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Immunological Properties of Two Polypeptides of Mason-Pfizer Monkey Virus

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Two polypeptides were isolated from the Mason-Pfizer monkey virus (MP-MV). The polypeptides, designated MP-MV p26 and MP-MV p15, were 26,000 and 15,000 molecular weight, respectively, based on gel filtration chromatography in the presence of 6 M guanidine hydrochloride. Radioimmunoassays developed for MP-MV p26 and p15 were, respectively, 10 and 100 times more sensitive than immunodiffusion and 1 and 10 times more sensitive than micro-complement fixation for the detection of MP-MV. Antigens of other reverse transcriptase-containing RNA viruses did not cross-react in either MP-MV radioimmunoassay. Further, antisera against these other viruses did not react with the radioiodine-labeled MP-MV polypeptides. The MP-MV radioimmunoassays should be useful in studying the natural occurrence of this virus and its relationship to primate tumors.

The Mason-Pfizer monkey virus (MP-MV) was isolated from a spontaneous mammary carcinoma of a rhesus monkey (6). This virus has morphologic characteristics of both RNA type C and type B viruses (14, 19), contains 60 to 70*S* RNA, and a reverse transcriptase (27). MP-MV appears to be unique with respect to its antigenic properties. Earlier studies by Nowinski et al. (19) failed to detect any immunological relationship between two soluble antigens of MP-MV and those of several type C viruses, mouse mammary tumor virus, visna virus, or simian foamy virus. More recently, the reverse transcriptase and the major virion polypeptide of MP-MV were found to lack detectable antigenic cross-reactivity with the respective proteins of other known reverse transcriptase-containing viruses (24, 29).

The possibility that viruses similar to MP-MV might exist in humans was raised by the isolation of a virus morphologically and antigenically very similar to MP-MV from brain cells of a patient with Creutzfeldt-Jakob disease (11, 12). More recently, viruses immunologically and biochemically resembling MP-MV have also been isolated from tumor cell lines of human origin (4, 9, 24, 36). These findings indicate either that this virus class may naturally exist in humans or that MP-MV may have been introduced as a laboratory contaminant (12, 24). Radioimmunologic techniques have been applied to the detection and quantitation of various polypeptides of type C viruses (21, 28, 30, 32, 33). In particular, type-specific immunoassays have been developed that very sensitively discriminate between type C viruses that can not be readily distinguished by other available methods (31, 35). In the present study, we report the isolation of two virion polypeptides of MP-MV and the development of radioimmunoassays for each. The assays detect nanogram quantities of these polypeptides and provide highly specific antigenic markers for MP-MV.

MATERIALS AND METHODS

Viruses. Purified MP-MV grown in either the original monkey tumor cell line, CMMT, or a human lymphocyte line, NC37, was generously provided by Pfizer Laboratories, Maywood, N. J., through the Resources and Logistics Segment, National Cancer Institute. Purified type C RNA viruses were obtained either from Electro-Nucleonics, Rockville, Md., or Pfizer Laboratories. The type C RNA viruses used, designated according to the species from which they were isolated, are as follows: woolly monkey virus; gibbon ape virus; Rauscher (R)-murine leukemia virus (MuLV); rat leukemia virus (RaLV); the Rickard strain of feline leukemia virus (FeLV); and the RD114 feline virus. The sources of these viruses have been described (1). Mouse mammary tumor virus (M-MTV) and simian syncytial ("foamy") viruses, serotypes 1, 3, and 7, were obtained from Meloy Laboratories, Inc., Springfield, Va., and R. L. Herberling, Southwest Primate Center, San Antonio, Tex., respectively, through the Resources and Logistics Segment, National Cancer Institute.

Isolation of MP-MV polypeptides. MP-MV was repurified by isopycnic centrifugation in a continuous linear sucrose gradient. The viral polypeptides were

separated by agarose gel filtration chromatography in the presence of 6 M GuHCl as previously described (34).

PAGE. Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed by the method of Laemmli (15). PAGE in the presence of 6.25 M urea at either pH 3.2 or 9.0 was carried out according to the procedures of Panyim and Chalkley (23) and Maizel (18), respectively.

Iodination of viral polypeptides. Viral polypeptides were radioactively labeled with ^{125}I by the chloramine T method of Greenwood et al. (10).

Antisera. Antisera prepared against type C viral antigens of MP-MV antigens were generously provided by R. Wilsnack (Huntingdon Research Laboratories, Baltimore, Md.) through the Resources and Logistics Segment, National Cancer Institute. These antisera were prepared by immunizing goats with detergent-treated virions. Anti-goat IgG was prepared in pigs. These immunization procedures have been described in detail (34). Antisera to simian foamy viruses, serotypes 1, 3, and 7, were obtained from R. L. Herberling.

Immunological procedures. (i) Radioimmunoassays for inter- and intraspecies antigenic determinants of p30 polypeptides of mammalian RNA type C viruses were carried out as previously reported (30). Radioimmunoassays for the p12 polypeptides of MuLV, the woolly monkey, and gibbon ape type C viruses have also been described (34, 35, S. Tronick et al., manuscript in preparation). (ii) For immunodiffusion the method of Ouchterlony was used (22). The gels contained 1% noble agar (Difco, Detroit, Mich.) dissolved in phosphate-buffered saline and 15 mM sodium azide. (iii) Micro-complement fixation was performed according to the method of Levine and Van Vanukis (16) except that the final reaction volume was 1.2 ml. Viruses were disrupted with 1% SDS and assayed at sufficient dilution ($<0.07\%$ SDS) to avoid lysis of the red cells.

Protein determinations. Proteins were quantitated by the method of Lowry et al. (17). Purified viral polypeptides, available only in microgram quantities, were electrophoresed on SDS gels, stained with Coomassie blue, and quantitated by densitometry using a Gilford linear transport. Standards were used which migrated approximately the same distance as the sample, since migration distance has been shown to markedly affect the linearity of calibration curves (8).

RESULTS

Isolation of MP-MV polypeptides. A profile of MP-MV polypeptides separated by PAGE-SDS is presented in Fig. 1A. The major polypeptide had a molecular weight (MW), relative to standards, of 26,000 consistent with the results of other laboratories (20, 24). Other major polypeptides had MW's of 65,000 and 41,000. Three less well-resolved polypeptides had MW's of 12,000 to 14,500, and two minor peaks had MW's of 22,000 and 20,000. To isolate individual polypeptides, the virus was disrupted with 6 M GuHCl in the presence of dithiothreitol (DTT) and chromatographed on a 6% agarose column. Fractions were pooled, dialyzed to remove GuHCl, and concentrated. Two polypeptides studied in detail in the present report were from the column fractions corresponding to 24,000 to 28,000 MW and 14,000 to 16,000 MW. When analyzed by PAGE-SDS, the MW of the larger polypeptide was 26,000, whereas the MW of the smaller was 20,000 (Fig. 1B and C). The MW's of small polypeptides of type C viruses have also been found to be higher when determined by PAGE-SDS as compared to gel filtration in 6 M CuHCl (5, 31). These polypeptides were iodinated by using chloramine T as described in Materials and Methods, and were analyzed by gel filtration chromatography in 6 M GuHCl. The elution profiles are shown in Fig. 2. The larger polypeptide eluted as a single peak at 26,000 MW (Fig. 2A). The smaller polypeptide eluted at a position corresponding to 15,000 MW (Fig. 2B) again in contrast to 20,000 as determined by PAGE-SDS. A minor 5,500 MW peak (Fig. 2B) probably represents a breakdown product, since the relative amount of radioactivity in this peak increased with time of storage of this preparation. Based on their elution from the agarose column, the MP-MV polypeptides are designated p26 and p15.

Each ^{125}I-labeled polypeptide was also examined by PAGE in the presence of 6.25 M urea. ^{125}I-p26 migrated as a single peak in the pH 3.2 system (Fig. 3A). In contrast, ^{125}I-p15, which did not move into the gel at pH 3.2, chromatographed as a single peak at pH 9.0 (Fig. 3B). These data suggest that p15 is an acidic protein. Both polypeptides had to be carboxymethylated in order to prevent the formation of aggregates (3), although some ^{125}I-p26 aggregated even after alkylation. The above results indicate that both p15 and p26 were sufficiently pure for use in radioimmunoassays.

Antibody precipitation of MP-MV polypeptides. An antiserum produced by immunizing a goat with Tween 80-ether-treated MP-MV was tested for its ability to bind p26 and p15. This antiserum maximally bound 98 and 80% of the acid-precipitable radioactivity of ^{125}I-p15 and ^{125}I-p26, respectively. The slopes of the binding curves differed markedly for each antigen indicating their immunologic dissimilarity (Fig. 4). Furthermore, the antiserum had a threefold higher titer for p26 (10% binding at a 1:100,000 dilution) than for p15 (10% binding at a 1:33,000 dilution). The characteristics of this antiserum are similar to those of antisera prepared against detergent-disrupted type C vi-

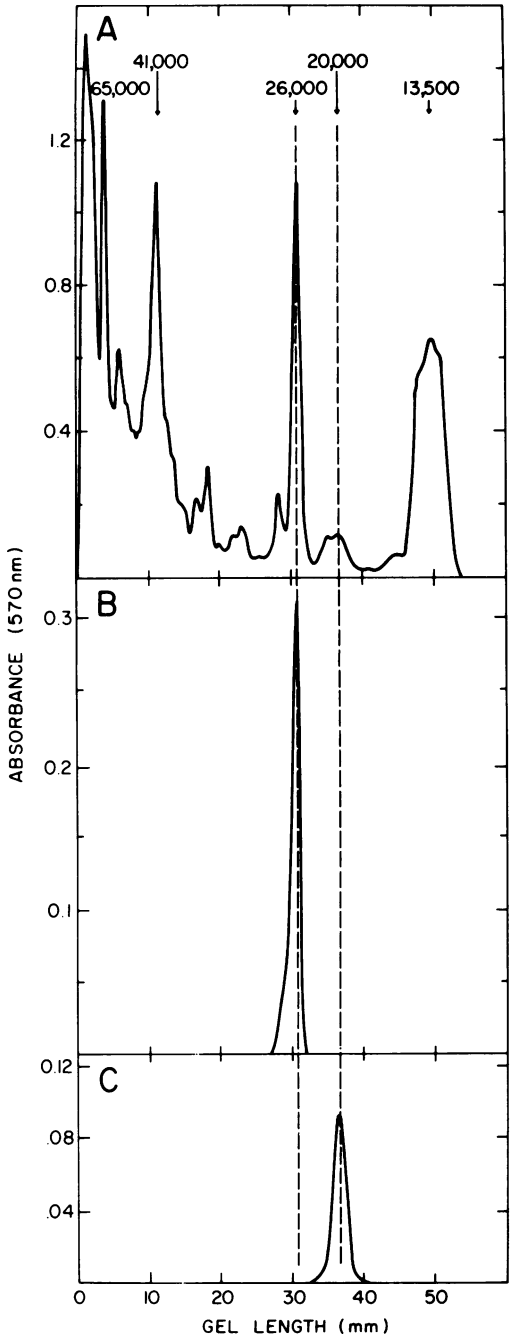

FIG. 1. *PAGE-SDS of MP-MV and of MP-MV polypeptides isolated by gel filtration in the presence of 6 M GuHCl. (A) MP-MV, 40 μg; (B) MP-MV polypeptide (1 μg) from column fractions corresponding to 22,000 to 28,000 molecular weight; (C) MP-MV polypeptide (0.5 μg) from column fractions corresponding to 14,000 to 16,000 molecular weight. The acrylamide concentration was 12%. Molecular weights were determined as described by Weber and Osborn (37) by using the standards bovine serum albumin (68,000), E. coli alkaline phosphatase (40,000), carbonic anhydrase (29,000), β-lactoglobulin (18,400), and lysozyme (14,300).*

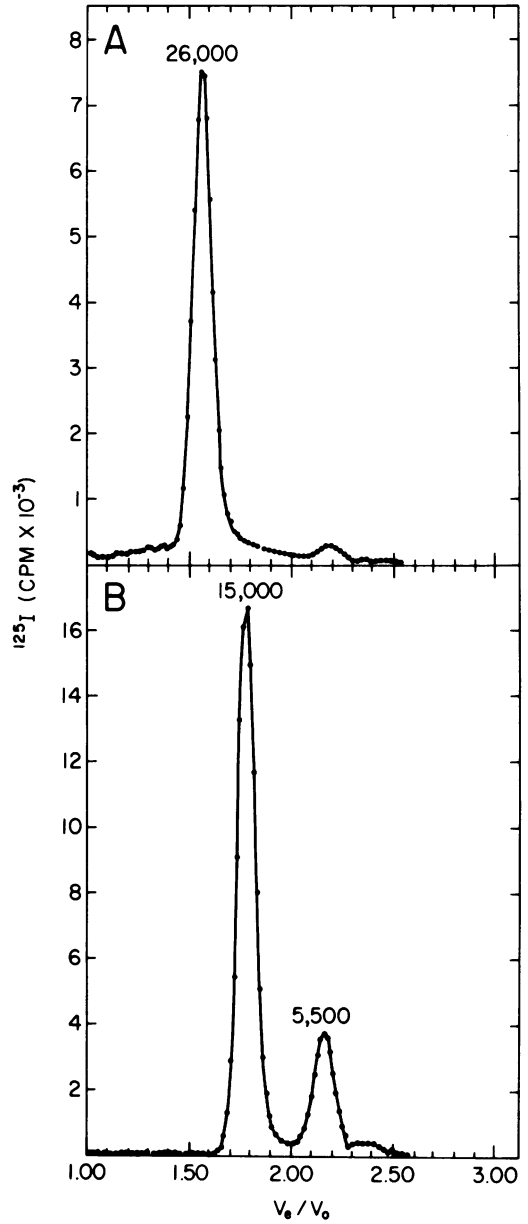

FIG. 2. *Gel filtration of iodinated MP-MV polypeptides in 6 M GuHCl on a 6% agarose column. (A) MP-MV polypeptide from Fig. 1B (26,000 daltons by PAGE-SDS); (B) MP-MV polypeptide from Fig. 1C (20,000 daltons by PAGE-SDS). Molecular weights were determined by plotting the distribution coefficient versus log MW (7) by using the standards listed in Fig. 1 and, in addition, cytochrome c (12,300) and insulin (2,900). V_e represents elution volume of each standard, and V_o designates the excluded volume, determined with blue dextran.*

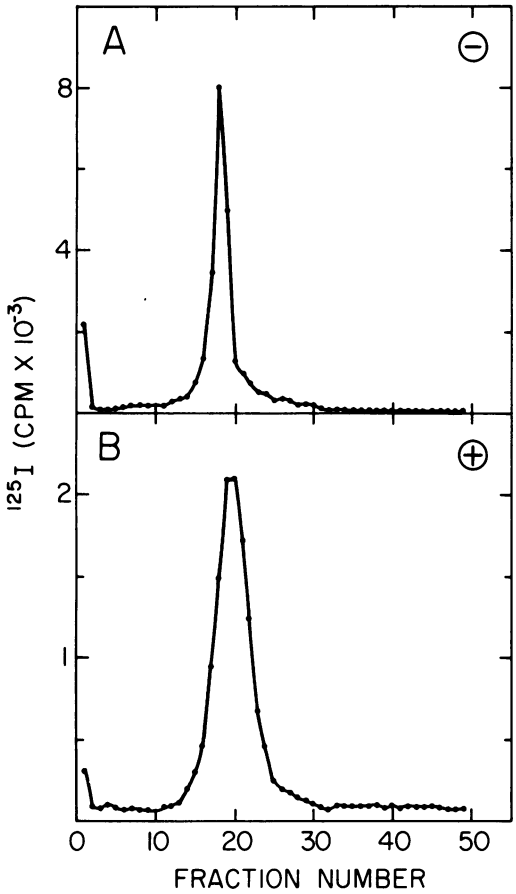

FIG. 3. *Analysis of ^{125}I-labeled MP-MV polypeptides by PAGE in the presence of 6.25 M urea. (A) ^{125}I-p26 at pH 3.2; (B) ^{125}I-p15 at pH 9.0. The acrylamide concentration was 10%. The iodinated polypeptides were carboxymethylated according to the method of Anderson et al. (3).*

ruses (34, 35) in that these latter antisera have higher titers but lower affinities for the higher MW (p30) polypeptide as compared to the lower MW (p12) antigen.

The specificities of antibody binding of ^{125}I-labeled p26 and p15 polypeptides were next examined (Table 1). Antisera prepared against several different reverse transcriptase-containing viruses were tested for binding ^{125}I-labeled MP-MV p26 and p15. Although anti-MP-MV serum bound both antigens, this same antiserum did not bind significantly the ^{125}I-p30 or ^{125}I-p12 polypeptides of either the woolly monkey virus or R-MuLV. Conversely, sera prepared against ether-disrupted type C viruses had high titers for binding the woolly monkey and MuLV ^{125}I-p30 antigens but were not significantly reactive against either MP-MV polypeptide. Similarly, antisera against simian foamy virus types 1, 3, and 7, and M-MTV, each of which had a high titer against its homologous virus as measured by complement fixation (data not shown), were nonreactive with the MP-MV polypeptides. These results demonstrate the specificity of the MP-MV ^{125}I-p26 and ^{125}I-p15 immunoprecipitation reactions and suggest the possibility of using such tests for detection of naturally occurring antibodies to this virus.

Competition radioimmunoassays for p12 and p26. Competition radioimmunoassays were developed for each antigen. Calibration curves were prepared by measuring the ability of unlabeled antigen to compete with ^{125}I-labeled polypeptide for limiting antibody (Fig. 5). In the p26 assay, 140 ng of p26 inhibited the binding of ^{125}I-p26 by 50%. The sensitivity of the assay, defined as 10% inhibition of binding, was 10 ng. In the p15 immunoassay, 8 ng of p15 inhibited binding by 50%, and 1 to 2 ng were readily detectable. Figure 5 also demonstrates the specificities of the assays. Unlabeled p15 did not compete significantly in the p26 assay. It should be noted that the p26 preparation competed in the p15 assay but was 14 times less effective than p15. This suggests a low level (around 7%) contamination of the p26 preparation with p15.

Specificity of competition radioimmunoassays for MP-MV p26 and p15. MP-MV grown

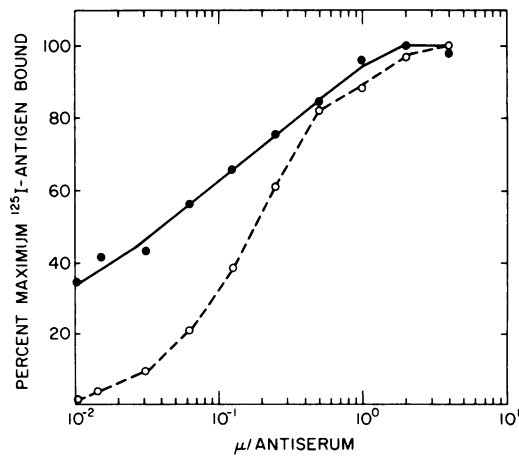

FIG. 4. *Binding of ^{125}I-p26 and ^{125}I-p15 by an antiserum prepared against detergent-disrupted MP-MV. The data have been normalized to the maximum percentage of binding of each antigen. This represents 80 and 98% of the acid-insoluble radioactivity of ^{125}I-p26 and ^{125}I-p15, respectively. A 10-ng amount (6,400 counts/min) of ^{125}I-p26 and 1 ng (6,500 counts/min) of ^{125}I-p15 were used in the assays. Symbols: ●, ^{125}I-p26; ○, ^{125}I-p15.*

TABLE 1. *Binding of ^{125}I-viral polypeptides by antisera prepared against reverse transcriptase-containing viruses*

Antiserum to	Antiserum titer for binding[a]					
	MP-MV		Woolly monkey		R-MuLV	
	p26	p15	p30	p12	p30	p12
MP-MV	5.0	4.5	<1.7	<1.7	<1.7	<1.7
Gibbon ape type C virus	<1.7	<1.7	5.9	4.7	4.3	<1.7
Woolly monkey type C virus	<1.7	<1.7	6.0	4.5	4.5	<1.7
FeLV	<1.7	<1.7	5.3	<1.7	5.6	<1.7
R-MuLV	<1.7	<1.7	5.3	<1.7	3.5	4.5
Simian syncytial virus (types 1, 3, and 7)	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7
M-MTV	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7

[a] The antiserum titer is defined as the log of the reciprocal of the antiserum dilution required to bind 10% (500 to 1,000 counts/min over background of 40 counts/min) of the iodinated antigen. Antisera to simian syncytial virus were positive in complement fixation assays for their respective viruses. The antiserum to M-MTV was positive in immunodiffusion assays with M-MTV.

in either human or monkey cells, several mammalian type C viruses, simian syncytial virus (serotypes 1, 3, and 7), and M-MTV were tested in the p26 and p15 immunoassays. Although MP-MV reacted in both assays, none of the other viruses showed detectable reactivity even at the highest concentrations tested (10 μg/ml) (Fig. 6). This result confirms and extends previous findings suggesting a lack of immunological relatedness between MP-MV antigens and those of other reverse transcriptase-containing viruses (19, 24, 29). Furthermore, it is clear that both the p15 and p26 immunoassays are highly specific for the detection of MP-MV.

Sensitivities of different immunological assays for the detection of MP-MV antigens. A comparison of the sensitivities of the competition radioimmunoassays for MP-MV p15 and p26 with other immunological methods for detection of MP-MV antigens is shown in Table 2. As little as 1 to 2 ng of p15 can be detected by radioimmunoassay; however, p15 did not give a positive reaction in immunodiffusion or complement-fixing assays. MP-MV p26 was detected as sensitively by the p26 radioimmunoassay and by micro-complement fixation, whereas 10 times more p26 was required to give a positive reaction in immunodiffusion assays. For detection of SDS-disrupted virus, the p15 immunoassay was most sensitive, detecting 5 ng of viral protein. The p26 assay and micro-complement fixation were each about 10-fold less sensitive, whereas immunodiffusion required at least 500 ng of viral protein for a positive reaction. Micro-complement fixation and immunodiffusion were also specific for MP-MV, since type C viruses (Table 2, unpublished data) were unreactive even with the addition of 30 to 50 μg of viral protein.

DISCUSSION

RNA viruses resembling MP-MV have now been isolated in several laboratories (4, 6, 9, 11, 25, 36). Although the biological activity in vivo of the prototype virus of this group, MP-MV, is not yet known, recent reports of its transforming activity in tissue culture (26) increase the likelihood that it may be involved in some forms of cancer. Biochemical comparisons of MP-MV with other reverse transcriptase-containing viruses have revealed that MP-MV contains a major polypeptide similar in size to that of type C viruses (26,000 versus 30,000 MW) whereas the major virion polypeptide of a type B virus, M-MTV, is 52,000 daltons (20). In contrast, the reverse transcriptase of MP-MV more closely resembles that of M-MTV with respect to molecular size and divalent cation requirements (2, 13).

Previous immunologic studies have indicated that the reverse transcriptase of MP-MV is unrelated to the type C or simian syncytial viral enzymes (29). Further, MP-MV antigens have failed to show cross-reactivity in assays for the major structural polypeptides of M-MTV and type C viruses (19, 24). The competition radioimmunoassays developed in the present studies have made possible the specific quantitation of two MP-MV polypeptides, p26 and p15. These assays were shown to be 10 to 100 times more sensitive than immunodiffusion, the technique primarily used in earlier studies for detection of MP-MV antigens (19, 24). Examination of several mammalian type C viruses and a mouse type B virus in the more sensitive competition radioimmunoassays revealed no immunologic relatedness to MP-MV. Furthermore, antisera prepared to type C, type B, and

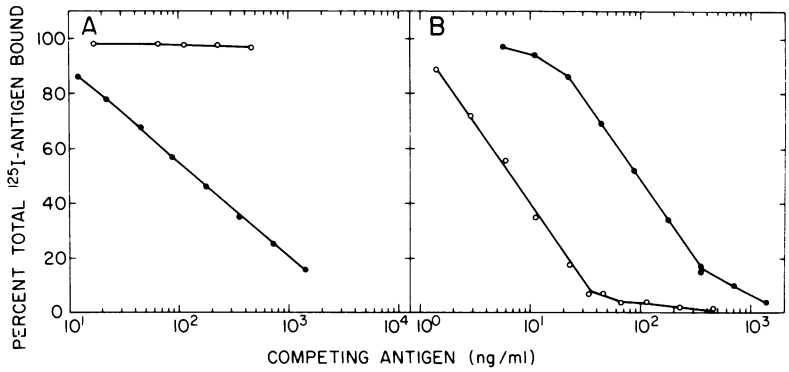

FIG. 5. *Competition radioimmunoassays for p26 and p15. (A) Unlabeled p26 and p15 were competed with ^{125}I-p26 (6,400 counts/min) for an amount of antibody, which, in the absence of competing antigen, bound 50% of ^{125}I-p26; (B) Competition of unlabeled p26 and p15 with ^{125}I-p15 (6,500 counts/min) for limiting antibody. p26, ●; p15, ○.*

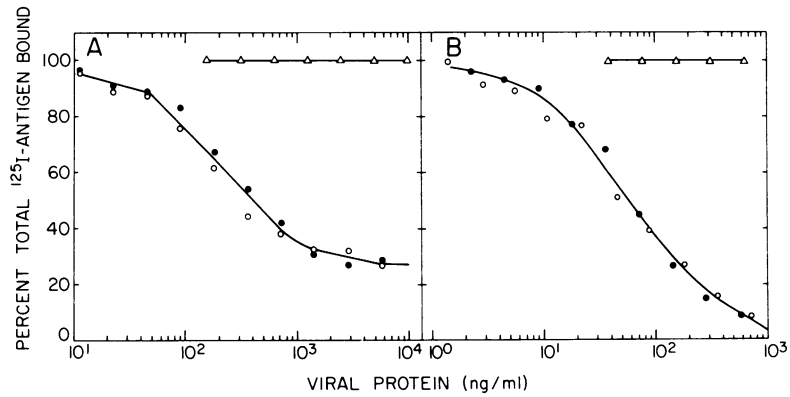

FIG. 6. *Cross-reactions of reverse transcriptase-containing RNA viruses in competition radioimmunoassays for MP-MV polypeptides. Viruses were disrupted with 1% SDS and assayed at dilutions such that the SDS concentration (≤0.1%) did not inhibit antibody-antigen complex formation. MP-MV grown in human lymphocytes (NC37), ●; MP-MV grown in the original monkey tumor cell line (CMMT), ○. M-MTV, R-MuLV, AKR-MuLV, RaLV, FeLV, RD114, woolly monkey, and gibbon ape viruses did not react in these assays and are represented by Δ. (A) p26 assay; (B) p15 assay. The details of the radioimmunoassays are described in Fig. 5.*

TABLE 2. *Comparison of sensitivities of immunological assays for detection of MP-MV antigens*

Assay	Viral antigen(s) (ng protein) detectable at end-point[a]			
	Purified antigens		SDS disrupted virus	
	MP-MV p15	MP-MV p26	MP-MV	Woolly monkey
Immunodiffusion	>210	90	500	>35,000
Micro-complement fixation	>210	10	40	>35,000
MP-MV p15	1-2	>1440	50	>10,000
MP-MV p26	>420	8	5	>10,000

[a] Immunodiffusion experiments were carried out at 37 C for 18 to 24 h by using undiluted antiserum. The end-point is defined as the least amount of antigen sufficient to give a precipitin line. Micro-complement fixation reactions utilized a 1:4,000 dilution of antiserum, and the end-point is the lowest level of viral antigen necessary to fix 10% of the maximum complement bound. The radioimmunoassays were performed as described in Fig. 5, and the end-point is defined as the amount of unlabeled antigen required to inhibit antibody binding by 10%. The same antiserum was used for each method.

simian syncytial viruses did not bind the radioactive MP-MV polypeptides. Thus, these findings confirm and extend previous studies indicating the lack of antigenic homology between MP-MV and other reverse transcriptase-containing viruses (19, 24, 29).

Micro-complement fixation was found to be comparable in sensitivity to the p26 radioimmunoassay, but was at least 10 times less sensitive than the p15 assay for virus detection. However, complement-fixing assays may not be suitable for testing cell extracts due to the presence of nonspecific complement-binding components which can obscure detection of low levels of viral antigens. In contrast, radioimmunoassay techniques such as those developed in this study have been quite useful in demonstrating the presence of nanogram amounts of viral polypeptides in normal mouse cells (32).

In addition to providing highly sensitive methods for measuring viral antigens, the availability of radioiodine-labeled MP-MV polypeptides provides an extremely sensitive method for antibody detection. Thus, significant binding of one viral antigen (p26) was obtained with an antiserum dilution as low as 1:100,000 (Table 1). In contrast, the concentrations of the same antiserum required to give detectable reactions with unlabeled p26 in micro-complement fixation and immunodiffusion tests were, respectively, 25 and 50,000-fold higher. Thus, tests of sera from primate species in radioimmunoprecipitation assays with MP-MV polypeptides may provide information concerning the natural occurrence of this virus and its relationship to neoplastic diseases.

Type-specific radioimmunoassays for a low molecular polypeptide (p12) of mouse type C viruses readily distinguish between different classes of otherwise closely related endogenous type C viruses of the mouse (31, 32), providing markers in studying the genetics of these viruses. Analogous methods have made it possible to discriminate between type C viruses isolated from two primate species, the gibbon ape and woolly monkey (35; S. Tronick et al., manuscript in preparation); these viruses possess immunologically indistinguishable reverse transcriptases and p30 polypeptides (25, 29, 35). If either or both MP-MV polypeptides can be shown to possess a high degree of antigenic type-specificity, it should be possible to determine whether MP-MV-like viruses isolated from human cells are distinguishable from MP-MV. This information could provide evidence regarding the identity of these new virus strains.

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