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Radioimmunoassay of Mammalian Type-C Viral Proteins: Interspecies Antigenic Reactivities of the Major Internal Polypeptide*

(gs-3/visna/Mason-Pfizer virus/syncytium-forming virus)

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ABSTRACT **Mammalian type-C viruses contain a major internal polypeptide of about 30,000 daltons that is characterized by both intraspecies and interspecies antigenic reactivities. Radioimmunoprecipitation assays were used for measurement of this protein; the assay was based upon interspecies reactivities of the protein. As little as 5 ng of the group-specific antigen of murine leukemia virus can be measured by radioimmunoprecipitation assays, thus providing an approximate 10,000-fold increase in sensitivity over the standard immunodiffusion procedure. The type-C viruses that were recently isolated from a woolly monkey and gibbon ape each have an interspecies type-C antigenic reactivity. The primate viruses, however, could be distinguished from the type-C viruses of murine, rat, hamster, and feline origin that were more highly related to each other. The interspecies reactivity of the 30,000-dalton polypeptide is an immunological marker of the mammalian type-C viruses, since even with this sensitive assay other mammalian viruses with RNA-dependent DNA polymerase activity did not contain the type-C interspecies antigen.**

The major internal polypeptide of mammalian type-C RNA viruses is the third-fastest migrating polypeptide in sodium dodecyl sulfate (SDS)–polyacrylamide gels, and has a molecular weight of about 30,000 (1, 2). This group-specific (gs) antigen displays an antigenic reactivity common to all strains of type-C virus from a given species, thus making it possible to determine the species of origin of a type-C virus. Geering *et al.* first described an antigen in feline leukemia virus (FeLV) that crossreacted with a murine leukemia virus (MuLV) antigen in gel diffusion studies that they termed group specific(gs)-3 (3). Studies by Schäfer *et al.* with FeLV suggested that the 30,000-dalton polypeptide contains the interspecies or gs-3 antigen common to mammalian type-C viruses; they proposed that the species-specific determinant(s) of the feline virus, or gs-1, resided only on a smaller polypeptide (15,000 daltons) (4). Gilden *et al.* and Oroszlan *et al.* have presented evidence that indicates that the 30,000-dalton polypeptides of MuLV and FeLV (5, 6) each contain distinct intraspecies and common interspecies reactivities.

The studies to be presented provide further evidence that the 30,000-dalton polypeptide from both murine and feline type-C viruses contain both intraspecific and interspecific reactivities. Further, by the appropriate selection of antisera in a radioimmunoassay procedure (7), it is possible to quantitate specifically the crossreacting moiety of a mammalian type-C viral polypeptide, and consequently to identify type-C viruses from all mammalian species examined.

Abbreviations: MuLV, murine type-C virus; FeLV, feline type-C virus; SDS, sodium dodecyl sulfate; gs, group specific; MVP, FVP, murine and feline viral protein.

* This is paper no. 2 of the series, no. 1 is ref. 7.

MATERIALS AND METHODS

The source of virus and methods of virus purification have been detailed (8, 9). Rauscher-MuLV and Moloney-MuLV were obtained from a tissue culture source prepared by Electro-Nucleonics, Bethesda, Md. Snyder–Theilen-FeLV and Rickard-FeLV were obtained from University Labs., Highland, N.J., and from Electro-Nucleonics. Cultures of woolly monkey (Lagothrix) and gibbon ape (Hylobates) cells infected with type-C virus were the gifts of Thomas Kawakami and his associates at the University of California, Davis, Calif. (10, 11). Supernatant fluids from these cultures were used to infect a strain of human embryonic lung cells, M413B, which were used as a source of large amounts of fluid necessary for the preparation of adequate quantities of purified virion antigens. The primate viruses were purified by the method described for Rauscher-MuLV (8); procedures for the disruption of the virus, Sephadex G-100 gel chromatography, polyacrylamide gel electrophoresis, and isoelectric focusing have been described (7).

Since other viral proteins, including RNA-dependent DNA polymerase (reverse transcriptase) (about 70,000 daltons), possess "group"- or species-specific antigenic reactivities (8, 9), we prefer the designation VP3(gs) for the "gs" antigen. This is based on the observation that the approximately 30,000-dalton polypeptide of all mammalian type-C viruses so far examined is the third-fastest migrating polypeptide from detergent-disrupted virions in SDS–acrylamide gel electropherograms. This designation thus provides a means of designating viral polypeptides based on physical properties independent of immunological criteria.

Robert Huebner, NCI, NIH, Bethesda, Md., supplied two pools of sera from hamsters that bore tumors induced by Schmidt–Ruppin strain of Rous sarcoma virus (SR-RSV) that reacted with several internal proteins of Rous sarcoma virus (12). Roger Wilsnack, Huntington Research, Baltimore, Md., kindly supplied sera prepared in goats by repeated immunization with a tween–ether-disrupted strain of Human-cell adapted-MuLV or Snyder–Theilen-FeLV and sera from Fischer rats that bore tumors that release Moloney-MuLV that reacted with murine VP3(gs) [MVP3(gs)] by complement-fixation and gel diffusion. Similar sera, which were

described elsewhere (13), give by gel diffusion lines of identity with rabbit or guinea pig sera prepared against highly purified VP3(gs) (unpublished data). Raymond Gilden, Flow Laboratories, Rockville, Md., supplied several sera described in publications from their laboratory that were prepared with electrofocused "gs" polypeptides (VP3) from mouse, rat, hamster, and cat in guinea pigs or rabbits (2, 6).

3- to 6-kg New Zealand White female rabbits were immunized with 40–80 μg of MVP3(gs) or feline (F)VP3(gs) in complete Freund's adjuvant prepared by emulsification in a high-speed homogenizer (Omnimixer, Sorvall, Hartford, Conn.) at 4°. Primary inoculations were administered into footpads, and subsequent booster immunizations (10–25 μg) were administered without adjuvant in hind legs by the subcutaneous route. After antibodies were detected, subsequent immunizations were administered intravenously. Three rabbits were immunized with Rauscher-MuLV VP3(gs) and five with Snyder–Theilen-FeLV VP3 [FVP3(gs)]. Trial bleedings were collected from the ear artery at about weekly intervals. Antisera against rabbit, goat, and guinea pig immunoglobulin G (IgG) were purchased from Meloy Laboratories, Springfield, Va. Anti-rat IgG was purchased from Hyland Labs, Costa Mesa, Calif. All antiglobulins were used undiluted.

Details of the radioimmunoprecipitation assay for antibody and antigen have been described (7), and the specific components and their concentrations in the particular assays are indicated in the legends. Iodination by the chloramine-T method (14) and determination of specific activities were as described (7). Both labeled MVP3(gs) and FVP3(gs) were 80–95% precipitable with excess antibody, and migrated as a single band in SDS–polyacrylamide gels. For antibody assays, labeled VP3(gs) is incubated with antibody for 3 hr at 37° and then for 14–18 hr at 4°. Antiglobulin is added, and after 1 hr at 37°, incubation is continued for 3 hr at 4°. The mixtures are then centrifuged at 10,000 × *g* for 15 min; supernatant counts were assayed by liquid scintillation (7). VP3(gs) antigen assays are similar, except that unlabeled antigen and a limiting amount of antibody (determined by prior titration) are incubated together for 2 hr before labeled antigen is added. The remainder of the VP3(gs)-antigen assay was identical to the assay for antibody. Titers of antibody are expressed as the reciprocal of the serum dilution that precipitated 10% or more of labeled MVP3(gs) or of the labeled FVP3(gs). Antigen titers were determined by reference to standard curves (7); where standard curves were not available, antigen titers were the reciprocal of the antigen dilution used. Protein concentrations were determined by the procedure of Lowry *et al.* (15); bovine serum albumin was used as a standard.

RESULTS

Coelectrophoresis of Intraspecies and Interspecies Reactivities. MVP3(gs) purified by gel chromatography and isoelectric focusing migrates as a single polypeptide in neutral SDS, and in acid or alkaline urea gels (7). As shown in Fig. 1, complement-fixing activity was recovered from the SDS and alkaline urea gels that corresponds to the visible bands. The intraspecies and interspecies reactivities, as determined by complement fixation, cochromatographed. For quantitation of the murine intraspecies reactivity, sera were used from rats with tumors that release MuLV that primarily react

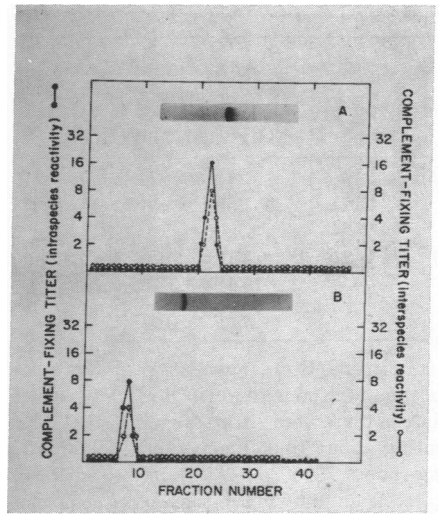

FIG. 1. Coelectrophoresis of intraspecies and interspecies antigenic reactivities. (*A*) 12 μg of purified MVP3 (gs) protein was applied to a 0.1% SDS–acrylamide (pH 7.2) gel as described (7). The gel was cut in 1.3-mm slices and antigen was eluted at 25° with 0.2 ml of 0.01 M Tris·HCl (pH 7.8); 25 μl of each fraction was assayed for complement-fixing activities with sera that measure murine species-specific [rabbit anti-VP3(MuLV)] or interspecies reactivity (anti-FVP3). The antiserum to VP3 (FeLV), prepared against disrupted ST-FeLV, produces a line of partial identity in gel diffusion between feline VP3 (gs) and murine VP3 (gs), as well as with other strains of mammalian type-C viruses (16). (*B*) 12 μg of purified MVP3 (gs) protein was applied to an 8.0 M urea–acrylamide gel (pH 8.7) as described (7), and assayed as in *A*.

with the murine VP3(gs), as measured by complement-fixation tests. To measure the interspecies reactivity either by complement fixation or gel diffusion, a goat antiserum was used. No complement-fixing activity was recovered from the region of the visible band in the acid urea gels. These results strongly suggest that MVP3(gs) contains both intraspecies and interspecies reactivities on the same polypeptide.

Antisera Prepared against MVP3 and FVP3. Purified VP3(gs) from MuLV and FeLV were used to immunize rabbits, and the sera were assayed for intraspecies and interspecies reactivities by titration of antisera with labeled MVP3-(gs) and FVP3(gs). Since the slopes of the precipitation curves with sera were linear between 15 and 70% of the antigen precipitated, quantitation of the relative affinity of a given serum for labeled MVP3(gs) against FVP3(gs) could be made (7). The data are summarized in Table 1. With three sera from rabbits immunized with MVP3(gs), the ratio of activity of anti-VP3(MuLV) with MVP3(gs) against FVP3(gs) varied from 256/1–640/1. Thus, antisera from rabbits immunized with MVP3(gs) had a predominantly *intraspecies* reactivity.

In contrast, sera from rabbits immunized with FVP3(gs) showed ratios of 1/1–4/1. Later sera (12–16 weeks) from the same rabbits had ratios as high as 64/1. The data show that sera prepared against purified MVP3(gs) or FVP3(gs) generally revealed a significantly greater degree of interspecies reactivity, indicating that the interspecies determinant of FVP3(gs) may be relatively more immunogenic in rabbits.

The above data also suggest that crossreacting antigenic reactivities were present in purified VP3(gs) from either

TABLE 1. *Assay for intraspecies and interspecies reactivities*

Immunizing antigen	Animal number	Antiserum titer against MVP3(gs)	Antiserum titer against FVP3(gs)	Ratio (intraspecies/interspecies)
MVP3(gs)	219	2560	10	256
	222	2560	5	512
	259	51,200	80	640
FVP3(gs)	241	100	400	4
	244	160	640	4
	270	5120	5120	1

Rabbit antiserum titers against MVP3(gs) and FVP3(gs) from animals immunized with purified MVP3(gs) or FVP3(gs), as described in the text, were titrated against [^{125}I]MVP3(gs) (0.10 ng representing about 10,000 cpm) and [^{125}I]FVP3(gs) (0.20 ng representing about 8000 cpm). Antigen–antibody complexes were separated from unbound antigen by precipitation with anti-rabbit IgG after an overnight incubation. The antiserum titer is defined as the reciprocal of the highest serum dilution that precipitates 10% or more of the [^{125}I]VP3(gs).

species. To determine whether the interspecies antigenic reactivity of VP3(gs) was uniformly distributed throughout all of the ^{125}I-labeled molecules, labeled VP3(gs) was precipitated with sera prepared against a homologous and a heterologous immunogen. As shown in Table 2, either type of antiserum [anti-VP3(MuLV) or anti-VP3(FeLV)] was able to bind nearly all of either murine or feline [^{125}I]VP3(gs). Although sera prepared against antigen from the homologous species had higher titers, more than 90% of MVP3(gs) and 80% of the FVP3(gs) was precipitable by antisera prepared against the heterologous antigen. These data indicate that the crossreacting determinants are present on essentially all the [^{125}I]labeled molecules.

Comparison of Intraspecies and Interspecies Reactivity in Other Sera. Although there is clear evidence of species-specific reactivity for a given mammalian type-C virus (2, 5), the above data would suggest that most sera prepared against VP3(gs) contain antibodies to crossreacting determinants on the VP3(gs) from other mammalian type-C viruses. Consequently, we have examined sera from several sources prepared against mammalian and avian type-C viral proteins in diverse species; representative examples are shown in Table 3. Rat serum of a high titer (by complement-fixation) from

TABLE 2. *Completeness of [^{125}I]VP3(gs) precipitation by heterologous sera*

Rabbit serum	Antiserum dilution tested	Antigen precipitated (%) MVP3(gs)	Antigen precipitated (%) FVP3(gs)
Anti-MVP3	1:10	92 (±4)	83 (±3)
	1:1000	78 (±2)	2 (±3)
Anti-FVP3	1:10	90 (±3)	83 (±2)
	1:1000	63 (±1)	81 (±3)
Unimmunized	1:1	0	0

Assays were performed as described in the legend to Table 1. The data shown represent the means and standard errors of three separate assays performed in duplicate.

TABLE 3. *Presence of interspecies antibody in sera of immunized animals*

Sera: Immunogen	Sera: Species	Titer against MVP3(gs)	Titer against FVP3(gs)
Moloney-MuLV (tumor)	Rat	16,000	80
Schmidt-Ruppin-Rous sarcoma virus (tumor)	Hamster	0	0
Rickard-FeLV T-E	Goat	32,000	32,000
Human-cell adapted-MuLV T-E	Goat	160,000	3,200
Rat leukemia virus-VP3(gs)	Guinea pig	80	20
Hamster leukemia virus-VP3(gs)	Guinea pig	20	10
Rickard-FeLV-VP3(gs)	Guinea pig	400	25,600

Assays were as described in the legend to Table 1. Antiglobulin of the appropriate species was added as second antibody. The anti-VP3 (HaLV) and anti-VP3 (RaLV) have comparable complement-fixing titers (about 1:256) against their homologous antigens. Sera from SR-RSV tumor-bearing hamsters has a complement-fixing titer of 1:128 against avian type-C virus.

Roger Wilsnack showed a predominantly intraspecies reaction for the murine VP3(gs) antigen. In contrast, a serum prepared in goats against MuLV had an unusually high intraspecies titer, and also showed considerable interspecies reactivity. Although titers against labeled rat or hamster polypeptides could not be determined by radioimmunoassay, both anti-VP3(rat leukemia virus) and anti-VP3(hamster leukemia virus) had low-titered reactions with labeled MVP3(gs) and FVP3(gs). The latter two sera have comparable complement-fixing antibody titers against the VP3(gs) from their respective species (Gilden, R., personal communication).

In the case of FVP3(gs), rabbits immunized in our laboratory with highly purified FVP3(gs) yielded sera with a high degree of interspecies reactivity (Table 1). As shown in Table 3, we have also examined sera from a goat immunized with FeLV disrupted with tween–ether, and from a guinea pig immunized with purified feline VP3(gs). The data show that the goat serum has a high degree of interspecies reactivity. On the other hand, the guinea pig serum and other anti-VP3(FeLV) have relatively greater species specificity, with intraspecies/interspecies ratios of 16-64/1, still significantly lower than the range noted with the highly species-specific antisera to MVP3(gs). In each case, the sera could quantitatively precipitate ^{125}I-labeled FVP3(gs). In contrast to the relatively high degree of crossreactivity among antisera to the mammalian type-C viruses, there was no evidence of crossreaction with antisera to avian type-C viruses. Similarly, in studies not shown here, normal sera from rabbits, cats, guinea pigs, monkeys, or man did not show evidence of anti-VP3(gs).

Detection of Antigenic Crossreactions with [^{125}I]FVP3(gs) and Anti-FVP3. The extensive immunological crossreactivity among mammalian type-C VP3(gs) serves as the basis for a quantitative *competition* radioimmunoassay for unlabeled antigens from various species.

With labeled FVP3(gs) and a limiting concentration of anti-FVP3, standard curves were constructed of the reaction with unlabeled purified FVP3(gs) (7). As shown in Fig. 2,

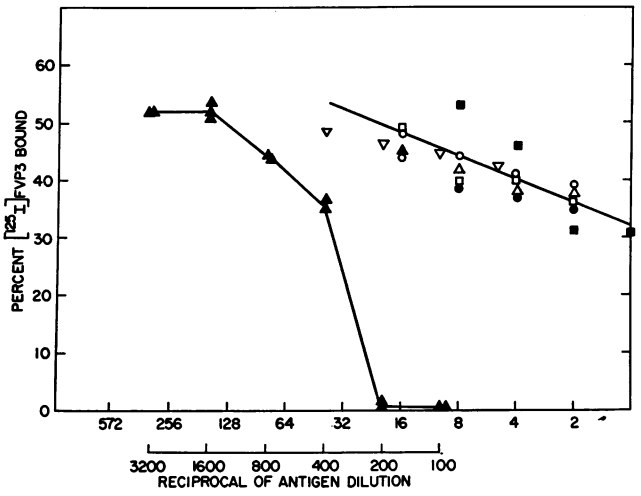

FIG. 2. Dose–response curve of homologous double-antibody radioimmunoassay for interspecies antigenic reactivity. Rabbit anti-FVP3 (7.4 mg/ml), at a final dilution of 1:7200, precipitated about 50% of ^{125}IFVP3 (0.2 ng represents about 8000 cpm). Unlabeled, purified Rickard-FeLV (▲——▲), disrupted by extraction with 10 volumes of ether, was used at concentrations shown on the *lower abscissa* (antigen dilution) (72 μg/ml undiluted). Other viral antigens also used as ether-disrupted, purified, virus preparations were Rauscher-MuLV (□——□), 72 μg/ml; Moloney-MuLV (○——○), 109 μg/ml: Rat leukemia virus (●——●), 48 μg/ml; hamster leukemia virus (■——■), 348 μg/ml; woolley type-C virus (△——△), 383 μg/ml; gibbon type-C virus (▽——▽), 211 μg/ml. Conditions are described in the *text*.

unlabeled FVP3(gs) can be detected at a concentration of about 1 ng per reaction, and progressive increments in unlabeled FVP3(gs) bound all available antibody. Normal cells and other RNA-containing viruses did not displace any antigen. In contrast, the type-C viruses from mouse, rat, hamster, woolly monkey, and gibbon ape were detectable by this assay. The slopes of the dose–response curves of the type-C viruses from species other than feline were similar to each other, but markedly different from the dose–response curve obtained when homologous feline virus was used as the competing antigen. Although this assay detects all type-C mammalian viruses, the disadvantage with this assay is that relatively large concentrations of heterologous VP3(gs) were required to register as positive. In the case where purified VP3(gs) was quantitated, it was found that 725 ng of MVP3(gs) was required for a significant displacement (>2 SD). Three different anti-FVP3 sera (prepared in rabbits or guinea pigs) gave quantitatively similar results. Thus, although it is possible to measure interspecies antigens in this way, the homologous assay for interspecies reactivity lacks adequate sensitivity.

Measurement of Interspecies Reactivities by [^{125}I]MVP3-(gs) and FVP3. With labeled murine VP3(gs) and anti-FVP3 that has an interspecies antibody of high titer, we have developed a sensitive interspecies antigen assay. In theory, the anti-FVP3 sera will only react with the heterologous or interspecies antigenic reactivities on the labeled MVP3(gs). Consequently, this system selectively measures antibodies with interspecies reactivities, thus increasing the specificity for the heterologous VP3(gs) determinants. When the anti-FVP3 serum is tested at a dilution that binds about 50% of the ^{122}I-labeled MVP3(gs), it is possible to measure the interspecies antigens of type-C viruses. As shown in Fig. 3, the system can detect an interspecies reactivity of rat, hamster, and cat viruses. All of these show dose–response curves parallel to each other and to the mouse VP3(gs); hence, the data from the heterologous antigen assay also suggest that these viruses are highly related. This heterologous assay also detects interspecies reactivity in the recently described primate type-C viruses. However, in order to detect displacement of labeled MVP3(gs) in the presence of the primate type-C viruses, 10-fold more primate than rodent viral protein must be added to the reaction to give a comparable displacement. Furthermore, primate VP3(gs) did not displace all of the antibody under the conditions used. Despite increasing the amounts of primate viral protein in the reaction mixture, the percent of bound MVP3(gs) did not exceed 50% of that of the rodent VP3(gs). In contrast to the homologous interspecies assay, the heterologous assay with [^{125}I]MVP3-(gs) and anti-FVP3 was at least 10-fold more sensitive in terms of the amount of primate viral protein required for detection.

Another comparison of the sensitivity differences between the two assays can be made by quantitation of the amounts of VP3(gs) detectable. By the homologus assay with [^{125}I]-FVP3(gs) and anti-FVP3, 725 ng of MVP3(gs) was required for detection; however, only 5 ng was required in the heterologous antigen assay. Since immunodiffusion, which has heretofore been the only available technique for detection of interspecies reactivities, requires a minimum of 50 μg of VP3(gs) per ml to register as positive, the radioimmune precipitation assay is about 10,000 times more sensitive than gel diffusion for detection of interspecies antigenic reactivities.

Other Mammalian Viruses that Contain RNA-Dependent DNA Polymerase. It was of interest to assay these RNA-

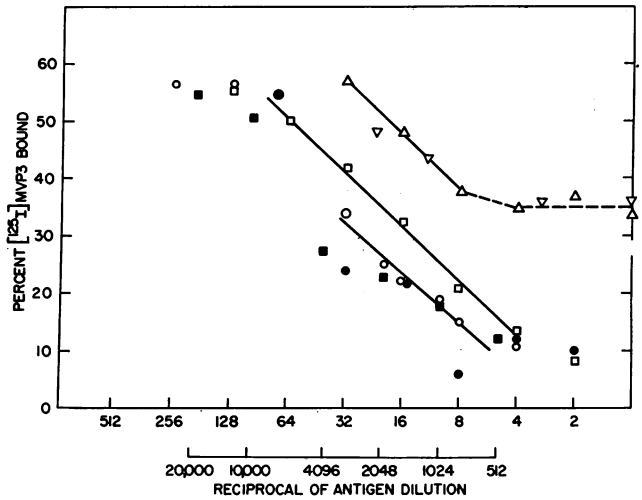

FIG. 3. Dose–response curves of heterologous double-antibody radioimmunoassay for interspecies antigenic reactivity. Goat anti-FVP3, 9.6 mg/ml, at a final dilution of 1:24,000, precipitated 55% of [^{125}I]MVP3 (0.2 ng representing about 6,000 cpm). MVP3 (gs) (□——□), 6.4 μg/ml and FVP3 (gs) ○——○), 8.5 μg/ml were purified preparations and were used at the dilutions indicated on the *upper scale*. The other viruses were as described in the legend to Fig. 3. The *lower scale* represents the dilution of hamster lukemia virus used.

containing viruses for interspecies type-C VP3(gs) antigens. All viruses were tested at protein and RNA-dependent DNA polymerase levels at least 10-fold greater than the minimal amount of primate type-C viral protein that registered as positive. In these assays, Mason–Pfizer monkey virus, feline, and primate syncytium-forming ("foamy") viruses had no detectable interspecies reactivity. Similarly, visna viruses did not have type-C VP3(gs) interspecies antigens. Since all preparations of murine mammary tumor virus have significant contamination with MuLV (unpublished data), we cannot rule out a crossreaction between type-C viruses and mammary tumor viruses.

DISCUSSION

All mammalian type-C viruses so far examined contain an internal polypeptide of molecular weight about 30,000 that is the third-fastest migrating band in SDS–polyacrylamide gels. This protein, termed the gs antigen, or in our studies VP3 (gs), has been shown by Gilden and his associates to have not only species-specific antigenic reactivities, but also interspecies reactivity common to all mammalian type-C viruses. We have prepared VP3(gs) from purified virions by gel chromatography and by isoelectric focusing in ampholytes. After these procedures, the 30,000-dalton MVP3(gs) migrated as a single band in three dissociating solvents in polyacrylamide gels, indicating that VP3(gs) is a single polypeptide chain (7). VP3(gs) from these gels contains both intraspecies and interspecies antigenic reactivities.

Both murine and feline VP3(gs) iodinated by the chloramine-T method were completely precipitable by heterologous antiserum to VP3(gs) prepared against purified VP3-(gs). These data strongly suggest that interspecies antigenic reactivities are present on the VP3(gs) molecule. Further supporting evidence for this view was the fact that all the antisera to VP3(gs) examined contained both intraspecies and interspecies antibodies in various titers. We have developed radioimmunoprecipitation assays that measure the crossreacting determinants of mammalian type-C viruses by competition of labeled and unlabeled antigen for a limiting amount of antibody. The most sensitive technique uses [^{125}I]MVP3(gs) and anti-FVP3. This "heterologous" radioimmunoassay provides a unique means of measurement of VP3(gs) in any mammalian species based on the interspecies antigen. Presumably, the interspecies reactions observed with the other viruses are also present on their VP3(gs) polypeptides. Clearly, the crossreactions being measured in the type-C viruses must reflect sequence homology with the MVP3(gs), in view of the high degree of purity in the labeled mouse antigen.

As reflected in the results of the interspecies assays, the rodent and feline type-C viruses appeared highly related to each other. This result is consistent with the recent studies of the N-terminal amino-acid sequence by Gilden and Oroszlan that demonstrate that the NH_2-terminal tripeptide sequences of the feline and murine VP3(gs) are identical (17). Precise delineation of cross-reactions among the mammalian type-C viruses will require further study; however, it is already apparent that the two primate type-C viruses have related, but immunologically distinct, interspecies reactivities.

The high degree of relatedness of type-C viruses provides the basis for rapid and quantitative measurements of the interspecies reactivity. Less than 10 ng of MVP3(gs) protein and less than 200 ng of any mammalian type-C *viral* protein could be detected by this assay. Only slightly less sensitive than the species-specific assays, the radioimmunoprecipitation assay for the interspecies antigen, as presently performed, is about 10,000 times more sensitive than gel diffusion, heretofore the only proven method of demonstrating this reactivity. The heterologous assay for VP3(gs) is analogous to the assay for human follicle-stimulating hormone (18). Both assays use antibodies directed against interspecies determinants and thus take advantage of the resultant higher specificity to measure homologous proteins from other species.

The specificity of the assay for type-C viruses was demonstrated by showing that even with 10 times the viral protein necessary for minimal detection of type-C interspecies reactivity in other type-C viruses, Mason-Pfizer monkey virus, syncytium-forming ("foamy"), and visna viruses yield negative results. Because of the specificity and sensitivity of the radioimmunoassay for a type-C viral protein, it should prove useful both in classification of isolates as type-C viruses and in detection of viral expression in tissues from species such as man from which no type-C isolate is yet available.

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