CHRONIC INFECTION OF BALB/C MICE WITH MURINE HERPESVIRUS 72 IS ASSOCIATED WITH NEOPLASM DEVELOPMENT

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Summary. – One hundred Balb/c mice were infected with murine herpesvirus strain 72 (MHV-72) and observed for 2.5 years for neoplasm development and virus presence in tumour as well as non-tumour tissues. Out of 13 neoplasm-bearing mice the virus was recovered from solid tumours (one lymphoma, two non-differentiated lymphoblastomas and two fibrosarcomas) of five mice and from the spleen of one mouse with lymphatic leukemia. The virus persisted frequently also in various organs of the neoplasm-bearing mice.

Key words: murine herpes virus; neoplasm; tumour; lymphoproliferative disease

Introduction

MHV is widespread among free-living rodents (Blaškovič *et al.*, 1980). Five MHV strains, designated MHV-60, 68, 72, 76 and 78 were isolated from pools of spleen, liver, kidneys and lungs; in addition, further strains were recovered from the lungs of sero-positive voles confirming the existence of persistent MHV infection in the nature (Mistríková and Blaškovič, 1985; Kožuch *et al.*, 1994). Serologic surveys demonstrated a 12% prevalence of neutralizing antibodies in the *Apodemus* and *Clethryonomis* species of ticks (Mistríková and Blaškovič, 1985).

During the acute phase of infection, MHV spreads to numerous organs (lungs, spleen, kidneys, thymus, bone marrow, and lymph nodes) by lymphatic and haematogenic routes (Rajčáni *et al.*, 1985). Its axonal spread along nerves was suspected, but not confirmed (Rajčáni *et al.*, 1987). The haematogenic spread of MHV is mediated by B-lymphocytes (Sunil-Chandra *et al.*, 1992a) and by adherent mononuclear cells (Mistríková *et al.*, 1994). Peripheral blood mononuclear cells may harbour the virus for long time periods; this is the reason why spleen is a frequent site of virus persistence (Sunil-Chandra *et al.*, 1992b). In addition, explantation studies clearly demonstrated the latency and/or persistence of MHV in the lungs and less frequently also in the kidneys of outbred as well as of Balb/c mice (Rajčáni *et al.*, 1985; Mistríková *et al.*, 1996). About 10% of laboratory mice persistently infected with the lymphotropic MHV-68 developed lymphoproliferative disease and tumors (Sunil-Chandra *et al.*, 1994). In this study, using virological and histological methods, we investigated tumours or other neoplasm which developed during 2.5 years of observation in Balb/c mice after infection with MHV-72.

Abbreviations: EBV = Epstein-Barr virus; in = intranasal; MHV-72 = murine herpesvirus strain 72

Materials and Methods

Virus. MHV-72 stock was prepared in Vero cells propagated in Eagle's Basal Medium (BEM) supplemented with 7% inactivated bovine serum, glutamine (3 g/l) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Its titer was up to 10^7 $TCID_{50}$/ml.

Mice. Six-week-old Balb/c mice were obtained from the breed of the Institute of Virology, Bratislava. One hundred mice were inoculated intranasally (in) under light anesthesia with 2 x 10^5 $TCID_{50}$ of MHV-72 in 0.02 ml per animal. Fifty non-infected mice served as controls.

Virus recovery from tumour and organ tissues. MHV-72 was recovered from the organs of tumour-bearing animals and from the tumour tissue by explantation (lungs, kidneys, liver, spleen,

and solid tumours) or by co-cultivation with indicator Vero cells (lungs, kidneys, liver, spleen, solid tumours, lymph nodes, bone marrow, thymus, peritoneal mononuclear cells, and peripheral blood mononuclear cells). All procedures were described in detail previously (Mistríková *et al.*, 1996).

Histology. Tumour tissues, enlarged lymph nodes, spleen and skin from mice were fixed in formalin diluted 1:10 with phosphate-buffered saline pH 7.2. After standard paraffin embedding, 5-7 μm-thick sections were stained with haematoxylin and erythrosin. Selected sections were stained for collagen according to van Gieson and with trichrome stain, and for mucopolysaccharides with mucicarmine.

Results and Discussion

Neoplasms developed in 13 of 100 MHV-72-infected mice between day 240 and 870 p.i. None of the uninfected control mice developed tumours or other neoplasms during that time period. Nevertheless, a development of coincidental tumours cannot be excluded in the infected mice. This was certainly the case of the mouse No. 6 with squamous cells carcinoma of the skin. No virus was isolated from this particular tumour, though persistent MHV-72 infection was clearly demonstrated in the spleen by explantation and from the bone marrow by co-cultivation. After excluding all the incompletly examined cases, there remains an evidence of five solid tumours with clear recovery of the virus from tumour tissue. MHV-72 was isolated from two non-Hodgkin lymphomas (Figs. 1 and 2), two fibrosarcomas and one non-differentiated lymphoblastoma (Fig. 3). In four of these mice a persistent or latent MHV-72 infection was demonstrated also outside of the neoplastic tissue (in the spleen, lungs, kidneys, thymus and peritoneal cells). In addition, one mouse (No. 13) developed chronic lymphatic leukemia. Its spleen, positive for the virus by explantation, was extremely enlarged at gross examination. Histologically, the normal structure of the red pulp was replaced with uniform prolipherating reticulum cells. The sinuses were filled with lymphoid blastic cells showing hyperchromatic nuclei and relatively few cytoplasm. The cases of virus-positive fibrosarcoma (mice No. 1 and 4) are of particular interest with respect to the report of Lee *et al.* (1995) on the occurence of non-lymphoid and non-epithelial neoplasms (non-differentiated leiomyosarcomas) in immunocompromised children associated with Epstein-Barr virus (EBV) infection. In an independent study, Chandra *et al.* (1992b) claimed that MHV is a B-lymphotropic gammaherpesvirus which causes lymphoproliferative disease similar to that caused by EBV in man. Studies of the genome of MHV-68, a strain isolated by Blaskovič *et al.* (1980), showed that its DNA had at least 18 genes with nucleotide stretches identical with those of the genes of EBV (Efsthathiou *et al.*, 1990). Also the polypeptides of MHV-72 indicate a pattern closely resembling that of other gammaherpesviruses (Reichel *et al.*, 1994). Furthermore, the gB gene of MHV-68 showed sequence identical with that of the gB gene

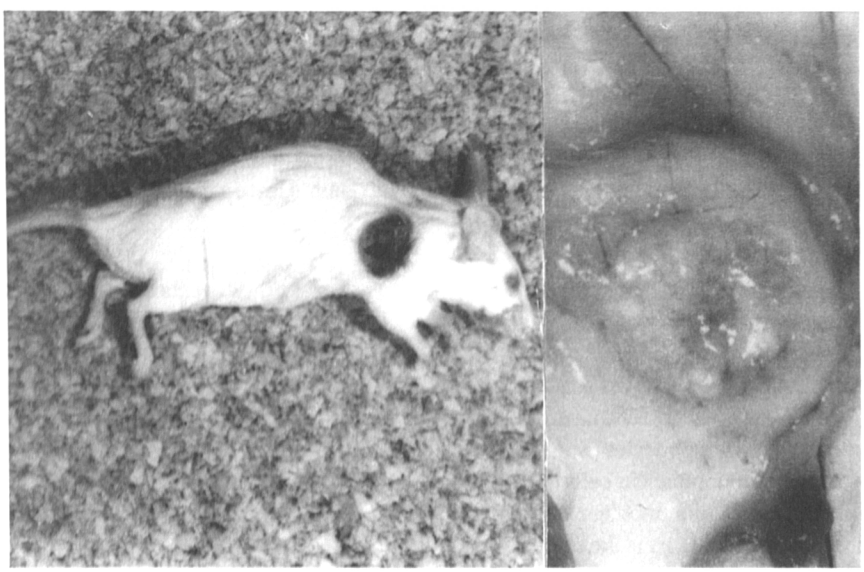

Fig. 1
Mouse No. 3 with lymphoblastoma
Left part: enlarged cervical lymph node tumour mass penetrating the skin with partial exulceration in the upper area of frontal shoulder. Right part: detail of the same tumour from the inner side of the skin.

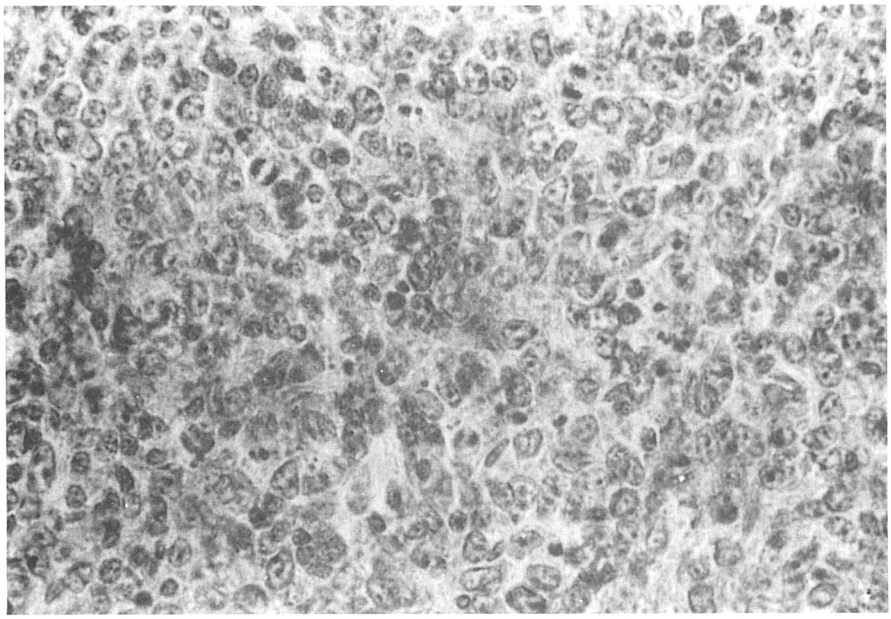

Fig. 2
Histology of the lymphoblastoma from Fig. 1
Round-shaped lymphoid cells with spare perinuclear cytoplasm and large circular nuclei of irregular size infiltrate the lymph node, penetrate its capsule and invade the sourrounding connective tissue. Several mitoses can be seen. (Magnification 660 x).

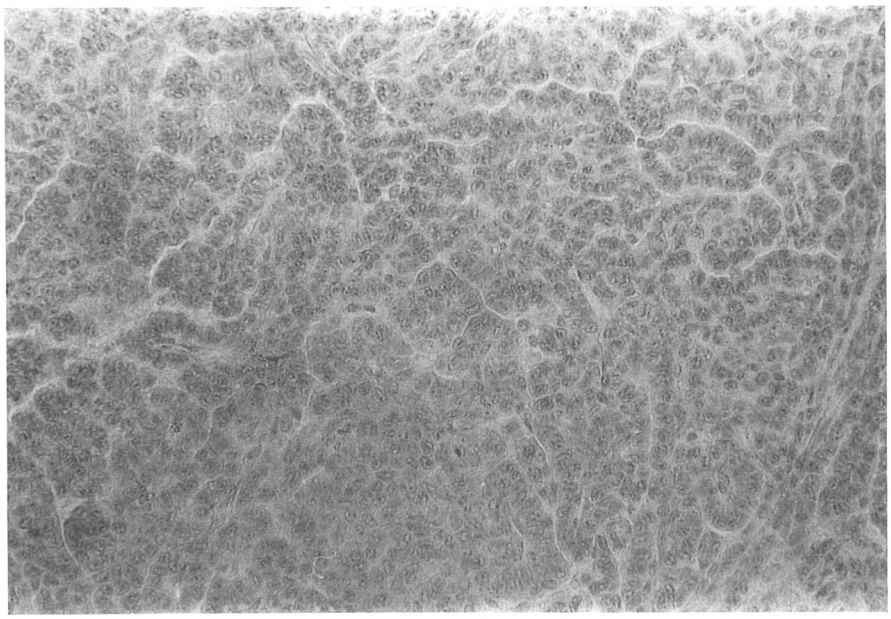

Fig. 3
Histology of non-differentiated lymphoblastoma of mouse No. 11
Non-differentiated atypical cells with round-shaped or slightly elongated nuclei contain more cytoplasm than those shown in Fig. 2. The cells occasionally form epitheloid structures, however, staining for mucin was repeatedly negative. Mitoses are clearly visible. (Magnification 320 x).

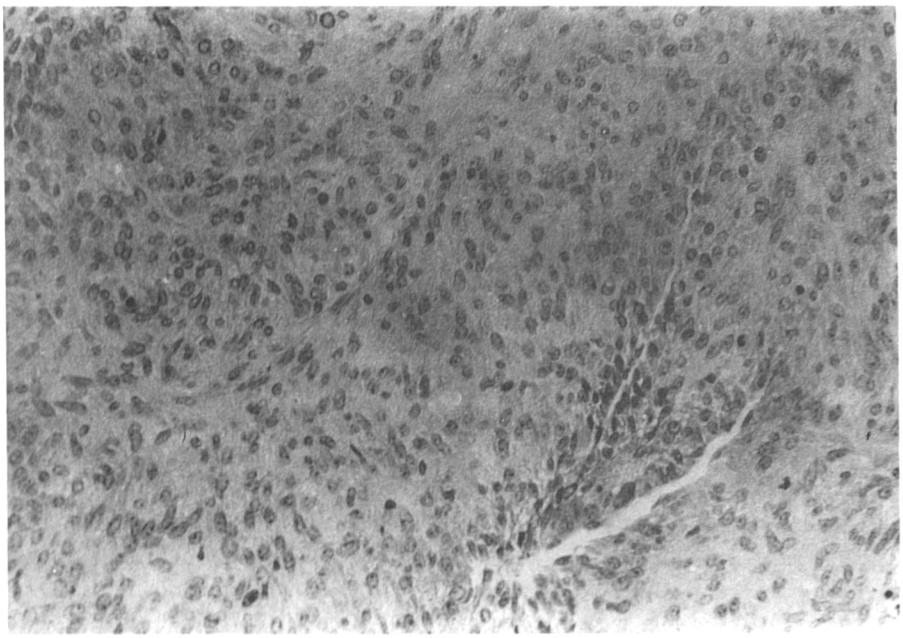

Fig. 4
Histology of fibrosarcoma of mouse No. 4
Poorly differentiated tumour consists of elongated cells with oval nuclei, which occasionally form whirl-like structures. Staining for myosin filaments was negative.

of EB virus coding for gp110 (Stewart *et al.*, 1994). Because of the similarity between EBV and MHV in gene sequences and biological effects (tumour formation), the experimental MHV infection may be an interesting model for studying the lymphoproliferative effect of gammaherpesviruses. In immunocompromised patients, EBV-associated lymphomas are not infrequent (Sugdan 1994). A more recent list of lymphoproliferative diseases, possibly associated with EB virus includes T-cell lymphomas, Hodgkin's disease, immunoblastic lymphomas and non-differentiated carcinomas (Ambinder and Mann, 1994). Further studies focused on the development of MHV-associated tumours in nude mice are being performed to elucidate the mechanism of the lymphoproliferative effect of gammaherpesvirus infections.

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Table 1. Development of neoplasms in Balb/c mice infected with MHV-72

Neoplasm-bearing mouse No.	Examination on day p.i.	Neoplasm localization	Type of neoplasm	Virus isolated from	
				Tumour	Various organs
1	240	cervical lymphs nodes	fibosarcoma,leukemia	+	lungs
2	270	abdominal cavity	non-diferentiated lymphoblastoma	–	ND
3	300	cervical lymphs nodes	lymphoblastoma	+	thymus, spleen, peritoneal cells
4	300	cervical lymphs nodes	fibrosarcoma	+	lungs, spleen
5	360	cervical lymphs nodes	ND	–	lungs, spleen, macrophages
6	390	cervical lymphs nodes	squamous cell carcinoma	–	spleen, bone marrow
7	480	cervical lymphs nodes	ND	ND	ND
8	490	hind limb	haemangioblastoma	ND	ND
9	540	cervical lymphs nodes	lymphoma	+	spleen, kidney
10	720	abdominal cavity	ND	–	lungs, bone marrow
11	750	mediastinal tumor	non-differentiated lymphoblastoma	+	ND
12	780	abdominal cavity	lymphoblastoma	–	lungs, spleen
13	870	peripheral blood	lymphatic leukemia	–	spleen, lungs

(+) = positive, (–) = negative, ND = not done.

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