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POLYPEPTIDES SYNTHESIZED IN RABBIT CELLS INFECTED WITH MURINE HERPESVIRUS (MHV): A COMPARISON OF PROTEINS SPECIFIED BY VARIOUS MHV STRAINS

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Summary. - Polypeptides synthesized in rabbit embryo fibroblasts (REF) infected with six isolates of murine herpesvirus (MHV) coming from different localities in ČSFR were compared by electrophoresis (SDS-PAGE). Altogether 28 virus-coded polypeptides with apparent molecular weights ranging from 240 kD to 16 kD were identified. While isolates MHV-60, MHV-72, and MHV-76 had identical polypeptide profiles, the isolates MHV-68 and MHV-78 lacked a polypeptide with apparent molecular weight of 46 kD, the rest of polypeptides being identical as well. The polypeptide profile of MHV Šumava A.f. was most different from that of other MHV isolates. This result is in agreement with some different biological properties of this isolate. At a high multiplicity of infection (40 $TCID_{50}$ per cell) first polypeptides with apparent molecular weights 125 kD and 84 kD were detected by 5-9 hr post-infection (p.i.). All other proteins appeared 14-18 p.i.

Key words: novel murine herpesvirus; virus polypeptides; polyacrylamide gel electrophoresis

Introduction

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.) and some of their biological properties were descsribed (Blaškovič *et al.*, 1980; Mistríková and Blaškovič, 1985). Electron microscopic studies of infected REF cells provided morphological evidence that the viruses belonged to the family *Herpesviridae* (Čiampor *et al.*, 1981). As the result of further biological, biochemical, and the pathogenetic studies (Svobodová *et al.*, 1982*a, b;* Blaškovič *et al.*, 1984; Rajčáni *et al.*, 1985; Stančeková *et al.*, 1987) has been found that

MHV showed some species properties of an alphaherpesvirus in phylogenetically different wild rodents; the sensitivity to this virus was under the limit of the species specificity (Mistríková and Blaškovič, 1985; Blaškovič *et al.*, 1987*a, b*).

Efstathiou *et al.* (1990*a, b,*) investigated the genome of MHV-68. They have identified nine genes which encode amino acid sequences with greater similarity to proteins of the gammaherpesvirus Epstein-Barr virus (EBV) than to the homologous products of the alphaherpesvirus varicella-zoster virus and herpes simplex virus type 1 or the betaherpesvirus human cytomegalovirus. In addition, the genome organization of MHV-68 is shown to have an overall colinearity with that of the gammaherpesviruses EBV and *herpesvirus saimiri.* They suggested to classify MHV-68 into the subfamily *Gammaherpesvirinae.* This points at the difficulties of classification of MHV whose biological behaviour is not quite typical for a gammaherpesvirus. In this presentation we compare the polypeptide profiles of the REF cells infected with six isolates of MHV and strains KOS and HSZP of the herpes simplex virus type 1 (HSV-1). Furthermore, the kinetics of the synthesis of virus polypeptides in REF cells infected with MHV-60 and MHV-76 were investigated.

Materials and Methods

Cells and media. Stable line of rabbit embryo fibroblast (REF) cells (kindly provided by Dr. D. Řezáčová, Institute of Sera and Vaccines, Prague) were cultured in Eagle's basal medium (BEM) supplemented with 5–10 % inactivated bovine serum (IBS), glutamine (3 g per 100 ml) and antibiotics (100 units of penicillin and 100 μg of streptomycin per ml). These cells were used throughout all experiments.

Viruses. Isolates of MHV coming from *Clethrionomys glareolus* (No. 60, 68, 72) and from *Apodemus flavicollis* (No. 76, 78, and Šumava A.f.) (Blaškovič *et al.*, 1980; Mistríková and Blaškovič, 1985) were compared with HSV-1 strain HSZP (Szántó *et al.*, 1972) and the prototype strain KOS.

Infection and labelling of the cells. Confluent cell monolayers (approximately 2 x 10^6 cells per flask) were infected at a multiplicity of infection (MOI) of 1, 10, or 40 $TCID_{50}$ per cell and kept for 60 min at 37 °C. After virus adsorption, the inoculum was removed and replaced with BEM supplemented with 5 % IBS. For labelling, the medium was replaced with 2 ml of medium BEM/10 (ten times reduced amino acids except of arginine and supplemented with 1 % IBS) containing 0.1 MBq/ml of ^{14}C-amino acid hydrolysate (ÚVVVR, Prague, specific activity 4 x 10^4 MBq/g). At the end of the labelling peiod the cells were rinsed with ice-cold phosphate-buffered saline (3 x 5.0 ml/flask) scrapped off, pelleted and stored frozen at –70 °C until subsequent polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Samples were analysed on 6 %, 8 %, 10 %, and 12 % polyacrylamide gels (cross-linked with methylenbisacrylamide) as described (Matis and Rajčáni, 1980). The gels were fixed and treated with 1 mol/l sodium salicylate to provide fluorographic enhancement (using Medix-Rapid X-ray film, Hradec Králové). For the molecular weight determination the following proteins were used: myosin, beta-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, and carbonic anhydrase (Sigma).

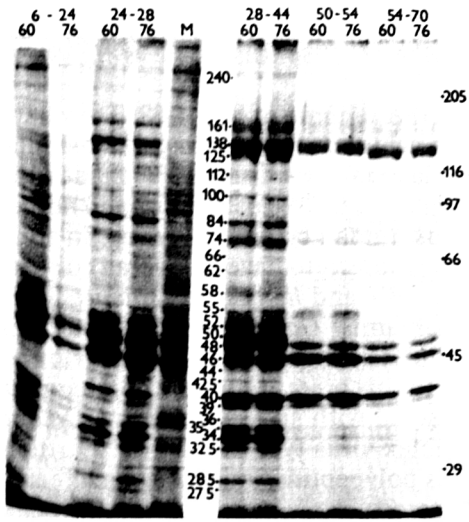

Fig. 1
Polypeptides synthesized in MHV-60 and MHV-76 infected cells

Virus-infected cells (60, 76) were pulse-labelled with ^{14}C-amino acids at various times post-infection (p.i.), the numbers at the top of the autoradiogram refer to time in hours. After labelling, the protein samples were analysed on an 8 % SDS-polyacrylamide gel. Mock-infected cells labelled for 16 hr (M). Apparent molecular weights x 10^{-3} of virus-induced polypeptides are shown in the middle and of the standard proteins on the right.

Results

MHV-60 and MHV-76 were chosen for detailed analysis of the polypeptide profile of a novel MHV. The MOI of 20 $TCID_{50}$ per cell was used and infected cells were labelled with ^{14}C-amino acid hydrolysate at various intervals post-infection (p.i.) (Figs. 1 and 2). 28 virus polypeptides with molecular weights ranging from 16 kD to 240 kD were demonstrated at both virus isolates. The following proteins were detected: 240, 161, 138, 125, 112, 100, 84, 74, 66, 62, 58, 55, 52, 50, 48, 46, 44, 42.5, 40, 39, 36, 35, 34, 32.5, 28.5, 27.5, 21, and 16 kD. No differences were found either in number of polypeptides or their molecular weights. We can conclude that MHV-60 and MHV-76 have identical polypeptide profiles.

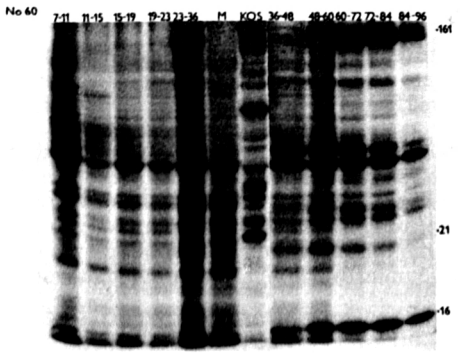

Fig. 2
Polypeptides synthesized in MHV-60 infected cells

Protein samples were analysed on a 10 % SDS-polyacrylamide gel. Cells infected with the strain KOS of HSV-1 and labelled with ^{14}C-amino acids from 4 to 20 hr p.i. (KOS). Apparent molecular weight x 10^{-3} of three virus-induced polypeptides is marked on the right. For further explanations see Fig. 1.

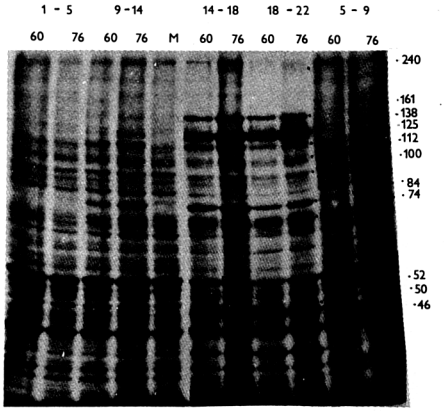

Fig. 3
The time course of the virus-induced polypeptide synthesis in MHV-60 nad MHV-76 infected cells

Cells were infected at a multiplicity of 40 $TCID_{50}$ per cell (60, 76) and pulse-labelled at various times p.i. Apparent molecular weights x 10^{-3} of virus-induced polypeptides are shown on the right. For further explanations see Fig. 1.

The kinetics of the synthesis of virus polypeptides of MHV-60 and MHV-76 was investigated in infected REF cells at a MOI of 1, 20, and 40 $TCID_{50}$ per cell. At a MOI of 1 $TCID_{50}$ per cell the polypeptides 138 kD and 84 kD were first detected 24-28 hr p.i. All other virus polypeptides were seen at 40-44 hr p.i. (not shown). At MOI of 20 $TCID_{50}$ per cell the first polypeptides occurred at 15-19 hr p.i. and the rest of polypeptides at 23-36 hr p.i. (not shown). At MOI of 40 $TCID_{50}$ per cell the first polypeptides (138 kD and 84 kD) were detected 5-9 hr p.i. and all others at 14-18 hr p.i. (Fig. 3).

Further we compared the polypeptide profiles of the REF cells infected with six isolates of MHV including MHV Šumava A.f. which was isolated from very distant locality (Mistríková and Blaškovič, 1985). The polypeptide profile of MHV Šumava A.f. is the most different from the other isolates of MHV, synthesizing an 84 kD polypeptide. In cells infected with the MHV Šumava A.f. a 79 kD polypeptide, as well as two new proteins (59 and 64 kD) were detected

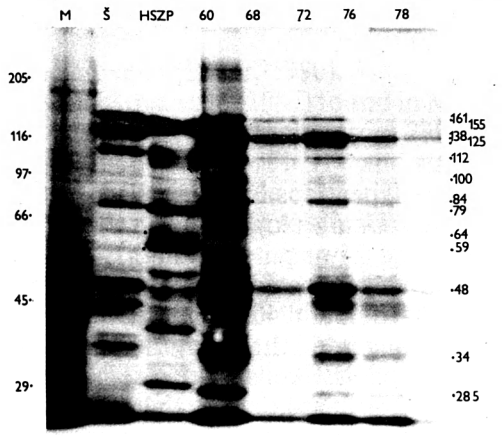

Fig. 4
Polypeptides synthesized in cells infected with various isolates of MHV

Cells were labelled from 72 to 96 hr p.i. and subjected to polyacrylamide gel electrophoresis. Apparent molecular weights x 10^{-3} of standard proteins are shown on the left and of the virus-induced polypeptides on the right. Cells infected with the isolate Šumava A.f. (Š), with the HSZP strain of HSV-1 (HSZP), with the individual isolates of MHV (60, 68, 72, 76, 78), mock-infected cells (M).

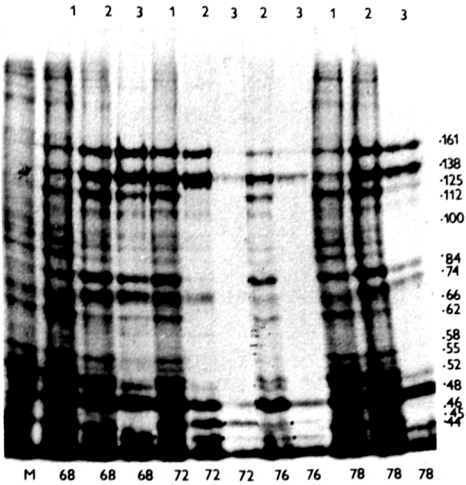

Fig. 5
Polypeptides synthesized in cells infected with various isolates of MHV
Protein samples were analysed on 6 % SDS-polyacrylamide gel. Isolates MHV-68, MHV-72, MHV-76, and MHV-78 were labelled at intervals from 48 to 72 (1), from 72 to 96 (2), or 96 to 120 hr (3), isolate 76 at intervals from 72 to 96 (2), or from 96 to 120 (3) hr p.i., respectively. For further explanations see Fig. 1.

(Fig. 4). Comparison of polypeptide profiles of MHV-68, MHV-72, MHV-76, and MHV-78 as illustrated in Fig. 5 shows that these isolates are identical with the exception of polypeptide 46 kD which is lacking in MHV-68 and MHV-78 infected cells. The same finding was obtained using a 12 % polyacrylamide gel.

Finally, we compared the polypeptide profiles of the strain KOS HSV-1 with MHV-76 (Fig. 6). A counterpart to the major nucleocapsid polypeptide (155 kD) of the strain KOS HSV-1 was the MHV-76 polypeptide 161 kD. Polypeptide 161 kD was common for all MHV isolates. The 125 and 138 kD polypeptides of MHV-76 seemed to correspond to the glycoproteins gB and gC of the strain KOS HSV-1. Also for the 112, 100, 84, and 74 kD polypeptides of MHV-76 counterparts in the polypeptide profile of cells infected with the strain KOS HSV-1 could be found. No counterpart to glycoprotein gD of the strain KOS HSV-1 in MHV-76 infected cells was detected. Further, two polypeptides (48 and 50 kD) synthesized in cells infected with MHV-76 were not detected in KOS-infected cells.

Discussion

In this communication, the synthesis of polypeptides in REF cells infected with six isolates of a novel MHV was analysed. Using a low MOI (1 $TCID_{50}$ per cell) the first virus polypeptides were detected 24 hr p.i. At a high MOI (40 $TCID_{50}$ per cell), these polypeptides were already detected by 5–9 hr p.i. Additional virus polypeptides were detected at 14–18 hr and 40–44 hr p.i., respectively. On the basis of these results, it can be concluded that the time course of virus polypeptide synthesis in the MHV-infected REF cells more resembled to that in *herpesvirus saimiri* infected cells (Randall, 1985) than to that in HSV-1

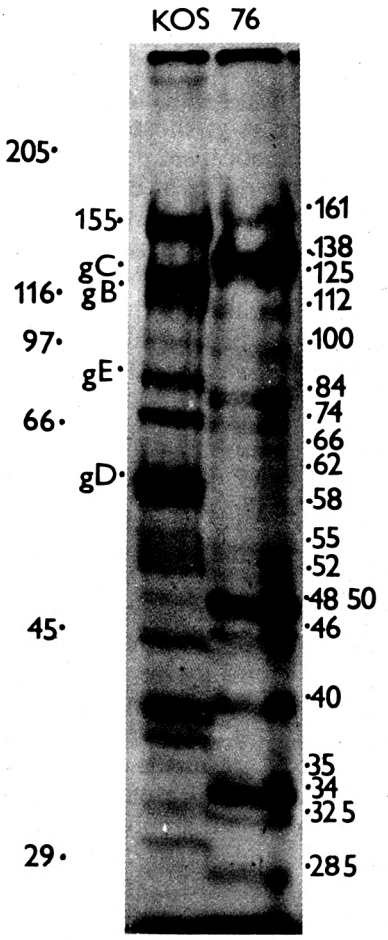

Fig. 6
Polypeptides synthesized in cells infected with the KOS strain of HSV-1 and with the MHV-76

Cells infected with the KOS strain were labelled from 4 to 20 hr p.i. Cells infected with the MHV-76 were labelled from 48 to 72 hr p.i. Apparent molecular weights x 10^{-3} of the standard proteins are indicated on the most left, of the major capsid polypeptide (ICP5), as well as the positions of individual glycoproteins of the KOS strain of HSV-1 on the left, and of the virus-induced polypeptides of MHV-76 on the right.

infected cells (Spear, 1980).

Stančeková *et al.* (1987) found in MHV about 24 structural polypeptides with a molecular weight ranging from 275 to 25 kD. Based on close similarity of the polypeptide profiles of MHV and strain HSZP of HSV-1 and on differences in the polypeptide profile of murine cytomegalovirus they supported the notion that MHV may belong to subfamily *Alphaherpesvirinae*. In MHV-infected REF cells 28 virus polypeptides with apparent molecular weights ranging from 240 to 16 kD were detected. In comparison with Stančeková *et al.* (1987), some differences in the number of detected virus polypeptides as well as in their apparent molecular weights were observed. The possible explanation is that Stančeková *et al.* (1987) analysed the polypeptides of purified viruses whereas in this communication the polypeptides synthesized in the MHV-infected REF cells

were investigated.

Some resemblance between the polypeptide profiles of MHV-76 and strain KOS HSV-1 has been found. Because of the possible glycosylation and phosphorylation of particular polypeptides, the unity of molecular weights of some polypeptides gt these compared viruses can be accidental. In addition, the major nucleocapsid polypeptide 155 kD HSV-1 and some other proteins have their equivalents at each of three herpesvirus subfamilies (Killington *et al.*, 1977; Honess and Watson, 1977; Modrow and Wolf, 1983; Landini and Michelson, 1988). This means that it is not possible to classify MHV into one of the subfamilies of herpesviruses on the basis of the polypeptide profiles only. In addition, the polypeptide profile of MHV has not yet been compared with the polypeptide profile of any gammaherpesvirus.

Efstathiou *et al.* (1990*a, b,*) suggested to group MHV-68 into subfamily *Gammaherpesvirinae* on the basis of the viral genome structure. Thus a comparison with the polypeptide profiles of certain gammaherpesviruses, namely with *herpesvirus saimiri*, will be desirable. Nevertheless, our results confirmed that the polypeptide profile of MHV has a characteristic feature of a herpesvirus polypeptide profile.

MHV Šumava A.f. was isolated from very distant locality and it differs from other isolates of MHV in some biological properties (Svobodová *et al.*, 1982a; Mistríková and Blaškovič, 1985). In addition, the studies on strain Šumava A.f. have shown that this virus genome has a mean G+C pair content of approximately 60 % and a size of 135 kb (Blaškovič *et al.*, 1988). On the other hand, MHV-68 genome consists of 188 kb of unique DNA having a G+C content of 45 % flanked by variable copies of an 1.23 kb repeat unit (Efstathiou *et al.*, 1990*a, b*). Our results confirm that the polypeptide profile of MHV Šumava A.f. was the most different from polypeptide profiles of other MHV isolates, too.

Comparing the polypeptide profiles of MHV isolates it has been shown that MHV-68 and MHV-78 unlike MHV-60, MHV-72, and MHV-76 are lacking the polypeptide 46 kD. This polypeptide is glycosylated and is accumulated in the cytoplasmic fraction (our unpublished results). The absence of this polypeptide could explain the antigenic differences among individual MHV isolates (Svobodová *et al.*, 1982*b*). MHV-68 and MHV-78 which are lacking the polypeptide 46 kD were isolated from phylogenetically different species of wild rodents (MHV-68 from *Clethrionomys glareolus* and MHV-78 from *Apodemus flavicollis)* similarly as isolates with this polypeptide. This confirms the conclusions of Blaškovič *et al.* (1987*b*) that the sensitivity to this virus is under the limit of the species specificity.

Further analysis of polypeptides present in the cytoplasmic and nuclear fractions of the MHV infected REF cells is currently under way.

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