): Cloning and molecular characterization of the murine herpesvirus 68 genome. *J. Gen. Virol.* **71**, 1355–1364.

Efstathiou S, Ho YM, Hall S, Styles CJ, Scott SD, Gompels UA (1990b): Murine herpesvirus 68 is genetically related to the gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. *J. Gen. Virol.* **71**, 1365–1372.

Ehtisham S, Sunil-Chandra NP, Nash AA (1993): Pathogenesis of murine gammaherpesvirus infection in mice deficient in CD4 and CD8 T cells. *J. Virol.* **67**, 5247–5252.

Flano E, Woodland DL, Blackman MA (2002) : A mouse model for infectious mononucleosis. *Immunol. Research* **25**, 3, 201–217.

Kožuch O, Reichel M, Leššo J, Remeňová A, Labuda M, Lysý J, Mistríková J (1993): Further isolation of murine herpesviruses from small mammals in southwestern Slovakia. *Acta Virol.* **37**, 101–105.

Kutlík I (1950): *Histologický laborant*. Práca, Bratislava, pp. 93–174 (in Slovak).

Macrae IA, Dutia BM, Milligan S, Browstein DG, Allen DJ, Mistríková J, Davison AJ, Nash AA, Steward JP (2001): Analysis of a novel strain of murine gammaherpesvirus reveals a genomic locus important for acute pathogenesis. *J .Virol.* **75**, 5315–5327.

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

Mistríková J, Remeňová A, Leššo J, Stančeková M (1994): Replication and persistence of murine herpesvirus 72 in lymphatic system in peripheral blood mononuclear cells of BALB/c mice. *Acta Virol.* **38**, 151–156.

Mistríková J, Rajčáni J, Mrmusová M, Oravcová I (1996): Chronic infection of BALB/c mice with murine herpesvirus 72 is associated with neoplasm development. *Acta Virol.* **40,** 297–301.

Mistríková J,Mrmusová M (1998) : Detection of atypical lymphocytes in a blood of Balb/c mice infected with murine herpesvirus 72: the analogy with EBV infection. *Acta Virol*. **42**, 79–82.

Mistríková J, Rašlová H, Mrmusová M, Kúdelová M (2000): A murine gammaherpesvirus. *Acta Virol*. **44**, 211–226.

Mistríková J, Moško T, Mrmusová M (2002): Pathogenetic characterization of a mouse herpesvirus isolate Šumava. *Acta Virol.* **46**, 41–46.

Mistríková J, Mrmusová-Šupolíková M, Rajčáni J (2004): Leukemia-like syndrome in Balb/c mice infected with the lymphotropic gamma herpesvirus MHV-Šumava: an analogy to EBV infection. *Neoplasma* (in press).

Mrmusová-Šupolíková M, Pappová M, Mistríková J (2003): Pathogenesis of murine lymphotropic gammaherpesvirus isolate 78. *Acta Vet*. **72**, 371–376.

Nash AA, Sunil-Chandra NP (1994): Interactions of murine gammaherpesvirus with the immune system. *Curr.Opin. Immunol.* **6**, 560–563.

Nash AA, Asherwood EJ, Stewart JP (1996): Immunological features of murine gammaherpesvirus infection. *Semin. Virol.* **7**, 125–130.

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

Rašlová H, Berebbi M, Rajčáni J, Sarasin A, Matis J, Kúdelová M (2001): Susceptibility of mouse mammary glands to murine gammaherpesvirus 72 (MHV-72) infection: evidence of MHV-72 transmission via breast milk. *Microb. Pathog.* **31**, 47–58.

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

Sunil-Chandra NP, Efstathiou S, Arno J, Nash AA (1992a): Virological and pathological features of mice infected with murine gammaherpesvirus 68. *J. Gen. Virol.* **73**, 2347–2356.

Sunil-Chandra NP, Efstathiou S, Nash AA (1992b): Murine gammaherpesvirus 68 establishes a latent infection in mouse B lymphocytes *in vivo*. *J. Gen. Virol.* **73**, 3275–3279.

Sunil-Chandra NP, Arno J, Fazakerley J, Nash AA (1994): Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68. *Am. J. Pathol.* **145**, 818–826.