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Comparisons of the Immunological Properties of Two Structural Polypeptides of Type C RNA Viruses Endogenous to Old World Monkeys

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Immunologically very closely related type C RNA viruses are endogenous to the domestic cat and to an old world primate, the baboon. In the present studies, radioimmunological techniques have been developed for detection of the 15,000 and 30,000 molecular weight (MW) polypeptides of each virus. The much more pronounced type-specific antigenic determinants of the lower MW polypeptides made it possible to readily differentiate these viruses from each other as well as from a type C virus isolate from a second baboon species. Normal rhesus monkey tissues were partially purified and shown to contain a reactivity with MW and immunological properties similar to that of the baboon virus 30,000 MW polypeptide. Despite a similar degree of purification, antigenic reactivity like that of the baboon virus 15,000 MW polypeptide was undetectable even in the broadest immunological tests available for this polypeptide. The present findings indicate that the immunological properties of two structural polypeptides of closely related viruses endogenous to primate and feline species have undergone different rates of antigenic change in the course of evolution within their respective host cell genome.

The application of radioimmunological techniques to the study of type C RNA viruses has led to the development of highly sensitive assays for their detection. Different virion polypeptides have been shown to possess specific immunological properties. The 30,000 molecular weight (MW) polypeptides of mammalian type C viruses contain group-specific antigenic determinants shared by virus isolates of the same species and interspecies antigenic determinants shared by all known mammalian type C viruses (8, 19, 20). In contrast, low MW polypeptides of 12,000 (33) and 15,000 (31) and the 70,000 MW glycoprotein (29; S. Hino, J. R. Stephenson, and S. A. Aaronson, J. Immunol., in press) have been shown to possess very pronounced type-specific antigenic determinants. Immunoassays that detect these latter determinants have made it possible to discriminate otherwise closely related virus strains of the same species and, thus, have been useful in identifying new virus isolates and studying their natural history (34, 38; J. R. Stephenson, R. K. Reynolds, S. R. Tronick, and S. A. Aaronson, Virology, in press).

Recently, type C viruses have been isolated from two species of baboons (2, 10, 13, 35). The p30's of these viruses have been reported to be closely related immunologically to that of a type C virus, designated RD114 (12, 18, 26), genetically transmitted in the cat (3, 6, 23). Molecular hybridization studies have detected genetic homology between the baboon virus and baboon cellular DNA, indicating that this virus is endogenous to the baboon (4). The close immunological relationship of endogenous baboon and RD114 cat viruses would not be expected based upon the degree of evolutionary divergence of primate and feline species (3). Thus, it is possible that one or both viruses become a part of its respective host cell genome more recently in evolution than the time at which the progenitors of the two species diverged.

In the present studies, investigation of the immunological relationships between the polypeptides of baboon and RD114-like viruses has led to the development of competition immunoassays for their 30,000 and 15,000 MW polypeptides. The latter are shown to possess very pronounced type-specific antigenic determinants which discriminate baboon and RD114 viruses as well as a type C virus isolate of a second baboon species. Cell extracts of another old world primate, the rhesus monkey, have been partially purified and analyzed for antigens cross-reactive with p30 and p15 of these

viruses. The results provide evidence for different rates of evolution of two structural polypeptides of the baboon-RD114 virus group.

MATERIALS AND METHODS

Cells and virus. Cells were grown in Dulbecco modification of Eagle medium supplemented with 10% calf serum (Colorado Serum Co., Denver Colo.). Type C RNA viruses included Rauscher murine leukemia virus (R-MuLV) (21), feline leukemia virus (22), Mason-Pfizer monkey virus (5), RD114 (18), and isolates from the woolly monkey (36), gibbon ape (14), *Papio cynocephalus* baboon (2, 13, 35), and *Papio hamadryas* baboon (10). These were obtained as sucrose density gradient purified preparations from Electro-Nucleonics Laboratories, Rockville, Md., or Pfizer, Inc., Maywood, N.J., through Jack Gruber, Office of Resources and Logistics, National Cancer Institute.

PAGE. Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed by the method of Laemmli (15).

Preparation of ^{125}I-labeled viral polypeptides. The 12,000, 15,000, and 30,000 MW polypeptides of *P. cynocephalus* baboon and RD114 virus were isolated by agarose gel filtration in 6 M GuHCl. Viruses were pelleted by centrifugation at 30,000 rpm, resuspended in 0.4 ml of 6 M GuHCl, 0.01 M dithiothreitol, 0.01 M EDTA (pH 8.5), heated at 40 C for 30 min and chromatographed on an agarose column (1.5 by 90 cm, Bio Gel A-5m, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) at a flow rate of 1.5 ml/h. Columns were calibrated using bovine serum albumin (68,000 MW), *Escherichia coli* alkaline phosphatase (40,000 MW), carbonic anhydrase (29,000 MW), β-lactoglobulin (18,400 MW) and lysozyme (14,300 MW) as standards. Fractions containing 1 ml corresponding to MWs of 30,000, 15,000, and 12,000 relative to the marker proteins were collected, appropriately pooled, dialyzed against 0.01 M Tris-hydrochloride (pH 8.0), and concentrated by lyophilization. The protein in each fraction was ^{125}I-labeled by the chloramine T method of Greenwood et al. (11) as previously described (32) and the radiochemical purity was determined by SDS-PAGE. The molecular weight designations, p12 (12,000), p15 (15,000), and p30 (30,000) used in the present study represent values obtained by GuHCl agarose gel filtration of the purified polypeptides (1).

Radioimmunoassays. Competition immunoassays for R-MuLV p30 and woolly monkey virus p30 have been described (32). Assays for p15 and p30 of *P. cynocephalus* and RD114 viruses were performed according to similar procedures. These assays measure the ability of unlabeled virus to compete with ^{125}I-labeled viral polypeptides for binding limiting concentrations of antibody. Antisera prepared by immunization of goats with Tween/ether-disrupted virus and swine anti-goat immunoglobulin G (38) were generously provided by R. Wilsnack through the Office of Resources and Logistics, National Cancer Institute. Protein concentrations were determined by the method of Lowry et al. (17).

Purification of p30 and p15 polypeptides from cell extracts. Methods developed for isolation of type C viral polypeptides from mouse cells (34) were used to purify type C viral antigens from virus-negative rhesus monkey cells. Briefly, cell extracts were prepared by homogenization of rhesus monkey liver in an equal volume of 0.01 M Tris-hydrochloride (pH 7.8). Trace amounts (10,000 to 12,000 counts/min) of ^{125}I-labeled RD114 p30 or p15 were added to approximately 1,000 mg of cell extract and applied to a Whatman DE52 column (1.5 by 25 cm) equilibrated with 0.01 M Tris hydrochloride–1.0 mM EDTA (pH 7.8). The column was washed with 100 ml of the same buffer, and the bound proteins eluted with 200 ml of a 0.01 M to 0.4 M KCl linear gradient. ^{125}I-RD114 p30 reactivity eluted at 0.07 to 0.09 M KCl, while the peak of ^{125}I-RD114 p15 occurred at 0.02 to 0.05 M KCl. Pooled fractions containing each of the two ^{125}I-labeled markers were dialyzed against 0.01 M Tris-hydrochloride (pH 8.0), lyophilized, and subjected to further purification by agarose gel filtration in 6 M GuHCl as described below.

RESULTS

Isolation and characterization of structural polypeptides of the baboon and RD114 viruses. Polypeptides of 12,000, 15,000, and 30,000 MW were isolated from *P. cynocephalus* and RD114 viruses by agarose gel filtration in 6 M GuHCl as described in Materials and Methods. Fractions corresponding to the 12,000, 15,000, and 30,000 MW regions were pooled and dialyzed. Samples were then ^{125}I-labeled at specific activities of 10 to 15 μCi/μg. By SDS-PAGE, each ^{125}I-labeled polypeptide migrated as single peak that contained at least 95% of the total radioactivity (Fig. 1). Their MWs were 13,000 18,000, and 30,000, respectively, by this method. When the same labeled polypeptides were subjected to agarose gel filtration in 6 M GuHCl, the MWs were 12,000, 15,000, and 30,000 relative to standards. Differences in MW determinations by SDS-PAGE and GuHCl agarose gel filtration have also been observed with low MW polypeptides of other RNA-containing viruses (7, 37). The viral polypeptides were designated p12, p15, and p30 based upon MW measurement by agarose gel filtration (1).

Some of the biochemical properties of p12 and p15 polypeptides of baboon and RD114 viruses were compared with those of similar MW polypeptides purified from a mouse type C virus, R-MuLV. The p12's of baboon and RD114 viruses possessed characteristics analogous to those of the mouse viral p15; each bound to phosphocellulose (pH 6.5) at 0.1 M KCl and showed a marked tendency to aggregate at low ionic strength (0.01 M Tris, pH 7.8) as shown by its elution in the void of

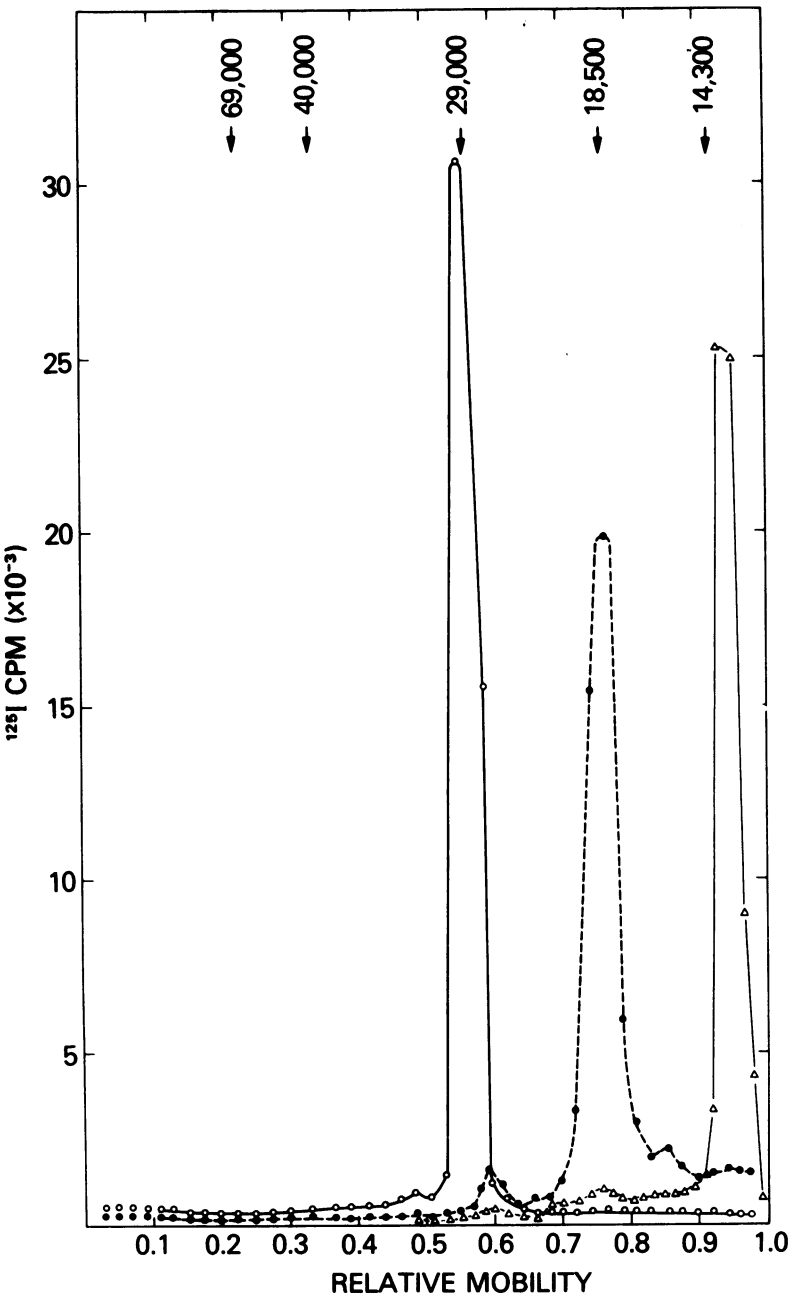

FIG. 1. *SDS-PAGE analysis of ^{125}I-labeled RD114-virus p30 (○), p15 (●), and p12 (△). Approximately 20,000 counts/min of each ^{125}I-labeled polypeptide were subjected to electrophoresis on 60-mm 12.5% acrylamide gels at 1.0 mA/gel for 4 to 5 h. After electrophoresis, samples were either stained with Coomassie blue or sliced into 1-mm fractions using a Savant autogeldivider and radioactivity was measured in a Packard gamma counter. Molecular weights were determined as described by Weber and Osborn (39), using bovine serum albumin (68,000 MW), E. coli alkaline phosphatase (40,000 MW), carbonic anhydrase (29,000 MW), β-lactoglobulin (18,400 MW), and lysozyme (14,300 MW) as standards.*

Sephadex G-200 under these conditions (data not shown). In contrast, baboon and RD114 viral p15's more closely resembled the p12 of mouse type C virus. Each eluted from phosphocellulose in 0.01 M KCl and showed no evidence of aggregation under similar conditions. Previous studies have demonstrated that, with mouse type C viruses, both p12 and p15 possess highly type-specific antigenic determinants (31, 33). However, because mouse virus p15 tends to aggregate, immunoassays utilizing it have generally been less sensitive than those for p12 (24, 31). Based upon the above biochemical properties of baboon and RD114, p12 and p15, it was decided to use the p15's of these viruses in attempts to develop type-specific immunoassays for this virus group.

Immunoprecipitation of ^{125}I-labeled p30 and p15 polypeptides of baboon and RD114 viruses. The relative abilities of anti-baboon virus and anti-RD114 sera to precipitate baboon and RD114 viral ^{125}I-labeled p15 and p30 polypeptides were compared. Both baboon and RD114 ^{125}I-labeled p30 polypeptides were precipitated at similar titers (1:60 to 1:125) by each antiserum (Table 1). In contrast, each ^{125}I-labeled p15 polypeptide was preferentially precipitated by its homologous antiserum. Around 50% precipitation of ^{125}I-labeled baboon virus p15 was achieved with antiserum to baboon virus at a titer of 1:160 and by anti-RD114 serum at 1:20. In contrast, ^{125}I-labeled RD114 p15 was precipitated by anti-baboon and RD114 sera at titers of 1:75 and 1:1100, respectively. Each ^{125}I-labeled polypeptide was over 90% precipitable by its homologous antiserum at high antibody concentration (data not shown).

A homologous immunoassay for baboon viral p30 was developed utilizing antiserum to baboon virus to precipitate its ^{125}I-labeled p30. A homologous immunoassay for RD114 p30 used anti-RD114 to precipitate its ^{125}I-labeled p30. *P. cynocephalus* baboon virus competed most efficiently in the homologous baboon virus p30 assay and to slightly lesser degrees in the homologous RD114 p30 assay and in a heterologous immunoassay utilizing anti-baboon virus to precipitate ^{125}I-labeled RD114 p30 (Fig. 2). RD114, on the other hand, reacted preferentially in the homologous assay for its p30 and slightly less well in the homologous baboon viral and heterologous p30 assays. Neither baboon virus nor RD114 showed detectable reactivity in a homologous immunoassay for woolly monkey virus or R-MuLV p30 (data not shown). These findings are consistent with previous reports (10, 27) indicating only minor differences between the antigenic determinants of the p30's of *P. cynocephalus* baboon and RD114 viruses.

Competition immunoassays for baboon and RD114 virus p15 polypeptides. Homologous competition immunoassays for the p15's of baboon and RD114 viruses were next developed. Baboon virus reacted very efficiently in a homologous immunoassay for baboon viral p15 and competed only very poorly in a homologous immunoassay for RD114 p15 (Fig. 3). RD114 virus showed a reciprocal pattern of reactivity, competing efficiently in the homologous RD114 p15 immunoassay but only to a very limited extent in the assay for baboon virus p15. Thus, *P. cynocephalus* baboon and RD114 viruses were readily distinguishable on the basis of the type-specific antigenic determinants of their p15 polypeptides.

Another isolate of an endogenous type C virus of cat cells is inducible from the Crandell line of cat embryo cells (6, 16, 23). The p30 of this virus has previously been shown to be immunologically indistinguishable from that of RD114 (26). The reactivity of the Crandell virus was compared with that of RD114 in type-specific immunoassays for RD114 and baboon viral p15. Crandell virus, grown in either human, rhesus

TABLE 1. *Antiserum precipitation of ^{125}I-labeled p30 and p15 polypeptides of baboon and RD114 viruses*

^{125}I-labeled viral polypeptide	Precipitation titer with antiserum against:[a]		Ratio:anti-baboon virus/anti-RD114 serum precipitation titer
	Baboon virus (*P. cynocephalus*)	RD114 virus	
p30			
Baboon (*P. cynocephalus*)	100	82	1.2
RD114	60	125	0.5
p15			
Baboon (*P. cynocephalus*)	160	20	8.0
RD114	75	1,100	0.07

[a] The precipitation titer represents the reciprocal of the highest final serum dilution at which 50% binding of ^{125}I-labeled viral polypeptide was obtained.

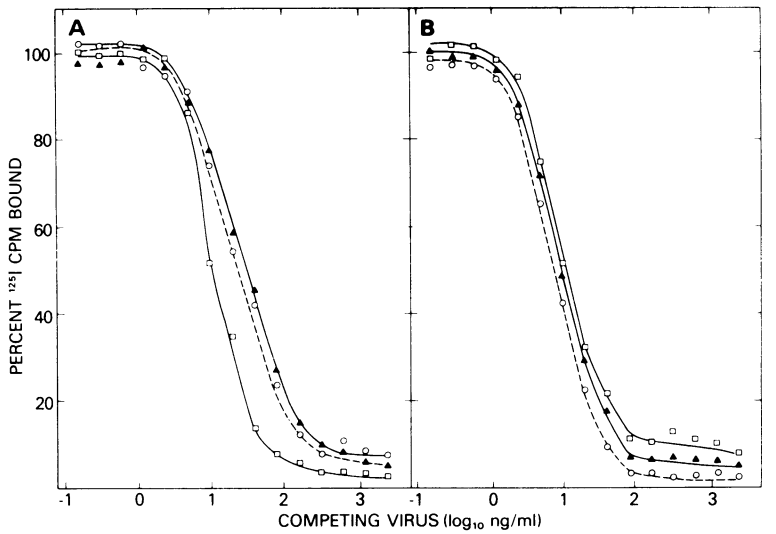

FIG. 2. *Comparisons of the immunological reactivities of P. cynocephalus baboon (A) and RD114 (B) viruses in radioimmunoassays for viral p30. Viruses were disrupted by incubation at 37 C for 30 min in 1% Triton and viral protein concentrations were determined by the method of Lowry et al. (17). Unlabeled viral antigens were tested at serial twofold dilutions for their ability to compete with ^{125}I-labeled p30 for binding limiting amounts of antiserum. Reaction mixtures contained 0.01 M Tris, 0.1 M NaCl, 0.15 M EDTA, 0.1% Triton X-100, and 1% bovine serum albumin (pH 7.8) in a volume of 0.7 ml. Antiserum and unlabeled competing antigen were incubated at 37 C for 1 h; ^{125}I-labeled antigen was then added, and the incubation was continued for 3 h at 37 C and a further 18 h at 4 C. After the addition of 0.025 ml of undiluted swine anti-goat immunoglobulin G to each tube, the mixture was incubated for 3 h at 4 C and centrifuged at 2,500 rpm for 15 min, and the resulting precipitate was measured for radioactivity. Assays included a homologous immunoassay using an antisera against RD114 p30 to precipitate ^{125}I-labeled RD114 p30 (○); a homologous immunoassay using an antisera against P. cynocephalus baboon virus to precipitate ^{125}I-labeled P. cynocephalus p30 (□); and a heterologous assay in which an antisera against P. cynocephalus baboon virus was used in combination with ^{125}I-labeled RD114 p30 (▲).*

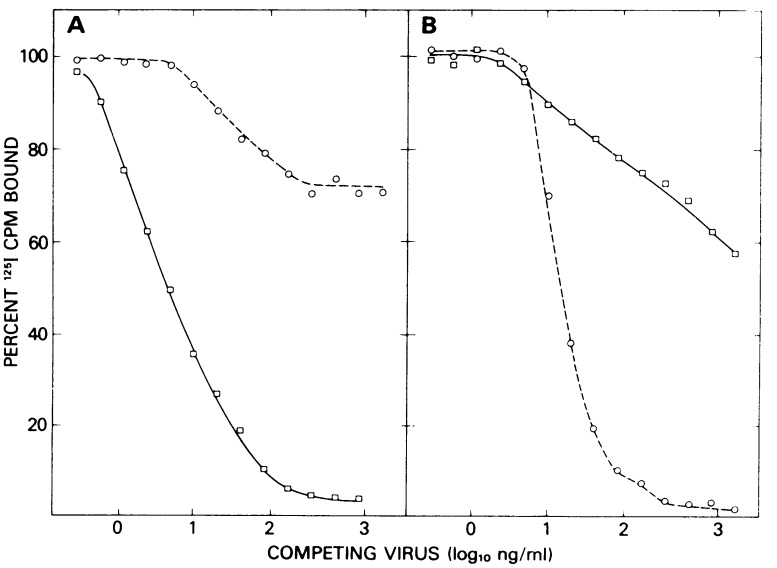

FIG. 3. *Reactivities of the P. cynocephalus baboon (A) and RD114 (B) viruses in homologous immunoassays for baboon and RD114 viral p15. Viruses were detergent-disrupted as described in the legend to Fig. 2 and tested in homologous immunoassays for the p15 polypeptides of RD114 (○) and P. cynocephalus baboon (□) viruses.*

monkey, or feline cells, reacted identically to RD114 in the homologous immunoassay for RD114 p15 (Fig. 4). In contrast, none of the Crandell virus preparations exhibited more than minor reactivity in the baboon viral p15 immunoassay. These findings demonstrate that the antigenic determinants of p15's of two different endogenous feline virus isolates were very similar to each other and were not altered by virus growth in cells of different species.

Antigenic determinants of the p15 polypeptide of a type C RNA virus isolate from a

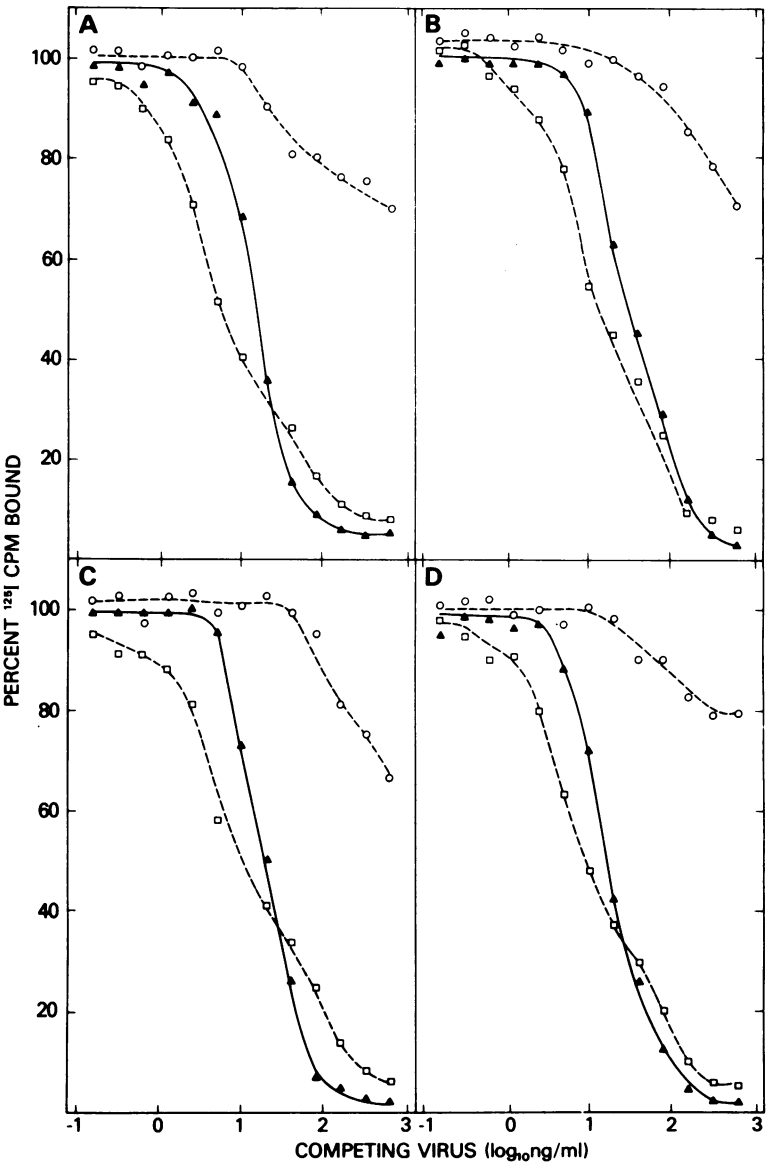

FIG. 4. *Comparison of immunological reactivity of p15 polypeptides of RD114 and Crandell cat virus after virus growth in different species. Viruses were detergent-disrupted as described in the legend to Fig. 2 and tested in homologous p15 immunoassays for the P. cynocephalus baboon (○) and RD114 (□) viruses and in a heterologous p30 immunoassay using antiserum to detergent-disrupt baboon virus to precipitate ^{125}I-labeled RD114 p30 (▲). Viruses tested included: RD114 grown in RD human cells (A); Crandell virus grown in human A673 cells (B); Crandell virus grown in FRH-1 rhesus monkey cells (C); Crandell virus grown in feline thymus cells (D).*

second species of baboon. In addition to virus isolates from *P. cynocephalus*, there has recently been an isolate from a second baboon species, *P. hamadryas* (10). It was possible to test whether the type-specific antigenic determinants of the baboon viral p15 were sufficiently pronounced to distinguish between endogenous type C viruses of two baboon species. *P. cynocephalus* virus reacted efficiently both in the homologous baboon viral and heterologous p15 immunoassay, but was not detectably reactive in the homologous RD114 p15 assay (Fig. 5A). In contrast, the *P. hamadryas* virus isolate was as reactive as *P. cynocephalus* virus in the heterologous p15 immunoassay (Fig. 5), but was only weakly reactive in either the homologous baboon or the RD114 viral p15 immunoassay. Other mammalian type C viruses tested, including feline leukemia virus, R-MuLV, and woolly monkey type C virus, were not reactive in any of these tests (data not shown).

Expression of p30 and p15 polypeptides in normal rhesus monkey tissues. There has been a recent report that antigens cross-reactive with baboon viral p30 were detectable at low levels in tumor tissue of another old world primate, the rhesus monkey (25). With mouse cells, methods for purification of cell-associated antigens cross-reactive with type C viral polypeptides have been developed (34). By sufficient purification of these antigens, it has been possible to demonstrate immunologically and biochemically that the antigens represented two type C viral polypeptides, p30 and p12, coordinately expressed in the normal mouse cell (35). Similar techniques were utilized in the present studies to attempt to detect and purify antigens cross-reactive with baboon viral p30 and p15 in normal rhesus monkey tissues.

When trace amounts of either ^{125}I-labeled baboon viral p30 or p15 were added to rhesus liver cell extracts, sequential purification by DEAE chromatography and agarose gel filtration led to an increase of 40- to 50-fold in the specific activities of the labeled markers (Tables 2 and 3). Immunological analysis of cell extract that comigrated with marker baboon p30 indicated that this antigen competed efficiently in the heterologous anti-baboon virus: ^{125}I-RD114 p30 immunoassay. Antigenic specificity was demonstrated by its lack of reactivity in immunoassays for p30's of other mammalian type C viruses (R-MuLV and woolly monkey type C virus). These results indicate that rhesus monkey cells expressed an antigen with immunological and biochemical properties similar to those

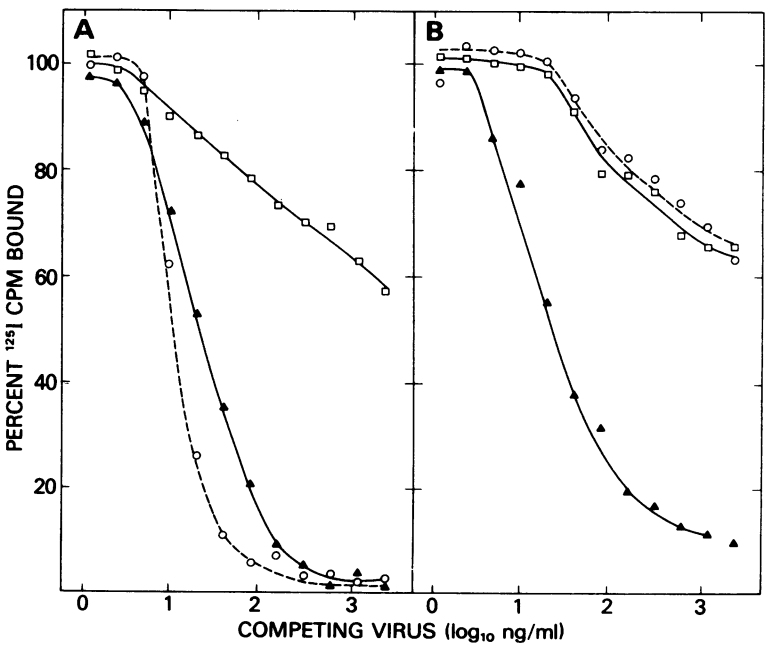

FIG. 5. *Comparison of p15 immunological reactivities of endogenous type C RNA viruses of the following baboon species: P. cynocephalus (A) and P. hamadryas (B). Viruses were detergent-disrupted as described in the legend to Fig. 2 and tested in homologous immunoassays for the p15 of P. cynocephalus baboon* (○) *and RD114* (□) *viruses and in a heterologous p15 immunoassay using antiserum to P. cynocephalus baboon virus to precipitate ^{125}I-labeled RD114 p15* (▲).

of baboon viral p30. In contrast, cell extract that co-chromatographed with ^{125}I-baboon p15 (Table 3) did not demonstrate detectable reactivity in the broadest immunoassay for p15 of the baboon virus group utilizing anti-baboon virus sera to precipitate ^{125}I-labeled RD114 p15 (Fig. 6). Antigenic reactivity in the p15 immunoassay was also undetectable in any other column fractions. This argues that the absence of reactivity was not the result of charge differences causing an antigenically related p15 to elute at a different salt concentration than the known viral p15's.

DISCUSSION

The present studies have investigated the immunological properties of structural polypeptides of type C RNA viruses endogenous to an old world primate, the baboon, and to the domestic cat. These viruses are indistinguishable by standard virologic techniques including neutralization and interference (12). Immunological analysis of their reverse transcriptases and major structural polypeptide, p30, have also failed to detect pronounced antigenic differences (12, 26). In the present report, the p30's of these viruses were shown to be very closely related as determined by homologous competition immunoassays. Because immunological tests for p30 have generally been less satisfactory in discriminating closely related type C viruses than assays for other viral polypeptides (29, 33), the 15,000 MW polypeptides were purified from baboon and RD114 viruses and immunoassays developed. These polypeptides were found to possess some biochemical characteristics analogous to those of 12,000 MW polypeptides of other type C viruses. Further, they exhibited sufficient type-specific antigenic determinants to make it possible to readily differentiate the two viruses. These immunoassays appear to be the best available immunological methods for discriminating baboon-RD114-like viruses.

The evidence indicates that baboon and RD114 viruses diverged evolutionarily as endogenous viruses of two very different progenitor species (3, 4). The present studies demonstrate that their structural polypeptides exhibited very different rates of evolution (i.e., antigenic change) during the course of their transmission within their respective host genomes. Antigenic characterization of analogous polypeptides of a type C virus isolate of a second baboon species, *P. hamadryas*, indicated that its p30 was immunologically indistinguishable from that of *P. cynocephalus*. However, the reactivity of *P. hamadryas* virus in homologous immunoassays for *P. cynocephalus* and RD114 viral p15's readily distinguished it from either test virus. This virus was still sufficiently related antigenically to be reactive in a heterologous immunoas-

TABLE 2. *Partial purification of viral p30 from rhesus monkey liver cell extracts*[a]

Material tested	Total protein (mg)	p30 antigenic reactivity			^{125}I-labeled p30 marker		
		Yield (ng)	Sp act (ng/mg)	Purification	Yield (counts/min)	Sp act (counts/min/mg)	Purification
Initial extract	1,820	9,100	5		8,736	4.8	
Extract following purification by:							
DEAE	339	7,800	23	4.6	7,200	21	4.4
Agarose gel filtration	13	2,660	205	41	3,190	245	51

[a] Trace amounts of ^{125}I-labeled RD114 p30 was added to liver cell extract and purification was performed by sequential DEAE ion-exchange chromatography and agarose gel filtration as described in Materials and Methods. Protein concentration was measured by the method of Lowry et al. (17).

TABLE 3. *Partial purification of viral p15 from rhesus monkey liver cell extracts*[a]

Materials tested	Total protein (mg)	^{125}I-labeled p30 marker (counts/min)	Sp act (counts/min/mg)	Degree of purification achieved
Initial extract	1,840	9,500	5.2	
Extract following purification by:				
DEAE	370	7,955	21.5	4.1
Agarose gel filtration	15	4,650	310	60

[a] Trace amounts of ^{125}I-labeled RD114 p15 was added to liver cell extracts, and purification was performed as described in the legend to Table 2.

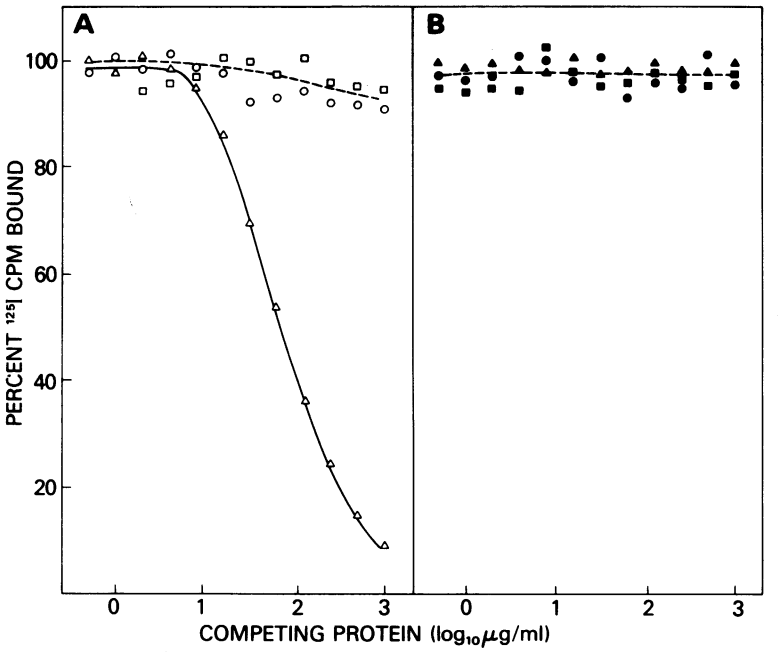

FIG. 6. *Immunological analysis of partially purified rhesus monkey liver extracts enriched 50- to 60-fold for cellular proteins comigrating through sequential ion-exchange chromatography and agarose gel filtration with tracer amounts of RD114 ^{125}I-p30 (A) and RD114·^{125}I-p15 (B). Immunoassays included assays for the p30 antigens of R-MuLV (○) and woolly monkey (□) type C viruses; a heterologous p30 assay using antiserum to P. cynocephalus baboon virus to precipitate ^{125}I-RD114 p30 (△); homologous immunoassays for the p15 antigens of RD114 (■); and P. cynocephalus baboon virus (●); and a heterologous p15 assay using antiserum to P. cynocephalus baboon virus to precipitate ^{125}I-RD114 p15 (▲).*

say for the antigenic determinants shared by RD114 and baboon viral p15's. These results indicate that there has been a more marked antigenic divergence during evolution of analogous low MW polypeptides than of the major structural polypeptides of the baboon-RD114 endogenous virus group. Whether differences in biological functions of viral p15 and p30's can in some way explain their different rates of antigenic change remains to be resolved.

A previous report detected antigens cross-reactive with p30 of the baboon-RD114 virus group in an ovarian carcinoma of a rhesus monkey, but not in several normal rhesus tissues (25). The specificity of the immunological reactivity was implied by the lack of activity in competition immunoassays for several other type C viral polypeptides. The present report extends those observations by demonstrating baboon-RD114-like virus p30 antigenic reactivity in normal rhesus tissues. Further, in the present studies the antigenic cross-reactivity has been purified more than 50-fold and shown to co-chromatograph in serial purification steps with known ^{125}I-labeled marker baboon viral p30. The purification procedures included a final sizing step under denaturing conditions which indicated a MW of around 30,000. Thus, the antigenic reactivity partially purified from normal rhesus tissues has properties consistent with those of the major structural polypeptide of a type C virus which is immunologically closely related to the baboon endogenous virus.

The absence of detectable p15 of either baboon or RD114 viruses in these same tissues despite similar degree of purification of marker p15 suggests that the p15 of a baboon-like virus endogenous to rhesus monkey may have diverged sufficiently in its immunological properties so that it is no longer cross-reactive even in the broadest immunoassays for this polypeptide. A second possibility is that p15, similar or dissimilar to that of baboon or viral p15, is not expressed at coordinate levels with viral p30 in normal rhesus liver.

There are several reports of antigens cross-reactive with mouse type C virus (30), baboon-RD114 virus (27, 30) and/or gibbon-woolly monkey type C virus (28, 30) in relatively crude extracts of human tissues. Extensive analysis of human tissues for type C viral antigens has demonstrated that apparently positive reactions, similar to those previously published, are often obtained with relatively crude tissue

extracts. However, attempts to purify these reactivities by methods such as those described above led to loss rather than enrichment of the immunological reactivity (J. R. Stephenson and S. A. Aaronson, submitted for publication). Whether the application of methods utilized in the present study can lead to the purification of type C virus-specific antigens from higher primates including man remains to be resolved.

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