MURINE GAMMAHERPESVIRUS 68 SERUM ANTIBODIES IN GENERAL HUMAN POPULATION

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Summary. – Previous studies using ELISA and virus neutralization test (VNT) have proved the presence of Murine gammaherpesvirus 68 (MHV-68) serum antibodies in sera of laboratory staff working with MHV-68, as well as in the general population. In this study, we investigated the incidence of serum antibodies to MHV-68 and to human herpesviruses presumably antigenically similar to MHV-68, as Herpes simplex virus 1 (HSV-1), Human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV), in general population using ELISA, VNT, and immunofluorescence assay (IFA). We also searched for the possible detection of false-positive reaction of MHV-68 antibodies due to cross-reactions between MHV-68 and the antibodies to the herpesviruses mentioned above. We found 16% of positive sera for MHV-68 antibodies by ELISA (titers of 1,600–102,400) and 4.5% by VNT and IFA (titers of 8–32). Tested human sera, either positive or negative for MHV-68 antibodies, were positive for antibodies to HSV-1, HCMV, and EBV (71/69%, 69/65%, and 66/49%, respectively). We concluded that the false-positivity of the sera for MHV-68 antibodies detected by ELISA was due to the non-specific cross-reactions with antibodies to antigenically similar EBV.

Key words: Murine gammaherpesvirus 68; virus neutralization test; ELISA; immunofluorescence test; Herpes simplex virus 1; Human cytomegalovirus; Epstein-Barr virus

Introduction

MHV-68 (*Murid herpesvirus 4, Mouse herpesvirus strain 68*) is a natural pathogen of free-living murid rodents. It was isolated from a bank vole (*Clethrionomys glareolus*) in Slovakia (Blaškovič *et al*., 1980). Molecular studies and sequencing of the genome have showed that it belongs to the family *Herpesviridae*, the genus *Rhadinovirus*, the species *Murid herpesvirus 4* (Fauquet *et al*., 2005; Virgin *et al*., 1997). Recently, the MHV-68 infection of inbred mice was established as a tractable and genetically manipulable model system for studying the pathogenesis of gammaherpesviruses and developing therapeutic strategies for them. However, a little is known about the MHV-68 natural host range, pathogenesis, and epidemiology.

In the population of wild rodents, MHV-68 is transmitted via air route through different excreta such as urine, saliva, tears and breast milk (Hricová, 2004). Different routes of transmission of MHV-68 in the nature together with the fact that the virus is not species-specific have led to the hypothesis that it could be transmitted to the other animal species living in the same biotope. Human herpesviruses HSV-1, HCMV, and EBV are widespread in human population due to their ability to establish a persistent infection and induce specific antibodies. The seroprevalence of HSV-1, HCMV, and EBV in adult population is about 80%, 85%, and 90%, respectively. As some studies suggested, Murine herpesvirus MHV-68 and human herpesviruses HSV-1, HCMV, EBV are presumably antigenically similar, what could lead to a serological cross-reaction (Stewart *et al*., 1999; Svobodová *et al*., 1982).

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Abbreviations: EBV = Epstein-Barr virus; HCMV = Human cytomegalovirus; HSV-1 = Herpes simplex virus 1; IFA = immunofluorescent assay; LCMV = Lymphocytic choriomeningitis virus; MHV-68 = Murine gammaherpesvirus 68; VNT = virus neutralization test

Mistríková *et al*. (2000) detected neutralizing serum MHV-68 antibodies in fallow deer (*Dama dama*), wild boars (*Sus scrofa*), deer (*Cervus elaphus*), sheep, and subsequently also in laboratory staff working with MHV-68 or wild rodents. Seven of 20 human sera (35%) of the latter group tested positive for MHV-68 antibodies by both VNT and ELISA. The presence of serum MHV-68 antibodies might be medically and epidemiologically important, especially in view of the knowledge that MHV-68 can persistently infect human cell lines and transform human B-lymphocytes *in vitro* (A.A. Nash, personal communication; Svobodová *et al*., 1982, 1987). Further study proved the seroprevalence of MHV-68 in human sera (16%) by both ELISA and VNT (Mistríková *et al*., 2006). We did not assume that such a high proportion of the population would get into contact with small rodents and contracted infection with MHV-68 and therefore we attempted to estimate the possible cross-reactions between antibodies to MHV-68 and antibodies to HSV-1, HCMV, and EBV in ELISA.

The aim of the presented study was to extend the examination of human sera by assaying the antibodies to HSV-1, HCMV, and EBV in addition to the detection of antibodies to MHV-68 using previously used assays ELISA and VNT. Moreover, we performed also IFA as the confirming assay. We examined 330 human sera sampled from general population.

Materials and Methods

Virus. MHV-68 was propagated in Vero cells (Svobodová *et al*., 1982).

Serum samples originated from patients of different hospitals in Slovakia with anonymous diagnoses, and from employees of the Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia. The sera were inactivated at 56°C for 30 mins and stored at -20°C until used.

ELISA for detection of MHV-68 antibodies was performed in a standard manner using the AffiniPure Goat Anti-Human IgG (Jackson Immunoresearch Laboratories, Pennsylvania, USA) as secondary antibody diluted 1:1,000 in PBS. A serum with MHV-68 antibodies was used as the positive control, while PBS served as the negative control. Detection of the antibodies against HSV-1, HCMV, and EBV was performed by ELISA using commercial kits (Human GmbH, Germany). Briefly, the tested serum diluted 1:100 (HSV-1 and HCMV) or 1:20 (EBV) was added to the antigen-coated microtiter plate wells. A_{492} was read in a Multiscan MCC/340 ELISA-reader.

VNT was performed in a standard manner using Vero cells (Mistríková *et al*., 1994). The human sera positive for EBV, HCMV, HSV-1, and negative for MHV-68 were used as the negative controls.

IFA was carried out in a standard manner using Vero cells infected with MHV-68. The swine anti-human IgG (Sevac) and swine anti-mouse IgG (Sevapharma) conjugated with FITC and diluted 1:1,000 in a blocking solution were used as the secondary antibodies. A tested serum was regarded as positive, when the specific fluorescence was observed in approximately 25% of cells and no fluorescence was seen in a negative control. A mouse immune (against Murine gammaherpesvirus 78) and non-immune sera were used as the positive and negative controls, respectively.

Results

ELISA as the most sensitive current method detected MHV-68 antibodies in 16.0% of sera, while VNT, as the most specific current method, did so in 4.5% only. Since the Western blot analysis turned out to be inappropriate for detecting MHV-68 antibodies in human sera, we chose IFA as the third reference assay. The IFA confirmed the presence of MHV-68 antibodies detected by VNT (4.5%), but not those detected by ELISA (16%) (Table 1, Fig. 1).

Table 1. MHV-68 antibodies in the human sera assayed by ELISA, VNT and IFA

Assay	Sera (No.)		Antibody titers	Positive sera (%)
	Total	Positive		
ELISA	330	53	1,600–102,400	16.0
VNT	330	15	8–32	4.5
IFA	330	15	8–32	4.5

The differences were found also in the levels of antibody titers against MHV-68 obtained by ELISA, VNT and IFA. The samples positive in all these methods (ELISA, VNT, IFA) are shown in Table 2. To investigate these differences in more

Table 2. Titers of MHV-68 antibodies in the human sera assayed by ELISA, VNT and IFA

Serum (No.)	Titer of MHV-68 antibodies		
	ELISA	VNT	IFA
1	12,800	8–16	8–16
2	1,600	8	8
3	12,800	16	16
4	6,400	8	8
5	1,600	16	16
6	51,200	8	8
7	6,400	8–16	8–16
8	102,400	8	8
9	51,200	8	8
10	12,800	16	16
11	6,400	8	8
12	25,600	16–32	16–32
13	6,400	16	16
14	102,400	16	16
15	25,600	32	32

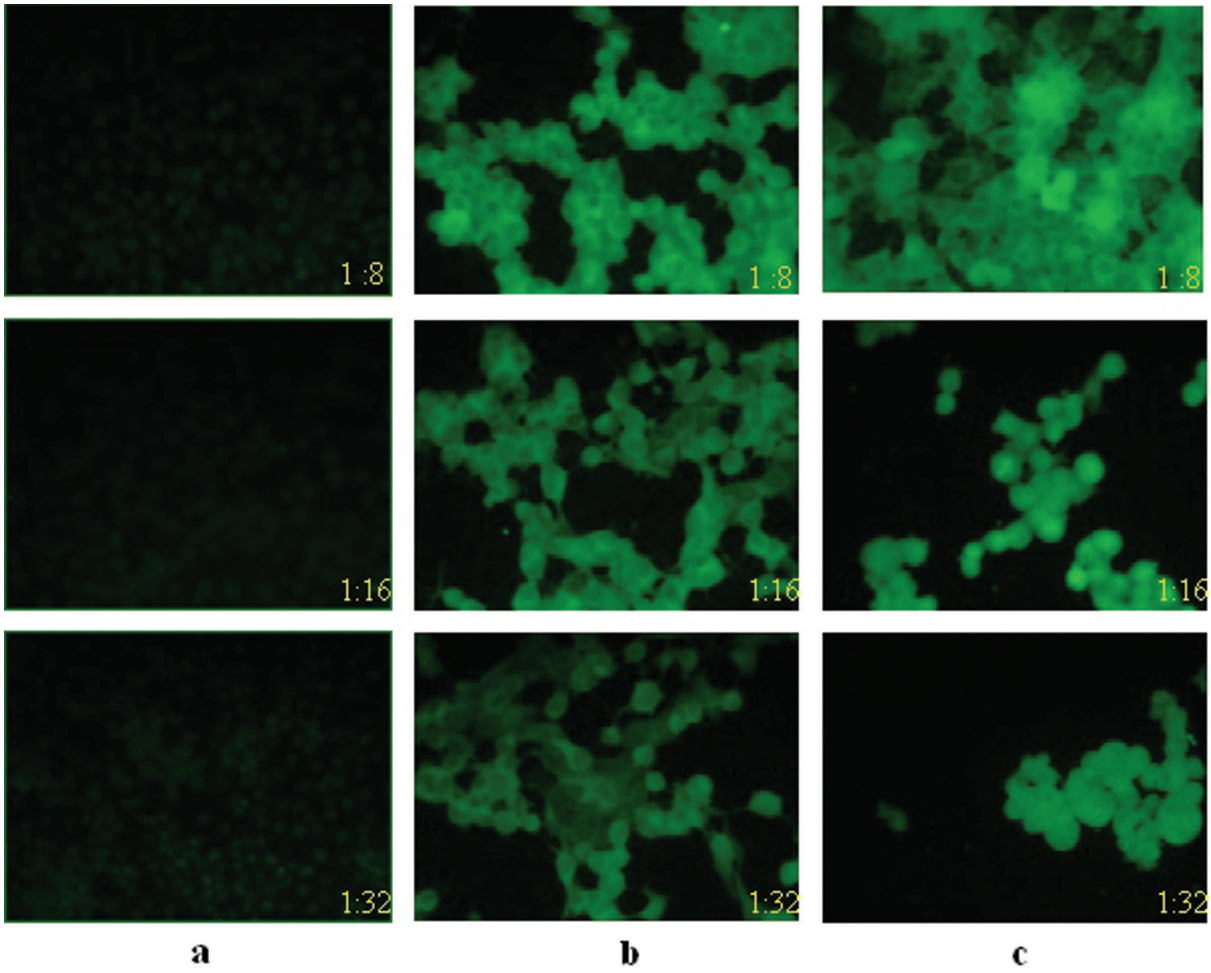

Fig. 1

IFA of MHV-68 antibodies in the positive human serum diluted 1:8, 1:16, 1:32

Negative control (a), positive control (b), serum sample (c).

Table 3. Presence of antibodies to HSV-1, HCMV, and EBV in human sera either positive or negative for MHV-68 antibodies

	HSV-1		HCMV		EBV	
	MHV-68-positive	MHV-68-negative	MHV-68-positive	MHV-68-negative	MHV-68-positive	MHV-68-negative
Number of tested sera	82	83	82	83	91	83
Number of positive sera or their expression in (%)	58 (71%)	57 (69%)	56 (69%)	54 (65%)	60 (66%)	40 (49%)

detail, we assayed IgG antibodies to HSV-1, HCMV, and EBV by ELISA in the extended group of sera either positive or negative for MHV-68 antibodies in ELISA (Table 3).

The obtained results showed that there was no difference in the levels for HCMV and HSV-1 antibodies between MHV-68-positive and MHV-68-negative sera. However, the level for EBV antibodies was markedly higher in MHV-68-positive (66%) than in MHV-68-negative sera (49%). This result indicated a false positivity for MHV-68 antibodies due to the presence of EBV antibodies and cross-reaction.

Discussion

The search for MHV-68 antibodies in human sera was based on the fact, that rodents represent an important reservoir for a number of microorganisms pathogenic for humans. The pathogenicity of MHV-68 for humans has not been studied yet. This virus belongs to the herpesviruses that are characteristic by colonizing almost all animal species to which they are usually not harmful. However, when transmitted to some other species, they may cause serious, sometimes fatal diseases (e.g. Herpesvirus simiae infecting humans and Pseudorabies virus infecting pigs). Although herpesviruses represent one of the most studied virus family, their circulation in the nature and epidemiological importance for humans are still quite unknown. This is also the case of recently isolated and previously unknown herpesviruses to which belongs MHV-68. In the nature this virus apparently circulates in reservoir-animals and is transmitted from their mothers transovarially, via saliva, milk, food contaminated with respiratory excrets, urine, or feces of other infected rodents (Štiglincová, personal communication; Blaškovič *et al*., 1987). It seems that this type of circulation is common in the hantaviruses and Lymphocytic choriomeningitis virus (LCMV) that use small rodents as natural hosts. The transmission of virus has been proved for MHV-68 via saliva, tears, urine, and mother milk (Hricová, 2004; Mistríková *et al*., 2002; Rašlová *et al*., 2001), for LCMV via urine and feces, and for hantaviruses via saliva, urine and feces (Lee *et al*., 1981). Similarly, a persistent infection of some tissues is common to MHV-68 and hantaviruses (Mistríková *et al*., 2000).

Several studies have proved a marked prevalence of MHV-68 in various phylogenetically distant species of murid rodents in Slovakia and UK (Becker *et al*., 2007; Telfer *et al*., 2007; Blasdell *et al*., 2003; Klempa *et al*., 2001; Blaškovič *et al*., 1987; Mistríková and Blaškovič, 1985). Furthermore, the MHV-68 infection of other animal species, namely fallow deer, wild boars, deer, sheep, foxes, and mouflons has been proved by detection of specific serum neutralization antibodies (Marková *et al*., 2007; Hamzová *et al*., 2005; Mistríková *et al*., 2000). Subsequently, serum MHV-68 antibodies were found in humans, particularly in the laboratory staff working with MHV-68 and infected rodents, in hunters, and in general human population (Marková *et al*., 2007; Mistríková *et al*., 2000, 2006; Hamzová *et al*., 2005).

In view of a relatively high prevalence of MHV-68 antibodies (16%) in the latter group assayed by ELISA, we attempted to exclude the possible false-positive results that could be caused by the cross-reactions of MHV-68 antigen with antibodies against other human herpesviruses, particularly HSV-1, HCMV, and EBV. The results obtained by VNT and IFA proved the false-positivity of the ELISA assay and the involvement of cross-reactivity of MHV-68 with EBV antibodies. A close similarity was confirmed between MHV-68 and EBV (Stewart *et al*., 1994; 1999; Virgin *et al*., 1997; Efstathiou *et al*., 1990). In addition, a high prevalence of antibodies against EBV was demonstrated in the general human population.

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