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Serological Evidence for Antigenic Differences Between the Mason–Pfizer Monkey Virus (MPMV) and an MPMV-like Virus (PMFV) Detected in a Malignant Permanent Human Cell Line*

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Abstract—*Mason–Pfizer monkey virus (MPMV) and an MPMV-like virus (PMFV) detected in a human continuous cell line were compared in immunofluorescence tests. Antisera against MPMV and PMFV revealed cross-reacting antigens in the cytoplasm of cells infected with either MPMV or PMFV. Immunofluorescence absorption showed that, in addition to the common specificities, both viruses have distinct antigens. MPMV and PMFV must therefore be regarded as different types of a closely related virus group.*

INTRODUCTION

THE PMF virus (PMFV, abbr. from German: permanente menschliche Fibroblasten, permanent human fibroblasts) was detected in a malignant permanent human cell line obtained after cultivation over a long period of fibroblasts from an embryo of a mother with generalized portio carcinoma [1].

According to morphological, biochemical and immunodiffusion analyses [1, 2], the PMFV is closely related to the Mason–Pfizer monkey virus (MPMV) which was isolated from a mammary carcinoma of a rhesus monkey [3]. Viruses similar to MPMV were also found in cell lines from normal rhesus lactating mammary gland (X381) and rhesus placental tissue (FTP-1) [4] and in a variety of human continuous cell lines including HeLa, A0 and HeP-2 (for references see [5] and [6]). According to serological and nucleic acid hybridization data, there is a close relationship between these viruses but not to type-C or type-B oncornaviruses [5, 6]. Because of common morphological, biochemical and immunological characteristics the viruses of this group are now classified as type-D viruses [7]. A subclassification of these type-D viruses does not yet exist. This seems to be of special interest since there is obviously some "evidence that at least some isolates may be the result of either viral or cellular contamination" [6]. In particular the different human isolates were a point of controversy because some of the virus-producing cell lines contain HeLa-markers [8] and nucleic acid hybridization and most serological investigations did not reveal differences between these viruses and MPMV [6, 9, 10]. Hitherto only a few papers mention the existence of antigenic differences between different type-D viruses [5, 11]. In this paper the PMFV was compared with the MPMV by the semiquantitative immunofluorescence absorption test and in addition to cross-reactivity distinct antigens were detected discriminating both viruses.

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MATERIALS AND METHODS

Cells and viruses

Tu 197 cells (human ovarial carcinoma line) were grown as monolayers in glass flasks in MEM supplemented with 5 or 10% heat-

inactivated normal calf-serum [1]. The same culture conditions were used for Tu 197 cells infected with PMFV or MPMV (designated Tu 197/PMFV and Tu 197/MPMV). NC 37 cells (normal human lymphocyte cell line) uninfected and infected with MPMV (NC 37/MPMV; obtained from Pfizer Inc., Maywood, N.J., through courtesy of Dr. J. Gruber, Office of Program Resources and Logistics, NCI) were grown in suspension in glass flasks in RPMI 1640 medium supplemented with 8% heat-inactivated fetal calf-serum.

Antisera

The following goat anti-MPMV sera were used (obtained from Huntingdon Research Center, Brooklandville, MD, through courtesy of Dr. J. Gruber, NCI):

2-S-0752 (anti-Tween-ether disrupted MPMV) and

2-S-0612 (anti-intact MPMV).

For indirect immunofluorescence with fixed cells (IF) the sera were diluted 1/40 (in PBS supplemented with calf serum and normal human AB-serum) and absorbed once with an equal volume of sedimented uninfected Tu 197 cells (frozen/thawed once) and a second time with uninfected NC 37 cells. After absorption the sera reacted in IF with MPMV- and PMFV-infected but not with uninfected cells and could be further diluted up to 1/16 or 1/32. Generally, the working dilution was 1/8.

Antisera against PMFV were produced in guinea pigs and rabbits with semipurified virus isolated from culture fluid of virus-producing cells by ultracentrifugation through a 30% sucrose cushion, and three times treatment with 15% NaCl (for 30 min) and subsequent centrifugation to remove non-viral proteins according to Traul *et al.* [12]. Nonidet P-40 disrupted virus was used for immunization. Two rabbits were immunized by subcutaneous injection at different sites of 500 μg of viral protein in Freund's complete adjuvant and three subsequent inoculations of 250 μg of protein without adjuvant at weekly intervals. Two guinea pigs were immunized in a similar way but 100 μg of viral protein were used per inoculation.

Since no decisive difference was observed in the reactivity of anti-disrupted MPMV and anti-intact MPMV sera no attempt was made to produce an anti-intact PMFV in addition to the anti-disrupted PMFV sera.

Specifically reactive sera were obtained after diluting them 1:5 (in calf serum and normal human AB-serum) and absorbing three times with an equal amount of Tu 197 cell sediment. The sera could be further diluted 1/2 and sometimes 1/4 but the specific reaction with infected cells was very weak compared with the anti-MPMV sera.

FITC-conjugated anti-sheep/goat, anti-guinea pig and anti-rabbit globulin sera (Staatliches Institut für Immunpräparate und Nährmedien, Berlin-Weissensee, GDR) were diluted 1/8 or 1/10 (in PBS supplemented with calf serum and normal human AB-serum) absorbed once with a mixture of Tu 197 and NC 37 cells and further diluted 1/2.

Indirect immunofluorescence test (IF)

The indirect immunofluorescence test with fixed cells was done according to the method of Hilgers *et al.* [13]. Eight small round filter paper pieces (5 mm in diameter) were spaced onto cleaned u.v.-light-permeable slides, and the slides were sprayed with Auto-Polish-spray (VEB Aerosol-Automat, Karl-Marx-Stadt, GDR) and permitted to dry. This treatment results in a layer of wax which is hydrophobic, aceton-insoluble and non-toxic for the cells used so far and can be easily washed away with xylol for cleaning. The filter paper was removed and 20 μl of a cell suspension with $1-2 \times 10^6$ cells/ml (prepared by trypsinization and washing in PBS) were placed onto each spot of the slide. The slides were dried at 40°C for 1 hr, fixed in aceton for 10 min at room temperature and stored at −20°C. For the test the slides were thawed, washed in distilled water and dried with a hairdryer. Each spot with fixed cells was incubated with 20 μl of the antiserum for 1 hr at 37°C in a moist chamber, washed twice with saline and twice in distilled water for 5 min and dried. The cells were then incubated with 20 μl of the FITC-conjugated antiglobuline serum for 1 hr at 37°C and washed again. Counterstaining was performed with 0.06% Evan's blue dye in distilled water for 5 min and the cells were washed another two times in distilled water and dried. The slides were mounted with glycerine-PBS and covered with a long coverslip. If not examined immediately the slides were stored at −20°C.

The examination was done under a Reichert fluorescence microscope with an HBO 200 high-pressure mercury lamp, a blue glass exciter filter BG 12 (50 mm in diameter, 3 mm thick, Jenaer Glaswerk, Schott u. Gen., Jena, GDR) and a GG 9 yellow barrier filter

(same factory) used for the microscope ocular. Positive cells showed a green cytoplasmic fluorescence, negative cells and the nuclei of positive cells were red because of the counterstaining.

Immunofluorescence absorption test (IFA)

The immunofluorescence absorption test (IFA) was done according to Hilgers *et al.* [13].

Absorptions were performed with cellular extracts from MPMV- and PMFV-infected cells. For the preparation of the extracts the trypsinized (in case of monolayers) and washed cells were sedimented, mixed with an equal volume of PBS and disrupted by 5 cycles of freezing and thawing. This mixture which contained about 60 million disrupted cells/ml was regarded as the undiluted extract (1/1). For IFA double dilutions of the extracts up to 1/64 were incubated with an equal volume of a standard dilution of the antisera overnight at 4°C. The standard dilution for the anti-MPMV sera (1/40 diluted and Tu 197-NC37-absorbed) was 1/4; that means the serum was 1/8 diluted after absorption. The anti-PMFV sera (1/5 diluted and Tu 197-absorbed) had to be absorbed without further dilution to give a final dilution of 1/2 after absorption.

After absorption the mixture was clarified by centrifugation for 10 min at 3000 *g* and then tested in IF (20 µl per spot) for residual antibody activity. For quantitative evaluation the percentage of fluorescent cells was estimated by counting 100–200 cells. To compare the absorbing effect of MPMV- and PMFV-containing cell extracts on anti-MPMV and anti-PMFV sera when tested against both infected cell types the decreased immunofluorescence after absorption was calculated as percentage of the IF in the positive control. This means that if the anti-MPMV serum reacted with 50% of the MPMV-infected cells and after absorption (with a special extract dilution) with 25% of the infected cells the decreased immunofluorescence was 50%.

RESULTS

Immunofluorescence with PMFV- and MPMV-infected cells

Anti-MPMV and anti-PMFV sera absorbed with calf serum and non-infected cells reacted specifically with the cytoplasm of both MPMV- and PMFV-infected but not with uninfected cells. Checking different passages of the permanently infected cell lines a high variation in the percentage of fluorescent cells (between 30 and 70%) was observed. In general no significant differences were observed in the percentage of fluorescent cells per passage when the different antisera were used. But in all tests with anti-PMFV sera the intensity of fluorescence was weak compared with anti-MPMV sera.

Comparison of PMFV and MPMV in immunofluorescence absorption

In the first experiments anti-MPMV serum (2-S-0752) was absorbed with either cell extracts from MPMV-infected NC37 or PMFV-infected Tu 197 cells and tested in IF against both cell types. In Fig. 1 the decreased immunofluorescence after absorption is plotted against the dilution of the cellular extract for one representative experiment.

As shown by the shape of the curves both MPMV- and PMFV-containing extracts absorbed the total antibody activity against PMFV-infected Tu 197 cells. But when tested against MPMV-infected NC37 cells there was a clear-cut difference between Tu 197/PMFV- and NC37/MPMV-absorbed serum. After absorption with NC37/MPMV extracts the antibody activity was removed but after absorption with Tu 197/PMFV extracts a positive reaction could still be detected against NC37/MPMV cells.

To avoid misinterpretation due to quantitative differences in the antigen concentration of the extracts the same extract pools and dilutions were used for at least one complete experiment. This means that the absorption shown e.g., in Fig. 1 was done with the same NC37/MPMV and the same Tu 197/PMFV extracts for both target cells.

In some experiments comparing the absorbing capacity of Tu 197/PMFV and NC37/MPMV extracts (which contain a high concentration of MPMV-induced antigens) differences of an obviously quantitative nature were also observed. This resulted in a higher absorption of anti-MPMV serum by NC37/MPMV extracts if tested against Tu 197/PMFV target cells. Since qualitative differences can be excluded in this test combination the distinct absorption curves can be only a result of quantitative differences. In such a case an antigen titer correction was done by bringing the curves arbitrarily to coincidence for the system: anti-MPMV se-

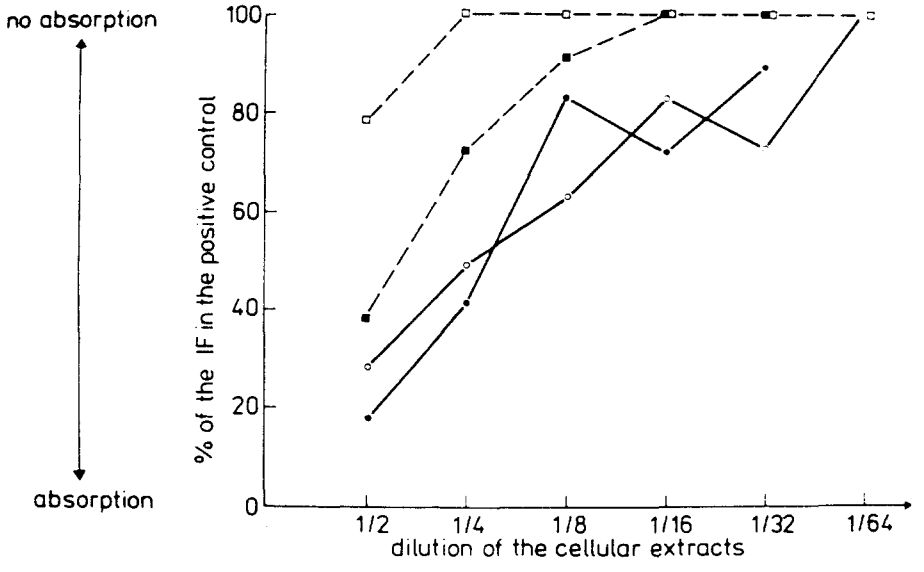

Fig. 1. Comparison of MPMV and PMFV in IFA with anti-MPMV serum (2-S-0752).
Unbroken lines: tests with Tu 197/PMFV cells, absorption with
●, *NC37/MPMV extract,*
○, *Tu 197/PMFV extract.*
Broken lines: tests with NC37/MPMV cells, absorption with
■, *NC37/MPMV extract,*
□, *Tu 197/PMFV extract.*

rum absorbed with both extracts and tested against Tu 197/PMFV cells which is equivalent to an equal antigen concentration in both extracts. If a titer correction of the same magnitude was performed for the tests with NC37/MPMV target cells a clear-cut difference remained between NC37/MPMV- and Tu 197/PMFV-absorbed serum indicating this as a qualitative phenomenon.

The presence of antigens detected by anti-MPMV sera (also shown with serum 2-S-0612) in NC37/MPMV but not in Tu 197/PMFV cells can be explained as evidence for genetic differences between both type-D viruses or by a possible expression of different antigens of the same virus in the different cells used for growing the virus. The presence of some different proteins in MPMV isolates grown in different cell types is known although it is not clear whether these proteins are coded for by the viral genome or are host cell-derived components [14].

Therefore, the experiments were repeated by using the same line for growing MPMV and PMFV to avoid any host cell factor which might be responsible for the antigen differences observed in the experiments described above. Thus, Tu 197 cells were also infected with MPMV and these cells used in IFA.

The comparison of Tu197/MPMV and NC37/MPMV in IFA tests using anti-MPMV sera (2-S-0612) showed no differences in the absorbing capacity of both extracts for antibodies reactive with both infected cell types (Fig. 2). If Tu197/MPMV and Tu197/PMFV cells were compared the same differences were observed as in the experiments with NC37/MPMV and Tu197/PMFV cells (Fig. 3). This means that the antigenic differences do obviously not depend on the cell type in which the virus is grown but rather reflect differences in the viral genome. The antigenic differences were also observed when using the anti-PMFV sera. These sera detected, in addition to the common components, some antigen(s) in PMFV-infected cells which cannot be absorbed by Tu197/MPMV extracts and are therefore not present in MPMV-infected cells (Fig. 4).

DISCUSSION

The results presented show that although MPMV and PMFV are closely related they have some antigenic differences which discriminate both viruses (Table 1). The sera and the technique used here do not allow a conclusion on which polypeptide these antigenic determinants are localized. Experiments of Stephenson done to compare different type-D

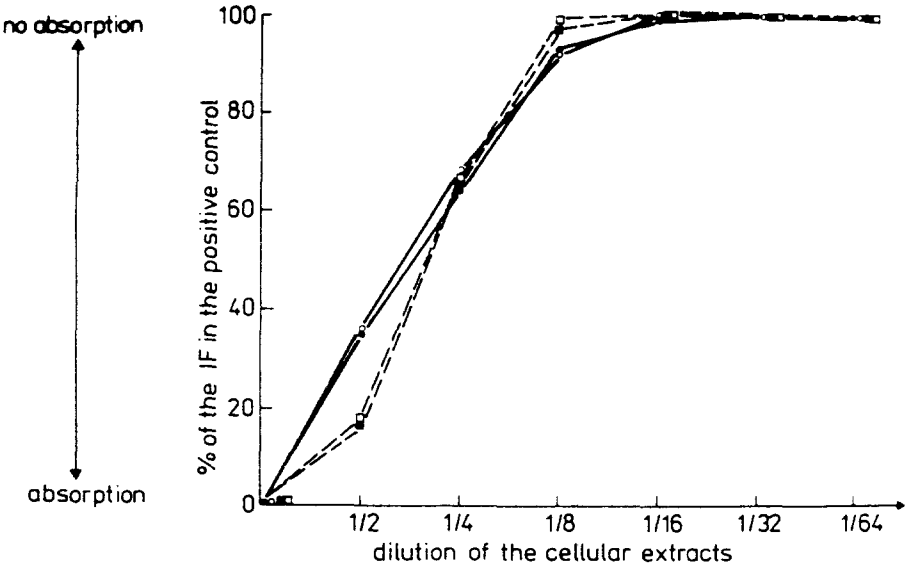

Fig. 2. Comparison of MPMV grown on different cells in IFA with anti-MPMV serum (2-S-0612).
Unbroken lines: tests with Tu 197/*MPMV cells, absorption with*
●, *Tu* 197/*MPMV extract,*
○, *NC*37/*MPMV extract.*
*Broken lines: tests with NC*37/*MPMV cells, absorption with*
■, *Tu* 197/*MPMV extract,*
□, *NC*37/*MPMV extract.*

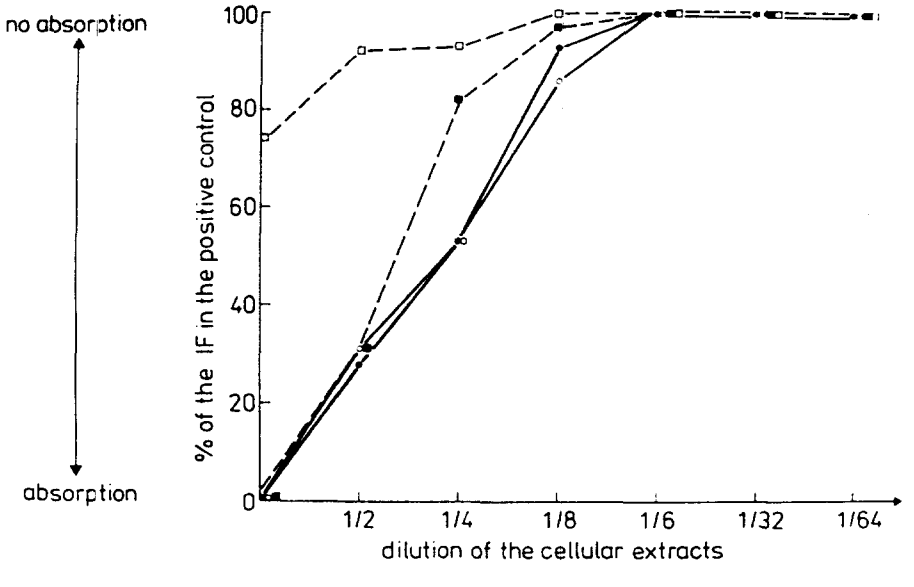

Fig. 3. Comparison of MPMV and PMFV in IFA with anti-MPMV serum (2-S-0612).
Unbroken lines: tests with Tu 197/*PMFV cells, absorption with*
●, *Tu* 197/*MPMV extract,*
○, *Tu* 197/*PMFV extract.*
Unbroken lines: tests with Tu 197/*MPMV cells, absorption with*
■, *Tu* 197/*MPMV extract,*
□, *Tu* 197/*PMFV extract.*

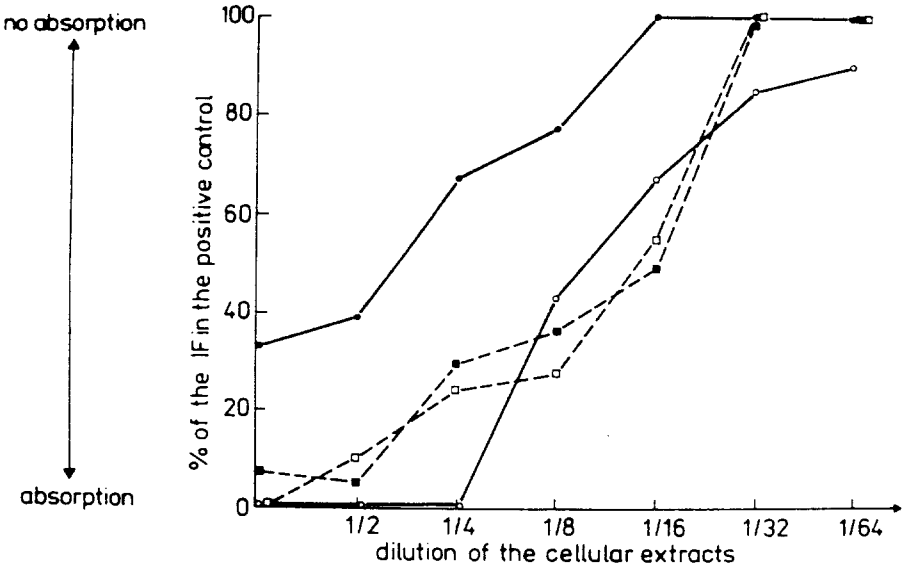

Fig. 4. Comparison of MPMV and PMFV in IFA with rabbit anti-PMFV serum. Unbroken lines: tests with Tu 197/PMFV cells, absorption with
●, *Tu 197/MPMF extract,*
○, *Tu 197/PMFV extract.*
Broken lines: tests with Tu 197/MPMV cells, absorption with
■, *Tu 197/MPMV extract,*
□, *Tu 197/PMFV extract.*

Table 1. Summary of the results of the experiments to detect antigenic differences between Mason–Pfizer monkey virus (MPMV) and PMF virus (PMFV) by indirect immunofluorescence absorption tests

	Antisera					
	Anti-MPMV not absorbed	Anti-MPMV absorbed Tu197/PMFV	Anti-MPMV absorbed Tu197/MPMV	Anti-PMFV not absorbed	Anti-PMFV absorbed Tu197/PMFV	Anti-PMFV absorbed Tu197/MPMV
Tu197/PMFV	+++*	–	–	+++	–	+
Tu197/MPMV	+++	++	–	+++	–	–

*Positive reaction in indirect immunofluorescence test with fixed cells.

viruses in competition radioimmunoassays showed that MPMV and PMFV have the same p27 but are different in the p15 (personal communication of J. R. Stephenson to A. Graffi).

Since there are some differences reported to be between MPMV and type-D viruses isolated from human cell lines [5, 11, 15] it would be of importance to know whether there are antigenic differences between the different human isolates and whether it is possible to detect some differences between the rhesus monkey isolates (MPMV, X381, FTP-1). This may give some hints at the human or non-human origin of the human isolates and at the question of contamination. It has been recently shown that MPMV is related to an endogenous type-D virus from langurs and that the MPMV obviously has been derived from these monkeys or a close relative by transspecies infection [16]. If a transmission of langur viruses had also occurred to man a more pronounced difference should be expected between MPMV and the human isolates. It can, therefore, be assumed that the human isolates are of rhesus monkey origin and that an infection of human cells with MPMV or a closely related virus took place either under natural conditions or in a cell culture laboratory. An answer to this question can probably only be received by searching for type-D virus activities in the human population. Hitherto there are some results which point to the presence of MPMV-activities in human beings with tumors or under immunodepression [11, 17–23].

But since there are also strong arguments against the distribution of MPMV-like viruses in man [10] further experiments have to clarify this situation. Such experiments will either help to give the human type-D viruses their own important place in the retravirus system or to unmask them as laboratory contaminations which only led their investigators on a wrong track.

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