DETECTION OF ABNORMAL LYMPHOCYTES IN THE BLOOD OF BALB/C MICE INFECTED WITH MURINE GAMMAHERPESVIRUS STRAIN 72: THE ANALOGY WITH EPSTEIN-BARR VIRUS INFECTION

J. MISTRÍKOVÁ, M. MRMUSOVÁ

Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B2, 842 15 Bratislava, Slovak Republic

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Summary. – We have followed the effect of murine gammaherpesvirus strain 72 (MHV-72) infection and immunosuppression on the differential white blood cell count of Balb/c mice. In both the acute and chronic phase of infection, abnormal lymphocytes resembling human B lymphocytes infected with Epstein-Barr virus (EBV) were detected. Immunosuppression had no significant effect on the haematological changes during the infection. Some of mice, which had developed tumours as a consequence of infection, showed splenomegaly, lymphadenopathy, leukocytosis and high percentage of immature blastic forms of leukocytes.

Key words: murine gammaherpesvirus; Balb/c mice; differential white blood cell count; abnormal lymphocytes

Introduction

The strains 60, 68, 72, 76 and 78 of MHV represent a group of related natural patogens of wild rodents. They have been isolated from organs of *Apodemus flavicollis* and *Clethrionomys glareolus* (Blaškovič *et al.*, 1980). These viruses are widespread in populations of free-living rodents, as demonstrated serologically by up to 12% anti-MHV antibodies prevalence (Mistríková and Blaškovič, 1985). When administered intranasally (i.n.), MHV-68 and MHV-72 established chronic infection at least in the lungs and spleens of outbred mice (Rajčáni *et al.*, 1985) and inbred Balb/c mice (Sunil-Chandra *et al.*, 1992b) and in adherent mononuclear cells Balb/c (Mistríková *et al.*, 1994). Inbred Balb/c mice chronically infected with MHV-68 developed a lymphoproliferative disease (LPD) with 9% frequency over a period of 3 years (Sunil-Chandra *et al.*, 1994). In accordance with these data, about 10% of Balb/c mice persistently infected with MHV-72 have developed LPD and solid tumours during 2.5 years (Mistríková *et al.*, 1996b). Histologically, these tumours were lymphomas, non-differentiated lymphoblastomas, fibrosarcomas, but haematological disorders like lymphatic leukemia were also observed. These observations suggested that the MHV-infected mice might be an important model for studying the pathogenesis of human LPD associated with gammaherpesviruses such as EBV. In man, the latter virus is causative agent of infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's lymphogranuloma. In addition, it can cause also some forms of leukemia. The link between infection with MHV-68 or MHV-72 and the growth of solid tumours was reported by Chandra *et al.* (1994) and Mistríková *et al.* (1996). However, the association between MHV infection and development of leukemias has not been proved yet. This led us to focus our attention on the analysis of differential white blood cell count of mice infected with MHV-72 and subjected to immunosuppression.

Abbreviations: BEM = Eagle's Basal Medium; EBV = Epstein-Barr virus; i.n. = intranasally; LPD = lymphoproliferative disease; MHV = murine gammaherpesvirus; PBS = phosphate-buffered saline; p.i. = post infection; s.c. = subcutaneously

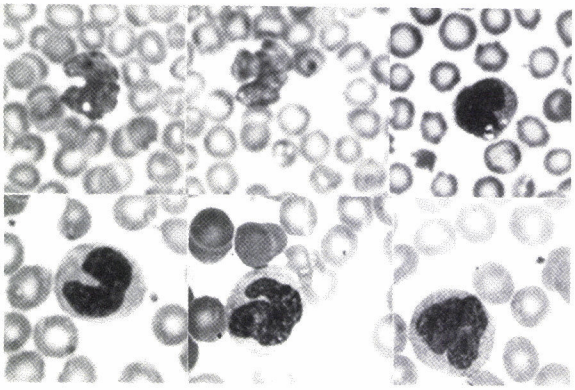

Fig. 1
Differential white blood cell count of Balb/c mice infected with MHV-72 (a) and EBV-infected patient in acute phase of infectious mononucleosis (b)
Abnormal morphology of lymphocytes. The nuclei are lobulated or kidney-shaped. The chromatin in coarse strands is irregularly distributed, giving a mottled appearance. More basophilic cytoplasm.

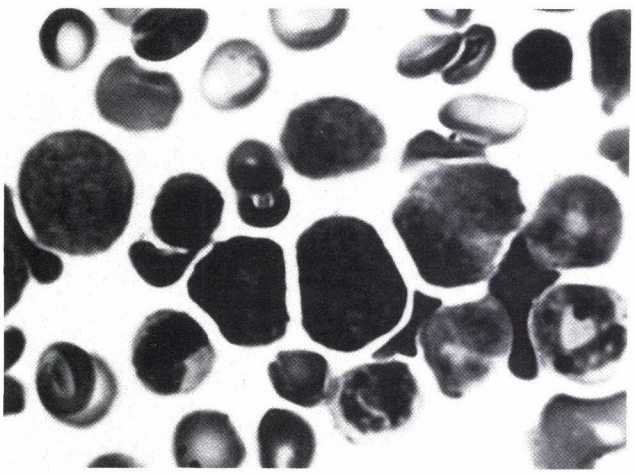

Fig. 2
Differential white blood cell count of Balb/c mice 980 days after MHV-72 infection
Leukocyte count was 70,000/µl and the percentage of blastic forms of lymphocytes was 84.

Materials and Methods

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

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

Immunosuppression. Immunosuppressive compound FR 900506 was a kind gift from Dr. M.A. Nalesnik, Pittsburgh Medical Center, Pittsburgh, PA, USA. The immunosuppression started 7 months p.i. The lyophilized substance freshly dissolved in PBS was inoculated subcutaneously (s.c.) to MHV-72-infected mice daily with exception of weekends for 4–6 weeks in a dose of 2 mg/kg/day (Yamamoto *et al.*, 1990).

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

Staining of blood elements. Blood smears were made immediately after blood collection. They were stained after fixation by air drying with May-Grünwald solution for 10 mins and Giemsa-Romanovski solution for 15 mins. Number of leukocytes was determined after 10 mins of staining with Türk solution.

Differential white blood cell count consisted of calculation of percentage of each kind of white blood cells.

Results and Discussion

The analysis of differential white blood cell count of healthy and virus-infected Balb/c mice subjected or non-subjected to immunosuppression was followed. The obtained results showed that the infection with MHV-72 was accompanied with major changes in the number of leukocytes and quality of differential white blood cell count in correlation with the duration of infection. In the acute phase of infection, abnormal lymphocytes (lymphoid monocytes) occurred in the blood with frequency of 9.2% or even higher in the immunosuppressed group of mice. These abnormal mouse lymphocytes were highly similar in shape to those observed in a patient suffering from infectious mononucleosis (Fig. 1). In the chronic phase of infection of mice, in addition to the splenomegaly (Figs. 3 and 4), we observed in the blood changes characteristic for leukemia: the number of leukocytes raising to 30,000 – 200,000, and the number of the blastic, immature forms of leukocytes increasing to 84% (Fig. 2). Compared to the control group, mice in the chronic phase of infection, either immunosuppressed or not, had slightly decreased numbers of leukocytes and lymphocytes associated with the increasing number of abnormal lymphocytes (Table 1). In contrast, mice in the chronic phase of infection which developed tumours had an about twenty times higher number of leukocytes, but a strongly reduced number of lymphocytes. In this group, the abnormal lymphocytes could be detected with five-times higher frequency than in the group of infected mice without tumours (Table 1). Abnormal lymphocytes observed in MHV-72-infected mice could be classified as lymphoblasts (developing stages during maturation of lymphocytes and monocytes), myeloblasts and promyelocytes (developing stages during maturation of neutrophile polymorphonuclear leukocytes) as well as in patients with myeloid leukemia. Whereas in all infected mice the frequencies of segment-

Fig. 3
Spleens of healthy (right) and MHV-72 -infected (left) Balb/c mice

Fig. 4
Balb/c mice 980 days after MHV-72 infection
Splenomegaly, lymphadenopathy, leukocytosis and blastic forms of lymphocytes.

Table 1. Effect of MHV-72 infection and immunosuppression on differential white blood cell count of Balb/c mice

Mice (number)	Leukocytes (number)	Monocytes (%)	Lymphocytes (%)	Abnormal lymphocytes and blastic forms (%)	Granulocytes (%)			
					Neutrophiles		Basophils	Eosinophils
					Segmented	Non-segmented		
Control (15)	7800	11	76	0	9.4	3.4	0.2	0.1
Infected (44)	6410	13	61.4	9.2	6.3	9.3	0.3	0.6
Infected and IS (44)	6330	6.5	64.2	10.8	6	10 9	0.4	1.2
Infected bearing tumours (17)	111850	11.3	37.3	48	6.5	9.9	0.3	0.4

IS = immunosuppressed

ed cells were reduced by one third in comparison to the control, the frequency of non-segmented cells increased about three times (Table 1). Infection with MHV-72 did not induce substantial changes in numbers of monocytes, basophils and eosinophils.

Demonstration of pathological changes in differential white blood cell count of MHV-infected mice confirmed our hypothesis on analogical biological properties shared by human herpesvirus EBV and murine herpesvirus. Association of the infection with MHV-68 or MHV-72 with LPD of mice and similarity of pathologies of MHV and EBV infections identifies these murine herpesviruses as valuable models for the study of human diseases associated with EBV.

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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.

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