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Structural Proteins of Mammalian Oncogenic RNA Viruses: Immunological Characterization of the p15 Polypeptide of Rauscher Murine Virus

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The immunological properties of the purified 15,000-dalton protein of Rauscher murine type-C virus were analyzed by radioimmunoassay. The majority of the antigenic determinants of this protein were found to be remarkably specific to Rauscher and Friend virus and to a lesser extent to Moloney virus. Determinants reactive with other murine viruses (group-specific) or type-C viruses of other species (interspecies) were also demonstrated but were minor components of the total antigenic specificities of the protein. The results provide evidence that the antigenic properties of this protein specify the Friend-Moloney-Rauscher subgroup of type-C viruses.

Type-C RNA viruses can be distinguished from each other on the basis of several biological properties: host range, tissue specificity, pathogenesis, interference, induction of neutralizing antibodies, and cell surface modification. Logically, such virus-specific biological properties must result from specific biochemical properties of the virion. One possible means of elucidating related biological and chemical properties of viral components is by immunochemical detection and analysis of type-specific differences of purified proteins. By such an approach we previously have shown that the Rauscher virus major envelope glycopeptides of 69,000 and 71,000 daltons (gp69/71) express strong type-specific antigenic reactivity (18, 19) and that these glycopeptides contain major determinants for specific virus neutralization (16). (The proposed conventions originated and were agreed upon at a meeting of approximately 50 of the active investigators in the field at the Sloan Kettering Memorial Institute for Cancer in June 1973. Viral polypeptides are designated according to their apparent molecular weight in thousands with abbreviation "p" for protein and "gp" for glycoprotein [1]). Virion proteins of 12,000 daltons (p12) and 30,000 daltons (p30) are also known to demonstrate type-specific antigenic determinants (17, 19), but as yet there are no assays associating these proteins with biological properties of type-C viruses.

We now report analysis of the Rauscher murine virus 15,000-dalton (p15) protein, another major structural component of this virus. This protein was found to express predominantly type-specific determinants and a subclass of group determinants common only to Rauscher, Friend, and Moloney viruses among the murine viruses tested. Other group and interspecies determinants were weakly reactive. The results provide evidence linking this protein of different murine C-type viruses with the recognized Friend-Moloney-Rauscher cell surface antigen and suggest that the analogous peptide of Gross virus will show equally strong subgroup specificities common to viruses demonstrating the Gross cell surface antigen.

MATERIALS AND METHODS

Viruses. Kirsten murine virus from NRK cells transformed by Kirsten sarcoma virus and productively infected with Kirsten leukemia virus, and AKR murine virus from a productively infected BALB 3T3 cell were propagated, harvested, and purified as previously described (2). The Kirsten virus (5) and AKR cultures were kindly provided by V. Klement, Univ. of Southern California, and G. Todaro, National Cancer Institute, respectively. Spleen focus-forming Friend virus was propagated in a spleen-derived transformed cell line FSD-1/F4 (11) which produces both spleen focus-forming and helper leukemia virus (8), and was kindly provided by M. Sveda of this institution. Moloney murine virus (104-6-5 and 362-52-94) was obtained from Electro-Nucleonic Laboratories, Inc. Mouse mammary tumor virus propagated in Mm5mt/cl clonal cell line (12) was obtained from D. Fine, Frederick Cancer Research Center. Rauscher murine virus propagated in a BALB/c mouse bone

marrow culture (JLS-V9) infected with Rauscher virus (21), the Rickard strain of feline virus propagated in the F-422 suspended cell culture derived from the thymus gland of a leukemic cat (14), the endogenous cat C-type virus RD 114 propagated in a human rhabdomyosarcoma cell line (7), and woolly monkey virus and gibbon ape viruses, both propagated in a human lymphoblastoid cell line NC-37, were all provided by S. Mayyasi, D. Larson, and P. Traul of the John L. Smith Memorial for Cancer Research. These viruses were propagated, harvested, and purified as previously described (19).

Antisera. Rabbit anti-Rauscher murine virus serum (4a-6) was obtained from animals injected with purified virus particles that had been degraded at 45 C for 1 min in 0.2% sodium dodecyl sulfate. For primary injections, 1 mg of purified virus was mixed with an equal volume of Freund complete adjuvant and injected into two footpads and multiple intradermal sites. Booster injections of approximately 350 μg of virus protein were given intramuscularly in Freund incomplete adjuvant at monthly intervals. Rabbit anti-Kirsten murine virus serum (no. 130) and rabbit anti-Rickard feline leukemia virus serum (no. 119) were prepared as above except that the viruses were degraded by incubation at 37 C for 15 min in a final concentration of 0.4% Triton X-100, and in some cases only 400 μg of purified virus particles was used for primary injections and 150 μg of virus protein was used for booster injections. Rabbit anti-baboon type-C virus serum was obtained from animals immunized with Triton X-100-disrupted M7 baboon virus and kindly provided by C. J. Sherr and G. Todaro of the National Cancer Institute. Goat anti-woolly monkey virus serum (no. 340) was prepared with purified virus propagated in NC-37 human lymphoblastoid cells and disrupted in 0.4% Triton X-100. Monospecific antisera against the three purified Rauscher virus proteins, gp69/71, p30, and p15, were prepared as described elsewhere (M. Strand and J. T. August, J. Biol. Chem., in press). All other anti-type-C virus sera, anti-Moloney murine virus (1S-166), anti-AKR murine virus (2S-296), anti-Theilen feline virus (1S-8), anti-rat type-C virus (3S-365), and anti-RD 114 virus (3S-72 and 3S-73) were obtained from goats immunized with purified virus particles disrupted by treatment with Tween-ether. Goat anti-rabbit immunoglobulin (Ig) serum (2S-526) and pig anti-goat IgG serum (4S-41) were prepared with IgG purified by ion exchange chromatography.

Radioimmunoassay. Purified proteins were labeled with ^{125}I as described by Hunter (3). The procedure was slightly modified for the p15 protein by adding 1 mg of crystalline bovine serum albumin immediately after the reaction was terminated so as to enhance recovery when the ^{125}I-labeled protein was separated from free iodine by Sephadex G-25 chromatography.

The reaction mixture for immunoprecipitation contained the following: 0.005 ml of normal rabbit or goat serum corresponding to the antiserum used, 1 to 8 ng of ^{125}I-labeled virus protein containing 2.5×10^4 to 10^5 counts per min per ng, and 0.01 ml of diluted rabbit or goat antiserum as indicated. The final volume was adjusted to 0.06 ml with TEN buffer (20 mM Tris-hydrochloride, pH 7.6, 1 mM EDTA, 100 mM NaCl) containing 2 mg of fraction V bovine serum albumin per ml. All antigen and antibody proteins were diluted in TEN buffer containing 20 mg of crystalline bovine serum albumin per ml. The reaction mixture was incubated at 37 C for 4 h after which 0.03 ml of goat anti-rabbit IgG or pig anti-goat IgG serum was added to precipitate the antigen-antibody complex. The mixture was then incubated an additional 12 h at 2 to 4 C. Cold TEN buffer (0.5 ml) was added, and the precipitate was collected by centrifugation at 4 C. The pellet was washed twice with 0.5 ml of TEN buffer, and the ^{125}I-labeled antigen present in the precipitate was measured in a gamma counter.

The competition radioimmunoassay reaction mixture was the same except that the dilution of antiserum added was that which precipitated approximately 50% of the labeled antigen, and the competitive inhibition of binding of the ^{125}I-iodinated antigen by unlabeled virus or virus protein was measured. Competing proteins were diluted in TEN buffer containing 20 mg of crystalline bovine serum albumin per ml and were added (0.01 ml) immediately before the antiserum. When virus particles were tested, Triton X-100 (0.4% final concentration) was added to the virus preparation and the suspension was incubated at 37 C for 10 min. The disrupted virus particles were then diluted in TEN buffer containing 20 mg of crystalline bovine serum albumin per ml and 0.4% Triton X-100.

When competing protein of cell extracts was measured by competition radioimmunoassay, it was necessary to increase the volume of the reaction mixture to 0.2 ml so as to facilitate addition of a large amount of protein. In this case, 0.15 ml of cell extract was added, previously diluted in TEN buffer containing 2 mg of crystalline serum albumin per ml and 0.2% final concentration of Triton X-100. The final volume of the reaction mixture was adjusted to 0.2 ml with TEN buffer containing 2 mg of crystalline serum albumin and 0.2% final concentration of Triton X-100. The reaction mixture was incubated for 14 h at 37 C, 0.04 ml of pig anti-goat IgG serum was added, and the incubation was continued for an additional 2 h at 37 C and for 14 h at 2 to 4 C.

Preparation of cell extracts. The cell culture fluid from approximately 10^7 cells propagated in a 75-cm^2 T flask was removed, and the cells were washed three times with phosphate-buffered saline (130 mM NaCl, 5 mM KCl, 5 mM dextrose, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$). A 10-ml volume of TEN buffer was then added, and the flask was incubated at 37 C for 5 min and then agitated to suspend the cells. The cells were collected by centrifugation at $2,000 \times g$ for 5 min, washed with TEN buffer, and suspended in approximately 0.2 ml of TEN buffer per 10^7 cells. Triton X-100 was added at a final concentration of 0.4% and the suspension was incubated at 37 C for 15 min and then frozen in dry ice-methanol and thawed at 37 C three times. The resulting suspension was centrifuged at $2,000 \times g$ for 10 min, and the supernatant was collected.

Purification of virus proteins. The Rauscher

type-C virus gp69/71, p30, and p15 proteins were purified by phosphocellulose column chromatography and Sephadex gel filtration as described elsewhere (Strand and August, J. Biol. Chem., in press).

RESULTS

Protein purity. Analysis of 10.5 μg of protein of the purified Rauscher murine virus p15 fraction used in these studies by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a single band of protein, indicating that the fraction was at least 90% homogeneous for p15 (Fig. 1).

For an even more sensitive assay of possible contamination by p30 or gp69/71, antiserum prepared against the purified p15 was tested by radioimmunoassay for precipitation of the other purified proteins. Whereas the serum precipitated 4 ng of ^{125}I-labeled p15 at a titer of 1:3000 final dilution, no precipitation of 1 ng of ^{125}I-labeled p30, or 2 ng of [^{125}I]gp69/71 occurred, even with concentrated serum (a final dilution of 1:12 in the reaction mixture). Moreover, neither anti-p30 nor anti-gp69/71 precipitated ^{125}I-labeled p.15.

Because, as shown below, the monospecific anti-p15 serum was strongly type specific, it could be argued that the apparent purity of the p15 was fortuitous in that the anti-p15 serum would not react with other proteins even if present. For this reason the possible contamination of p15 was tested as well by competition radioimmunoassay using anti-feline virus serum, known to be strongly reactive with the interspecies determinants of Rauscher p30 and gp69/71 (19). No competition of antibody binding to purified p15 was detected with p30 or gp69/71, indicating that there was no detectable contamination of the p15 by these proteins or cross-reactivity of the interspecies determinants of those three proteins (Fig. 2).

Possible contamination or cross-reactivity with the murine p12 was also tested with purified ^{125}I-labeled p12 kindly provided by S. Tronick and S. Aaronson of the National Cancer Institute. The ^{125}I-labeled p12 was readily precipitated by antiserum prepared against degraded whole Rauscher murine virus, but not by our anti-p15 serum.

Shared group and interspecies antigenic

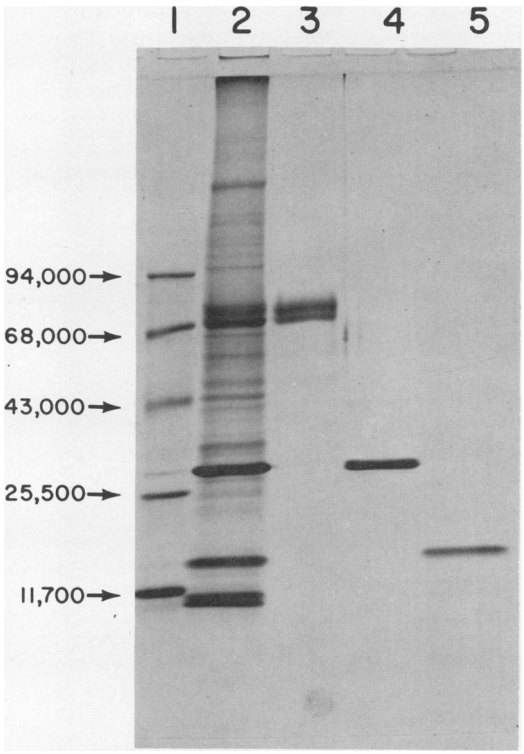

FIG. 1. *Polyacrylamide gel electrophoresis of Rauscher virus proteins. Electrophoresis in a linear 5 to 20% polyacrylamide gel gradient in the presence of 0.1% sodium dodecyl sulfate was carried out as described by Baum et al. (1). (1) Standard proteins; cytochrome c (3.3 μg; molecular weight 11,700), chymotrypsinogen (3.3 μg; molecular weight 25,500), ovalbumin (4.2 μg; molecular weight 43,000), bovine serum albumin (2.5 μg; molecular weight 68,000), phosphorylase A (6.6 μg; molecular weight 94,000). (2) Rauscher murine virus (80 μg of protein). (3) Purified Rauscher virus gp69/71 (9.5 μg of protein). (4) Purified Rauscher virus p30 (3.4 μg of protein). (5) Purified Rauscher virus p15 (10.5 μg of purified protein).*

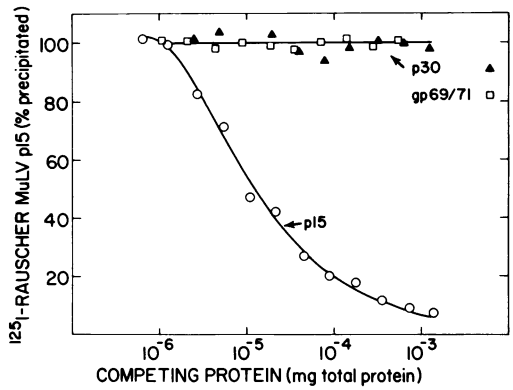

FIG. 2. *Analysis of interspecies determinants of Rauscher murine virus purified proteins, gp69/71, p30, and p15. The assays were performed as described in Materials and Methods for competition radioimmunoassay with 8 ng of ^{125}I-labeled Rauscher purine p15 protein (31,000 counts per min per ng) and goat anti-Theilen feline virus serum at 1:3,000 final dilution. The competing proteins, purified Rauscher murine virus gp69/71, p30, and p15 were added as indicated.*

determinants. The nature of the antigenic determinants of Rauscher p15 was tested by precipitation of [^{125}I]p15 using antisera prepared against a variety of other C-type viruses (Table 1). To provide a standard for the relative activity of these sera, each was also tested with the Rauscher p30, and where possible with the homologous p30 from the virus the antiserum was prepared against.

The data show that antisera against other murine viruses (heterologous group antisera) or other species of mammalian viruses (heterologous interspecies antisera) precipitate the Rauscher p15, thus indicating the presence of group and interspecies antigenic determinants as properties of the protein.

The ratios of the titer against p30 as compared to p15 varied markedly with the different antisera. Without an extensive study of a larger number of antisera, the basis for these different titers is unknown and may reflect either the virus used as immunogen or the response of the animal. In all cases, however, the anti-p30 titer was greater than the anti-p15 titer, presumably resulting from the greater concentration of p30 in disrupted virions and possibly greater relative immunogenicity.

Friend-Moloney-Rauscher subgroup specificity of the p15 protein. Radioimmunoassay analysis of the extent of type specificity or shared group determinants of the Rauscher p15 was carried out by measuring the competition by several murine viruses in the homologous assay system containing ^{125}I-labeled Rauscher p15 and anti-Rauscher p15 serum.

With monospecific goat anti-Rauscher p15 serum and 6 ng of ^{125}I-labeled p15 antigen, 50% competition was obtained with 5.5 ng of unlabeled p15 standard and 33 ng of Rauscher murine virus (Fig. 3). Friend virus bound almost all of the anti-p15 antibodies, indicating close homology between the p15 proteins of this and Rauscher virus. Moloney virus also bound almost 50% of the antibodies, indicating that some but not all antigenic determinants of the Rauscher virus p15 are shared by the Moloney virus protein. In contrast, AKR and Kirsten viruses showed little or no competition, indicat-

TABLE 1. *Titers of different antisera for Rauscher murine virus p15 and p30*

Anti type-C virus serum	Antiserum titer[a]			Ratio p30:p15
	Homologous p27 p28 or p30 proteins[b]	Rauscher murine virus p30[b]	Rauscher murine virus p15[b]	
Homologous[c]				
Anti-Rauscher murine	49,000	49,000	600	82
Heterologous group[c]				
Anti-Moloney murine		96,000	240	400
Anti-AKR murine		60,000	5,600	11
Anti-Kirsten murine		9,000	250	36
Heterologous interspecies[c]				
Anti-Rickard feline		6,000	150	40
Anti-Theilen feline	3,600,000	180,000	6,000	30
Anti-Wister rat		10,000	<12	>1,000
Anti-RD 114 (3S73)	100,000	4,800	300	16
Anti-RD 144 (3S72)	24,000	1,200	300	4
Anti-woolly monkey		200	<12	>17
Anti-baboon (M-7)	3,000[d]	600	30	20

[a] Antiserum titer is given as the reciprocal of the highest dilution which resulted in precipitation of 50% of ^{125}I-labeled antigens. The analysis was carried out by radioimmunoassay as described in Materials and Methods. The amount and specific activity of the labeled antigens was as follows: 1 ng of Rauscher murine virus p30 (3.8×10^4 to 5.6×10^4 counts per min per ng), 4 ng of Rauscher murine virus p15 (4.2×10^4 counts per min per ng), 2 ng of Theilen feline virus p27 (5.4×10^4 counts per min per ng), 2 ng of RD 114 p28 (2.5×10^4 counts per min per ng).

[b] ^{125}I-labeled antigen.

[c] The classification of antisera into homologous or heterologous groupings is related to the Rauscher protein antigens.

[d] A homologous protein is one derived from the same virus the antiserum was prepared against. An exception is the assay with anti-baboon serum; this titer was obtained by use of ^{125}I-labeled RD 114 p30 as antigen as this protein is closely related to the baboon virus p30 (C. J. Sherr and G. J. Todoro, Virology, in press).

ing that there are very few cross-reactive group determinants between the p15 proteins of these viruses and Rauscher virus. The differences in the concentrations of viruses required for competition indicate that there is either a reduced concentration of cross-reactive determinants in the different viral p15 proteins or a reduced concentration of the protein itself. Control studies showing that the absence of competition by the AKR and Kirsten viruses is attributable to the absence of shared determinants and not to absence of the p15 protein per se were provided by specific analysis of the group and interspecies determinants of these proteins, as shown below.

To confirm these findings, the same assay was carried out with broadly reactive anti-Rauscher murine serum prepared against disrupted virus. Results similar to those shown in Fig. 2 were obtained; in this case approximately 60% of the available antibodies were bound by Moloney virus and the maximum competition at 100-fold greater concentrations of AKR and Kirsten viruses was approximately 20% (data not shown).

Rickard feline virus and RD 114 virus were also tested in these assays and in keeping with the other results showed no competition for antibody binding, indicating that few if any of the antibodies were directed at interspecies antigens shared with these viruses (data not shown).

The Rauscher p15 protein thus appears to correspond antigenically to the observed Friend-Moloney-Rauscher subgroup of viruses previously categorized on the basis of cell surface antigens and cytotoxicity of virus-induced tumor cells (9).

Analysis of the group antigenic determinants. Although the competition between p15 proteins of Rauscher and AKR or Kirsten viruses was slight, the presence of some shared group determinants was indicated by the precipitation of ^{125}I-labeled Rauscher p15 by anti-AKR or anti-Moloney virus serum (Table 1). Specific analysis of these p15 group determinants common to Rauscher, AKR, and Kirsten viruses was carried out by use of the heterologous group radioimmunoassay. Determinants bound by anti-AKR serum, and thus common to Rauscher and AKR virus p15 proteins, were shared as well by Kirsten virus but poorly by Moloney virus (Fig. 4). Thus the p15 protein contains a second, weak subclass of group determinants common to Rauscher, AKR, and Kirsten viruses.

It is noteworthy that determinants of the AKR virus p15 that compete with the ^{125}I-labeled Rauscher p15 show a much greater affinity for the homologous anti-AKR antibodies than do the heterologous proteins. Such greater affinity of homologous antigens and antibodies is commonly observed as seen below (Fig. 5A) and in previous studies (19).

Analysis of interspecies antigenic

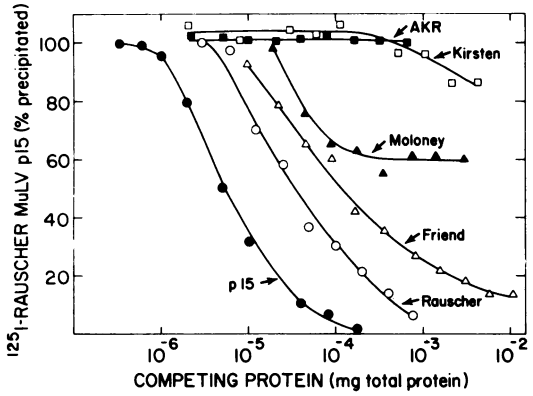

FIG. 3. *Analysis of type- and group-specific determinants of Rauscher virus p15 with monospecific anti-Rauscher virus p15 serum. The competition radioimmunoassay was performed as described in Materials and Methods with 6 ng of ^{125}I-labeled Rauscher virus p15 (38,000 counts per min per ng) and goat anti-Rauscher virus p15, 1:1,500 final dilution. The competing Triton X-100-disrupted viruses were added as indicated.*

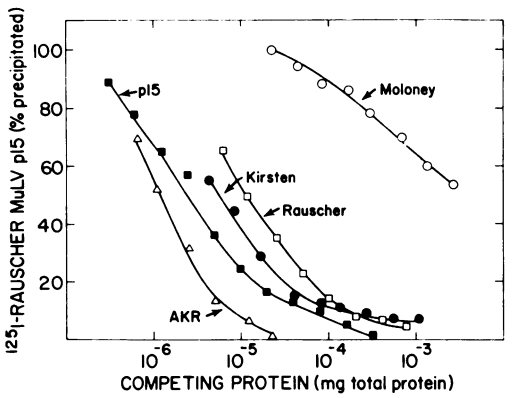

FIG. 4. *Analysis of group determinants of Rauscher virus p15 with anti-AKR serum. The competition radioimmunoassay was performed as described in Materials and Methods with 6 ng of ^{125}I-labeled Rauscher virus p15 (26,500 counts per min per ng) and goat anti-AKR virus serum, 1:6,000 final dilution. The competing Triton X-100-disrupted viruses were added as indicated.*

determinants. Despite the fact that the interspecies determinants of the Rauscher p15 were a very minor component of the antigenic specificities of the protein, these determinants were readily demonstrated by precipitation of the protein with anti-Theilen feline virus or anti-RD 114 virus serum. These determinants were analyzed by use of the heterologous interspecies assay system. With ^{125}I-labeled Rauscher p15 and anti-Theilen feline virus serum it was evident that the determinants common to Theilen virus and Rauscher p15 were also present in AKR, Kirsten, and Moloney viruses (Fig. 5A). Thus, as would be expected from studies of the gp69/71 and p30 proteins (19), the interspecies components are the most rigorously conserved antigenic determinants of the protein. The concentration of the determinants in Moloney virus appeared to be about 10-fold less than in the other viruses; this and the other comparative experiments in this study all suggest that the concentration of the p15 protein in Moloney virus was less than in other murine viruses. In addition, the affinities of the homologous anti-feline virus antibodies for the feline virus protein were greater than the affinities for the other cross-reactive proteins. Such patterns have commonly been observed in other experiments of this and earlier studies (18, 19) and are attributed to a spectrum of cross-reactive antigens and antibodies with differing affinities.

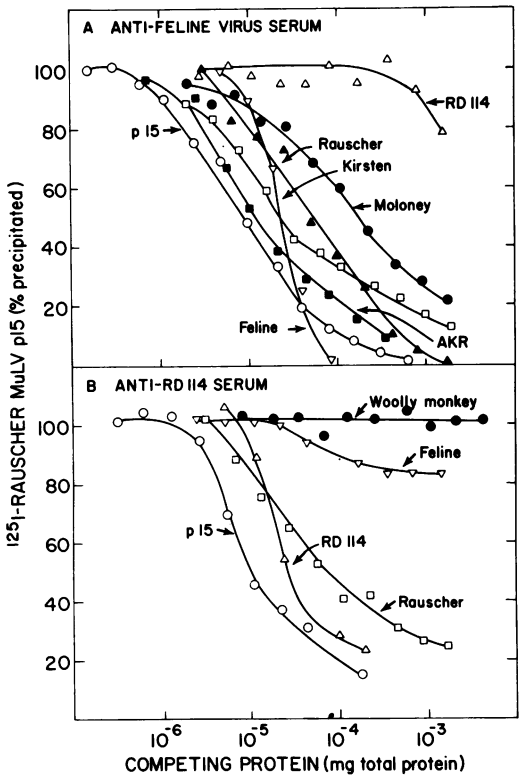

FIG. 5. *Analysis of interspecies determinants of Rauscher virus p15 by competition radioimmunoassay. The assays were performed as described in Materials and Methods. (A) 8 ng of ^{125}I-labeled Rauscher virus p15 (38,000 counts per min per ng) and goat anti-Theilen feline virus serum, 1:3,000 final dilution and (B) 6 ng of ^{125}I-labeled Rauscher virus p15 (26,500 counts per min per ng) and goat anti-RD 114 virus serum (3S72), 1:360 final dilution. The competing Triton X-100-disrupted viruses were added as indicated. Symbols for (A): □, Kirsten; ▽, feline.*

RD 114 virus showed very little competition in this assay containing Rauscher p15 and anti-feline virus serum, suggesting that there are at least two classes of interspecies determinants, those common to feline virus and others to RD 114 virus. This interpretation was confirmed by the results of a reciprocal experiment, using anti-RD 114 virus serum; whereas RD 114 virus competed equally as well as Rauscher virus, as expected, there was little competition with feline virus, and no detectable inhibition with woolly monkey virus (Fig. 5B). Gibbon ape and mouse mammary viruses were also tested in this assay and showed no competition (data not shown).

Relative antiserum titers against the interspecies determinants of murine virus proteins. The relative antiserum titers for the interspecies determinants of the gp69/71, p30, and p15 proteins common to murine and feline C-type viruses was analyzed by radioimmunoassay of the precipitation of each by anti-feline virus serum (Fig. 6). The antibody titer of this serum was greatest for p30 followed by gp69/71 and p15 in that order. In our experience, although the antibody titers of different sera vary, this relative order of titers is that commonly found.

Viral coding of murine virus p15. The type specificity of the antigenic determinants of the p15 protein gives strong indications that this protein is virus coded. As further evidence for this conclusion, we have found that the p15 is not detected in as much as 0.1 mg of protein of uninfected NRK cells or of NRK cells transformed by Kirsten murine sarcoma virus but not productive of virus particles (2), whereas the protein is readily detected in NRK cells producing Kirsten virus (Fig. 7). As presented elsewhere, it also has been found that, with the homologous assay system, using ^{125}I-labeled

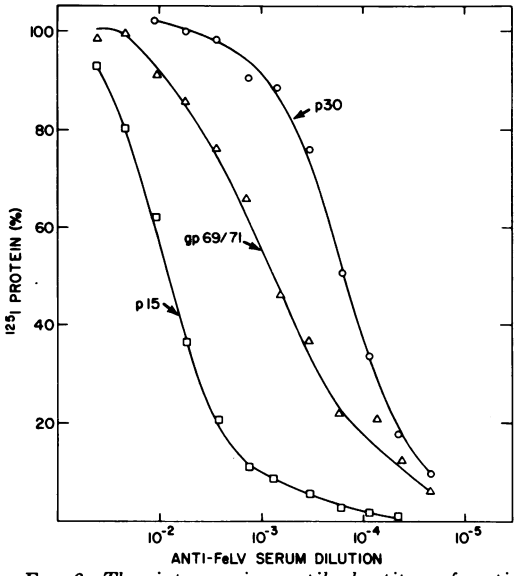

FIG. 6. *The interspecies antibody titer of anti-Theilen feline virus serum for Rauscher virus gp69/71, p30, and p15 proteins. Antibody titer was measured by radioimmunoassay as described in Materials and Methods. The labeled antigens were as follows: 4 ng of ^{125}I-labeled p15 (4.5×10^4 counts per min per ng), 1 ng of ^{125}I-labeled p30 (3.6×10^4 counts per min per ng), and 2 ng of ^{125}I-labeled gp69/71 (6.7×10^4 counts per min per ng). Anti-Theilen virus serum was added as indicated.*

p15 antigen and anti-p15 serum, Rauscher viruses propagated in mouse, rat, or human cells were equally effective in competing for the type-specific antibodies (Strand and August, J. Biol. Chem., in press). It is concluded that the Rauscher murine leukemia virus p15 is virus coded.

DISCUSSION

A well known category of virus-specific antigens are the cell surface antigens of mouse leukemia cells demonstrated by the cytotoxicity of serum from mice bearing virus-induced tumors or infected with murine type-C virus (9). Two major subgroups of these determinants have been defined from the pattern of cross-reactivity of antibodies prepared with various strains of mouse type-C viruses: (i) Gross (G) cell surface antigens found with leukemic cells and cells of normal lymphoid tissues of AKR, C58, PL, and F high-incidence leukemia strains and (ii) Friend-Moloney-Rauscher antigens on leukemia cells infected by these viruses. These determinants are cross-reactive among themselves but not with antigens or serum showing G specificity (10). The Friend-Moloney-Rauscher antigen was found in the sera of viremic mice as well as on the cell surface (20), and the antigenic activity was also found with disrupted virions, suggesting that it is a virion protein (6). Our present findings provide evidence that this protein is the p15, as this is the only viral component known to express Friend-Moloney-Rauscher subgroup specificity among those purified components that have been examined, the gp69/71 (19), p30 (19), and p12 (17). Moreover, in experiments carried out in collaboration with M. Friedman and F. Lilly of this institution, it was found that the monospecific anti-p15 serum was highly cytotoxic for spleen cells of Friend virus-infected mice, and that the purified p15 blocked cytotoxic antibodies of known anti-Friend-Moloney-Rauscher specificity. There also is evidence from the recent studies of Ihle et al. (4) that the p15 is localized at the viral or cell surface as it appears to be one of the peptides reactive with antibodies naturally present in mice. These findings lead us to conclude that the Friend-Moloney-Rauscher subgroup antigens are properties of the p15 protein and to speculate that the Gross antigen is a property of the analogous protein of the Gross-type virus.

In agreement with our earlier hypothesis (19), studies of the immunochemical properties of four purified structural proteins of type-C RNA tumor viruses have shown that all except possibly the p12 contain each of the three classes of

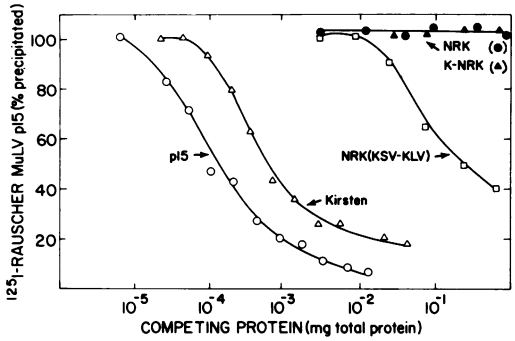

FIG. 7. *Analysis of p15 synthesis in normal rat kidney (NRK) cells. The assays of cell extracts were performed as described in Materials and Methods using 8 ng of ^{125}I-labeled Rauscher virus p15 (38,000 counts per min per ng) and goat anti-Theilen virus serum, 1:3,000 final dilution. The competing purified p15, Kirsten virus, and cell extracts of uninfected NRK, nonproductive NRK cells transformed by Kirsten sarcoma virus (K-NRK), and NRK transformed by Kirsten sarcoma virus and productively infected with Kirsten leukemia virus [NRK(KSV-KLV)] were added as indicated.*

antigenic determinants, type, group, and interspecies, and that the proteins vary markedly in the relative reactivity of these determinants. The gp69/71 major envelope glycopeptides have strongly reactive type and group, and weak interspecies determinants (19). The p30 major core protein has strong group (2a, 19) and interspecies (2a, 13, 19) specificities, and weak type specificity (17, 19). As described herein, the p15 is almost totally type and subgroup specific. The p12 protein was found to express strongly reactive type and moderately reactive group determinants; interspecies determinants were not detected (17). The viral reverse transcriptase has also been studied and as measured by antibody inhibition of enzyme activity, bears both group and interspecies determinants (15).

These different proteins and the spectrum of determinants they encompass provide reagents for increasingly detailed characterization of different C-type viruses. For example, by making use of the interspecies determinants, one can examine the extent of relatedness of this property for specific proteins between viruses of different species. Similarly, use of the type-specific determinants of the different proteins allows a detailed comparison of the different murine viruses in addition to the currently available typing based chiefly on virus neutralization. This is particularly true for the strongly type-specific proteins, as previously shown by Stephenson et al. for the p12 (17), and now even more so for the p15. The specificity of the p15 protein is manifest not only in the type- and subgroup-specific determinants but also in the different classes of group and interspecies determinants that were demonstrated. With the limited number of viruses employed in this study, two distinct classes of interspecies determinants and two of group determinants were evident. We would predict that these are not the only such different classes but that use of other antisera and competing viruses in the competition radioimmunoassay would reveal other classes of group and interspecies determinants as well, reflecting a wide spectrum of antigenic determinants of the p15 and other viral proteins.

One possible complication of these studies is that the Rauscher virus employed for protein purification might possibly contain endogenous BALB viruses as it was propagated in JLS-V9, a cell line derived from BALB/c mice. Fortunately, as the p15 protein is so strongly type specific, such a possibility can now be tested by use of cloned preparations of BALB virus as the source of competing protein in the radioimmunoassay analysis of the present purified Rauscher virus p15.

A question raised by the present experiments concerns the concentration of p15 in different types of murine virus. With each of the different assays, the ratio of p15 to total virus protein was lower for Moloney virus than for any of the other viruses employed. Even in an assay with ^{125}I-labeled Rauscher p15 antigen and anti-Moloney virus serum, where the determinants bound were selectively those common to the Moloney p15, a greater concentration of Moloney virus than of the others was required (data not shown). This could not be attributed to impurity of the virus preparation as the concentrations of gp69/71 and p30 were the same in all of the different preparations of murine viruses employed (19). The apparent explanation is that, although Moloney virus p15 has determinants closely similar to those of Rauscher p15, these determinants are present in low concentration. In contrast, AKR and Kirsten viruses have determinants that are only weakly cross-reactive with the Rauscher p15, but are present in higher concentrations. This may also explain why the antiserum prepared against Moloney virus had such low activity with the p15 protein as compared to the p30.

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