Chemical and Immunological Characterization of the Major Structural Protein (p28) of MMC-1, a Rhesus Monkey Endogenous Type C Virus: Homology with the Major Structural Protein of Avian Reticuloendotheliosis Virus

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The major core protein (p28) of MMC-1, an endogenous type C virus of the rhesus monkey (*Macaca mulatta*), was purified and subjected to structural and immunological analyses. The NH₂-terminal amino acid sequence of MMC-1 p28 showed extensive homology to the sequences of the major structural proteins (p30) of known mammalian type C viruses. Similarly, interspecies antigenic determinants shared by all the above viral proteins were detected in MMC-1 p28. Competition radioimmunoassays together with the results of statistical analysis of the primary structure data provided evidence that MMC-1 p28 is not more closely related to primate type C viruses of the *Papio* genus than to those isolated from rodents, cats, or New World monkeys. MMC-1 p28 was found to be closely related structurally to the p30 protein of the avian reticuloendotheliosis virus (REV-A), a horizontally transmitted type C virus of putative mammalian origin. In addition, MMC-1 p28 and REV-A p30 shared a specific subset of antigenic determinants not present in any of the other avian or mammalian type C viruses studied. These findings suggest that MMC-1 and REV may have a common evolutionary origin.

Type C RNA viruses are widely distributed in nature and are often genetically transmitted in an unexpressed form as endogenous viruses (1, 12, 33). Efforts to detect these viruses in primates have led to isolates from both New and Old World monkeys (18, 29, 32, 34, 35). Rabin and co-workers obtained an endogenous virus of rhesus monkeys (*Macaca mulatta*) which they designated MMC-1 (27). Immunologically and by nucleic acid hybridization, this virus closely resembled the endogenous type C virus from stump-tail monkeys (*M. arctoides*) isolated by Todaro et al. (34) and designated MAC-1. The relationship of MMC-1 virus to other previously isolated type C viruses has not as yet been explored in detail.

The most sensitive techniques for studying the relationships among type C viruses have involved structural comparisons of their major internal proteins and analysis of the antigenic determinants shared among specific groups of viruses. These approaches have yielded complementary information and have indicated closer relationships among type C viruses of the rodents (mice, rats, and hamsters) as opposed to primate-derived RD114 and baboon endogenous

viruses (BaEV). The present studies were undertaken to determine NH₂-terminal amino acid sequence of the MMC-1 major internal protein for comparison with those previously determined for the major structural proteins of representative type C viruses. In the course of these studies, we found evidence that the structural relationship of the p30 of horizontally transmitted avian reticuloendotheliosis virus type A (REV-A) is closer to that of MMC-1 than of other well-known type C viruses. In addition, we characterized the antigenic determinants of MMC-1 p28 regarding their immunological cross-reactivity with those of other known retroviral proteins and found that the major structural proteins of MMC-1 and REV-A share antigenic determinants not shared with other groups of type C viruses.

MATERIALS AND METHODS

Viruses. The endogenous type C virus of rhesus monkeys (*M. mulatta*), designated MMC-1 (27), was grown in either an 816A cell line derived from an esophageal carcinoma of a rhesus monkey (28) or canine fetal thymus cells. REV type T was originally obtained from H. Bose (University of Texas, Austin).

REV-A was grown in a continuous line of transformed chicken bone marrow cells as described (11, 30). Viruses were purified by sucrose density gradient centrifugation according to standard methods and were obtained from the Viral Resources Laboratory of the Frederick Cancer Research Center, Frederick, Md.

Protein purification. MMC-1 p28 ($M_r \approx 28,000$) was purified by isoelectric focusing, using two different procedures. In the first procedure, purified virus was disrupted by Tween 80-ether, and the soluble proteins were electrofocused in 1% ampholine (pH 3 to 10) and sucrose gradient as previously described (21). In the second procedure, purified virus was disrupted with sodium dodecyl sulfate (SDS)-urea. SDS was removed by Dowex 1-X-2, and the soluble proteins were electrofocused in 1 to 6 M urea gradient in the presence of 1% ampholine (pH 3 to 10) (21). After electrofocusing, gel filtration was used as a second step for purification. REV p30 was purified by phosphocellulose ion-exchange chromatography as previously reported (8).

Antisera. Antiserum to MMC-1 p28 was prepared by intramuscularly inoculating a goat with equal doses of purified protein (500 $\mu\text{g}/\text{injection}$) with complete Freund adjuvant at 10-day intervals. Fourteen days after the third injection, blood was collected, the serum was tested for the presence of antibody, and, since potent precipitating antibody was present, the animal was bled 2 days later. Other antisera used in radioimmunoassays were available from previous studies (2-5).

Amino acid analysis. Amino acid analyses were performed on a Durrum 500 analyzer on protein samples which were hydrolyzed in vacuo at 100°C for 24, 28, and 72 h with 6 M HCl containing 0.1% phenol. The data were analyzed by a computer program (7) as previously described (15).

NH₂-terminal sequence analysis. Automated Edman degradation (10) was performed with an updated Beckman sequenator model 890B, using the dilute Quadrol protein program (Beckman 0721772B) with modifications as previously described (22).

The thiazolinone derivatives of amino acids in each sequenator cycle were converted to the phenylthiohydantoin (PTH) derivatives with 0.2 ml of 1 N HCl at 80°C for 10 min. The samples were then lyophilized, dissolved in methanol, and analyzed by high-performance liquid chromatography, using the Waters Associates fatty acid columns and propionic acid gradient as previously described (14).

Radioimmunoassays. The major internal proteins of a variety of retroviruses were purified by a combination of ion-exchange and gel filtration chromatography as previously described (5). Purified viral proteins were labeled to high specific activity (50 to 200 $\mu\text{Ci}/\mu\text{g}$) with ^{125}I by the chloramine T method (13). The purity of radiolabeled probes was higher than 90% as determined by both electrophoretic and immunological criteria (3). ^{125}I -labeled retroviral proteins were immunoprecipitated with appropriate antisera elicited in either goats or rabbits against sucrose-banded viruses or with anti-MMC-1 p28 goat serum. Briefly, 10,000 cpm of ^{125}I -labeled antigen was incubated at 37°C for 3 h with twofold serial dilutions of the antiserum in a 0.20-ml reaction mixture containing 10 mM Tris-hydrochloride (pH 7.8), 10 mM NaCl, 1 mM

EDTA, 0.4% Triton X-100, and 0.6% bovine serum albumin. After an overnight incubation at 4°C, 0.025 ml of undiluted swine, anti-goat, or anti-rabbit immunoglobulin G was added and incubated for another 3 h at 4°C. Samples were diluted to 1 ml with the above buffer, except for the omission of bovine serum albumin, and the radioactivity in the precipitate was measured in a Searle 1285 gamma counter.

Competition radioimmunoassays were performed similarly, except that twofold serial dilutions of the competing antigen were preincubated for 1 h at 37°C with an antiserum dilution able to precipitate 25% of the input ^{125}I -labeled antigen. Protein concentrations were determined as described by Lowry et al. (19).

RESULTS

Physicochemical characterization and amino acid composition of the major internal protein of MMC-1 type C virus. The purity of the MMC-1 major internal protein, as prepared by isoelectric focusing and gel filtration chromatography, was confirmed by SDS-polyacrylamide gel electrophoresis. With this technique (Fig. 1), the molecular weight (M_r) was found to be $\sim 28,000$ (p28). The isoelectric point of MMC-1 p28, determined as described in Materials and Methods, was found to be at pH 6.7 (major molecular species).

Table 1 shows the compositional data for

FIG. 1. SDS-polyacrylamide gel electrophoresis of purified MMC-1 p28. Molecular weight markers: cytochrome c (11,700), myoglobin (17,200), carbonic anhydrase (29,000), ovalbumin (45,000), and bovine serum albumin (69,000).

TABLE 1. Amino acid composition of MMC-1 p28

Amino acid	No. of residues/ mol
Lysine	13
Histidine	2
Arginine	20
Aspartic acid	29
Threonine	18
Serine	16
Glutamic acid	33
Proline	16
Glycine	15
Alanine	19
Valine	10
Methionine	6
Isoleucine	7
Leucine	27
Tyrosine	7
Phenylalanine	10
Total	248

MMC-1 p28. The total number of amino acids calculated by the computer program was 248. The molecular weight calculated from the compositional data (without cysteine and tryptophan) for MMC-1 p28 (28,408) is in excellent agreement with the value for M_r determined by SDS-polyacrylamide gel electrophoresis.

NH₂-terminal amino acid sequence of MMC-1 p28. Two independent Edman degradations were performed on MMC-1 p28, and the results were essentially identical. Figure 2 shows the yields of the various amino acids at each sequenator cycle of one of the runs. Unambiguous identification of amino acid residues was possible up to residue 28, except at positions 14, 24, and 25, where histidine, serine, and aspartic acid, respectively, were tentatively assigned. The NH₂-terminal amino acid sequence of MMC-1 p28, as based on the quantitative data presented in Fig. 2, is given in Fig. 3.

Sequence homology of MMC-1 p28 with other mammalian type C virus p30's. Figure 4 compares the NH₂-terminal sequence of MMC-1 p28 with those of the major structural proteins of prototype type C viruses, such as Rauscher murine leukemia virus (R-MuLV), feline leukemia virus (FeLV), gibbon ape leukemia virus (GaLV), and BaEV. The alignments were made by the computer program ALIGN of Dayhoff. This program is based on the mutation data matrix for scoring amino acid comparisons as described (9). Table 2 summarizes the results of the statistical analyses. On the basis of the computer-generated alignment scores, it can be concluded that MMC-1 p28 is more closely related to GaLV p30 than to the p30 of any of the other mammalian type C viruses compared.

Homology between the NH₂-terminal

amino acid sequences of MMC-1 p28 and REV p30. The p30 of REV has been shown to possess structural properties that closely resemble mammalian, rather than avian, type C viruses (16). In addition, it has been shown that REV-A p30 shares antigenic determinants with the major structural protein of prototype mammalian type C viruses (3, 8). In the present study, REV-A p30 was analyzed in three independent sequenator runs. Figure 5 shows the quantitative data for this protein as determined for the third run. Among the initial 36 residues, assignments could not be made at positions 32 and 35. Figure 6 gives the NH₂-terminal amino acid sequence of REV-A p30. The alignment of REV-A p30 with MMC-1 p28 (Fig. 7) showed extensive homology. Sixteen residues out of 28 possible comparisons were found to be identical, correspond-

FIG. 2. Yields of amino acids obtained by degrading 9 nmol of MMC-1 p28. Equal portions of each cycle were analyzed by high-performance liquid chromatography. Yields were normalized to 100% of the sample.

ing to an approximate 57% homology. Statistical analysis yielded an alignment score of 7.8 (Table 2). Alignments between REV-A p30 sequence and those of other mammalian p30 retroviral proteins (Fig. 7) showed a lesser degree of homology (Table 2).

It should be noted that the REV-A p30 sequence as determined in this study (Fig. 5 and 6), although close to that published by Hunter et al. (16), exhibited some differences. For in-

stance, Hunter et al. (16) reported glutamic acid and alanine in position 8. In our initial analysis, we also found some PTH glutamic acid but no PTH alanine (data not shown) at cycle 8. On repetition, both of our two recent analyses yielded PTH arginine at cycle 8 (Fig. 5 and 6), where glutamic acid was seen again, but the quantitative data (Fig. 3) indicated that it represented a carryover from residue 7. Hunter et al. (16) used the spot test to identify arginine.

5 10 15
Pro-Leu-Arg-Glu-Ile-Gly-Ser-Leu-Asp-Asp-Thr-Gly-Leu-(His)-Arg-
20 25
Leu-Met-Tyr-Trp-Pro-Phe-Ser-Thr-(Ser-Asp)-Leu-Tyr-Asn-

FIG. 3. *NH*₂-terminal sequence of MMC-1 p28. Tentative assignments are shown in parentheses.

	1	5	10	15	20	25
MMC-1	P L R E I G S L D D T G L H R L M Y W P F S T S D L Y N					
R-MuLV	P L R - - - L G G N G - - Q L Q Y W P F S S S D L Y N					
Common	P L R		L	G	L Y W P F S	S D L Y N
	1	5	10	15	20	25
MMC-1	P L R E I G S L D D T G L H R L M Y W P F S T S D L Y N					
FeLV	P L R E G P N - - - - - N R P Q Y W P F S A S D L Y N					
Common	P L R E			R	Y W P F S	S D L Y N
	1	5	10	15	20	25
MMC-1	P L R E I G S L D D T G L H R L M Y W P F S T S D L Y N					
BaEV	P L R T V N - - - - - R T I Q Y W P F S A S D L Y N					
Common	P L R				Y W P F S	S D L Y N
	1	5	10	15	20	25
MMC-1	P L R F I G S L D D T - G L H R L M Y W P F S T S D L Y					
GaLV	P L R A I G P P A E P N G L V P L Q Y W P F S X A D L Y					
Common	P L R	I G		G L	L Y W P F S	D L Y

FIG. 4. Alignment of MMC-1 p28 with mammalian type C virus p30's. The one-letter code for amino acids is: A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; X, unidentified; Y, tyrosine.

TABLE 2. Summary of statistical analyses of relatedness of MMC-1 p28 and REV-A p30 to other mammalian type C virus p30 sequences

Sequences aligned	Identities/possible matches	No. of breaks	Alignment score ^a	Probability of score
MMC-1/R-MuLV	16/22	2	7.6	0.15×10^{-13}
MMC-1/FeLV	15/22	1	7.6	0.15×10^{-13}
MMC-1/BaEV	13/21	1	7.7	0.68×10^{-14}
MMC-1/GaLV	17/28	1	9.9	0.20×10^{-22}
REV/R-MuLV	13/22	1	6.5	0.4×10^{-10}
REV/FeLV	14/22	2	6.1	0.5×10^{-9}
REV/BaEV	13/21	2	6.5	0.4×10^{-10}
REV/GaLV	13/27	3	6.4	0.8×10^{-10}
REV/MMC-1	16/28	0	7.8	0.3×10^{-14}

^a ALIGN program of Dayhoff (9); 300 random runs.

FIG. 5. Yields of amino acids obtained by degrading 12 nmol of REV-A p30. Equal portions of each cycle were analyzed by high-performance liquid chromatography. Yields were normalized to 100% of the sample.

5 10 15 20
 Pro-Leu-Arg-Glu-Thr-Gly-Glu-Arg-Asp-Met-Gly-Gly-Arg-Pro-Met-Arg-Thr-Tyr-Val-Pro-
 25 30 35
 Phe-Thr-Thr-Ser-Asp-Leu-Tyr-Asn-Trp-Lys-Asn- X -Asn-Pro- X -Phe-

FIG. 6. *NH₂-terminal sequence of REV-A p30.*

	1	5	10	15	20	25
REV p30	P L R E T G E R D M G G R P M R T Y V P F T T S D L Y N					
R-MuLV p30	P L R - - - - - L G G N G Q L Q Y W P F S S S D L Y N					
Common	P L R		G G		Y P F	S D L Y N

	1	5	10	15	20	25
REV p30	P L R E T G E R D M G G R P M R T Y V P F T T S D L Y N					
FeLV p27	P L R E G P N N - - - - R P Q - - Y W P F S A S D L Y N					
Common	P L R E			R P	Y P F	S D L Y N

	1	5	10	15	20	25
REV p30	P L R E T G E R D M G G R P M R T Y V P F T T S D L Y N					
BaEV p30	P L R - T V N R T T Q - - - - - Y W P F S A S D L Y N					
Common	P L R T	R			Y P F	S D L Y N

	1	5	10	15	20	25
REV p30	P L R E T G E R - D M G G - R P M R T Y V P F T T S D L Y N					
GaLV-p30	P L R A I G P P A E P N G L V P L Q - Y W P F S X A D L Y N					
Common	P L R	G		G P	Y P F	D L Y N

	1	5	10	15	20	25
RFV p30	P L R E T G E R D M G G R P M R T Y V P F T T S D L Y N					
MMC-1 p28	P L R E I G S L D D T G L H R L M Y W P F S T S D L Y N					
Common	P L R E	G D	G		Y P F	T S D L Y N

FIG. 7. *Alignment of REV-A p30 with mammalian type C virus p30's. The one-letter code for amino acids is given in the legend to Fig. 4.*

We identified each amino acid by high-performance liquid chromatography, which is quantitative and more sensitive than the spot test. In all three analyses performed, we unambiguously identified threonine instead of glycine in position

5 and glycine instead of threonine in position 6. At position 26, we invariably found leucine instead of isoleucine. Other differences in our assignments include methionine instead of arginine, proline in place of alanine, and serine in-

stead of threonine in positions 15, 20, and 24, respectively. The possibility that the discrepancies may be due to strain differences is not ruled out.

Radioimmunoassay for MMC-1 p28. The major structural protein of MMC-1 type C virus was next characterized immunologically. For this purpose, we developed a homologous competition radioimmunoassay by precipitating ^{125}I -labeled MMC-1 p28 with limiting amounts of antibodies elicited against MMC-1 p28. MMC-1 p28 possessed type- or group-specific antigenic determinants, or both, that differed from those of all other retroviruses tested (Table 3).

Antigenic relationships between MMC-1 and other mammalian retroviruses. The antigenic relationships of MMC-1 p28 with the corresponding proteins of other mammalian retroviruses were next analyzed in a series of competition radioimmunoassays. We observed that antiserum to MMC-1 p28 precipitated the major structural proteins of mouse (R-MuLV) and baboon endogenous (M7) type C viruses (Table 3). This made it possible to develop anti-MMC-1 serum/ ^{125}I -R-MuLV p30 and anti-MMC-1 serum/ ^{125}I -BaEV p30 interspecies im-

munoassays. All tested mammalian type C viruses competed in both assays, indicating that the interspecies antigenic determinants shared by MMC-1 and type C viruses isolated from baboons were those conserved throughout type C virus evolution (Table 3).

MMC-1 cross-reacted in a broadly reactive competition radioimmunoassay capable of detecting the major structural proteins of all known mammalian type C, but not type B or D, retroviruses. These findings confirmed that MMC-1 was a member of the type C retrovirus genus. MMC-1 failed to compete in immunoassays specifically designed to detect antigenic determinants shared by rodent type C viruses (anti-R-MuLV serum/ ^{125}I -FeLV p30) or horizontally transmitted primate type C viruses (anti-GaLV serum/ ^{125}I -simian sarcoma-associated virus p30). In addition, MMC-1 p30 did not possess antigenic determinants shared by type C viruses isolated from different species of the *Papio* genus. Considering the close evolutionary relationship between the *Macaca* and *Papio* genera of Old World primates, these results suggested that MMC-1 virus had an evolutionary lineage distinct from that of BaEV.

TABLE 3. Immunological relationships between MMC-1 and representative viral isolates of the major retrovirus genus

Virus ^a	Competition ^b in radioimmunoassays ^c				
	Anti-MMC-1/ MMC-1 p28	Anti-MMC-1 ^d / R-MuLV p30	Anti-MMC-1 ^d / BaEV p30	Anti-MMC-1/ REV-A p30	Anti-REV-A/ MMC-1 p28
MMC-1	100	100	100	100	100
Mammalian type C					
R-MuLV	<5	100	95	<5	<5
FeLV	<5	100	100	<5	<5
SSAV	<5	95	90	<5	<5
GaLV	<5	90	90	<5	<5
RD114	<5	60	100	<5	<5
BaEV	<5	45	95	<5	<5
Avian type C					
REV-A	<5	55	60	100	100
RSV	<5	<5	<5	<5	<5
Primate type D					
MPMV	<5	<5	<5	<5	<5
Po-1-Lu	<5	<5	<5	<5	<5
SMRV	<5	<5	<5	<5	<5
Mouse type B, MMTV	<5	<5	<5	<5	<5
Unclassified					
VRV	<5	<5	<5	<5	<5
GPV	<5	<5	<5	<5	<5

^a Abbreviations not defined in text: SSAV, simian sarcoma-associated virus; RSV, Rous sarcoma virus; MPMV, Mason-Pfizer monkey virus; SMRV, squirrel monkey retrovirus; MMTV, mouse mammary tumor virus; VRV, viper retrovirus; GPV, guinea pig retrovirus.

^b Displacement of the ^{125}I -labeled viral proteins achieved by 20 μg of isopynically purified retroviruses when tested as competing antigens in each of the indicated double-antibody competition radioimmunoassays.

^c Schematic representation of a competition radioimmunoassay in which a limiting amount of antiserum elicited against a given type C virus (e.g., anti-MMC-1) was used to precipitate ^{125}I -labeled type C viral protein (10^4 cpm) (e.g., MMC-1 p28).

^d Similar results were obtained in the corresponding reciprocal competition radioimmunoassays: anti-R-MuLV serum/ ^{125}I -labeled MMC-1 p30 and anti-RD114 serum/ ^{125}I -labeled MMC-1 p28.

Immunological cross-reactivity between MMC-1 p28 and REV-A p30. In view of the similarities observed in the amino acid sequences of MMC-1 p28 and REV-A p30 proteins, we examined the nature of their immunological cross-reactivity. These proteins shared antigenic determinants not found in any other retrovirus tested (Table 3). Thus, MMC-1 p28 exhibited three distinct subsets of antigenic determinants: (i) specific determinants not detected in other type C viruses tested; (ii) broadly reactive antigenic determinants present in the major structural proteins of known mammalian type C viruses as well as the avian REV group; and (iii) a subset of antigenic determinants specifically shared with REV-A p30.

REV-A p30 has been found previously to share antigenic determinants with the corresponding protein of OMC-1, an endogenous retrovirus of owl monkeys (2). To date, no broad interspecies antigenic cross-reactivity between OMC-1 and other mammalian type C viruses has been detected. It was of interest to examine whether the determinants common to REV-A p30 and MMC-1 p28 were related to those shared by REV-A p30 and OMC-1 p30. No immunological cross-reactivity was detected between these different subsets of antigenic determinants (Table 4). Thus, four different subsets of antigenic determinants were identified in REV-A p30: those shared by members of the REV group; those in common with mammalian type C viruses; and those specifically shared with either MMC-1 or OMC-1 primate endogenous viruses.

DISCUSSION

MMC-1 (27), the single type C virus isolate of rhesus monkeys (*M. mulatta*), is representative of a highly related group of endogenous primate

viruses including isolates from *M. arctoides* (34) and *Colobus polykomos* (29). These are each represented in multiple copies in the host cell genome and are clearly distinguished from the other primate type C family represented by BaEV.

A detailed phylogenetic tree of the MMC-1 virus family in relation to other mammalian type C viruses cannot be proposed at this time because of insufficient data; however, the current data indicate a clear delineation from other subgroups based on amino acid sequence comparisons and immunological tests with one viral protein. Thus, the only mammalian type C virus interspecies-specific radioimmunoassay in which MMC-1 p28 competed was one capable of detecting all mammalian p30's. Statistical analyses of the primary structure data also indicated that MMC-1 is not more closely related to primate type C viruses of the *Papio* genus than to type C viruses isolated from rodents or cats. Although similar analysis provided evidence for a higher degree of homology between the NH₂-terminal sequences of MMC-1 and GaLV p30's than between MMC-1 and other mammalian p30's, antigenic determinants specifically shared with GaLV p30 were not detectable.

Early ultrastructural and biochemical studies suggested that REV was more closely related to mammalian type C viruses than to viruses of the avian leukosis-sarcoma complex (20, 36). Primary structure analysis (16) and immunological studies of the major internal structural protein (3, 8) and of reverse transcriptase (6) of REV have established an evolutionary linkage between members of the REV group and mammalian retroviruses. The statistical analysis of primary structure homology clearly establishes that REV-A p30 is more closely related to MMC-1 p28 than to other mammalian type C p30's (Table 2). The calculations are strongly influenced by the need for gaps or insertions to maintain positional homology. These are generally major events in protein evolution occurring at a much lower frequency than simple substitutions. Thus, the lack of any such events in the MMC-1 p28-REV p30 comparison is highly significant. In similar fashion, MMC-1 p28 and REV-A p30 shared interspecies antigenic determinants not found in the major structural proteins of the other retroviruses tested. Even though the reciprocal cross-reactivity in interspecies immunoassays may represent only a single antigenic determinant, and thus six to seven amino acid residues, such reactions, except in artificial systems, generally reflect a high degree of underlying relatedness (26).

Our previous experience with retrovirus p30's has been that this level of relationship will be in

TABLE 4. *Antigenic relationships among MMC-1, OMC-1, and REV-A retroviruses*

Competition radioimmunoassay	Competition by ^a :			
	MMC-1	REV-A	OMC-1	R-MuLV
Anti-REV-A/[¹²⁵ I]R-MuLV p30	+	+	-	+
Anti-R-MuLV/[¹²⁵ I]REV p30	+	+	-	+
Anti-REV-A/[¹²⁵ I]OMC-1 p30	-	+	+	-
Anti-MMC-1/[¹²⁵ I]REV-A p30	+	+	-	-
Anti-REV-A/[¹²⁵ I]MMC-1 p28	+	+	-	-

^a Displacement of the [¹²⁵I]-labeled viral proteins achieved by 20 µg of isopically purified MMC-1, REV-A, OMC-1, or R-MuLV in each of the indicated competition radioimmunoassays. +, >90% displacement; -, <20% displacement.

agreement with other methods of interval comparisons. However, molecular hybridization analyses have not yet revealed any relationship between REV-A and other mammalian viruses, nor have sequences related to the viruses of REV complex been found in cellular DNA of avian or mammalian species (17, 30). These data await reevaluation with probes representing specific regions of the various viral genomes.

At present, the retroviruses defy a definitive scheme of taxonomic interrelatedness. There is increasing evidence of genetic relatedness among various subfamilies (2, 4, 22, 25, 31) with suggestive evidence of intragenic recombination, as for the envelopes of certain primate type C and D viruses (31). As complete structures become available from both protein and nucleic acid sequencing studies, these relationships should be more accurately defined.

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