JOURNAL OF VIROLOGY, Jan. 1974, p. 171-180

Vol. 13, No. 1
Printed in U.S.A.

Structural Proteins of Mammalian Oncogenic RNA Viruses: Multiple Antigenic Determinants of the Major Internal Protein and Envelope Glycoprotein

METTE STRAND AND J. T. AUGUST

Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Received for publication 29 August 1973

The antigenic determinants of two purified protein constituents of mammalian C-type RNA viruses, the major structural protein of about 30,000 daltons, and the membrane glycopeptides of about 70,000 daltons were examined by competition radioimmunoassay. By the appropriate choice of antiserum and competing proteins, it was possible to distinguish type-specific, group-specific, and interspecies determinants. Both of the viral constituents were found to contain each of these three classes of antigens. The results suggested that the majority of the determinants of the major structural protein were group specific, 5% to 30% were interspecies, and a small fraction were type specific. In the case of the envelope glycopeptides, the chief determinants were type and group specific, and a small fraction were interspecies.

Three classes of antigenic determinants present in virions of C-type RNA viruses have been defined: type specific, group specific, and interspecies. Type-specific antigens, which distinguish different viruses derived from the same species of animal, have been characterized chiefly by studies of virus neutralization (11, 18, 23, 38). Some type-specific antigens believed to be components of the viral envelope glycoproteins have been recovered in partially purified glycoprotein fractions released from virions by treatment with detergent (7, 9, 46). Group-specific antigens have been identified as those that are common to different viruses of the same species (2-4, 6, 10, 12, 16, 20, 21, 26, 28, 30, 40, 41). It appears now that there can be more than one group of viruses in a given species of animal; the newly isolated RD-114 and CCC viruses, which appear to be endogenous C-type viruses of the cat, have been found to contain a group-specific antigen different from that of previously reported feline C-type viruses (26). Several viral components showing group-specific antigenicity have been detected by immunodiffusion analysis of degraded virions or by partial purification of the viral proteins (10, 12, 16, 20, 21, 26, 28, 30, 40, 41). Included among these is the major protein of the C-type viruses with a molecular weight of about 27,000 to 30,000 which has been extensively purified and characterized and is probably an internal element of the virion (19, 29, 31, 33, 34, 39). Interspecies antigens, first described by Geering et al. (14, 15), are defined as those that are common among C-type viruses of different animal species. At this time, three components of mammalian C-type viruses are known to contain such determinants: the major internal protein (17, 32, 35, 36, 40, 41), the viral RNA-dependent DNA polymerase (1, 42), and two glycopeptides which appear to be elements of the viral envelope (45; M. Strand and T. August, unpublished observations).

We now report additional studies of the antigenic properties of purified components of C-type particles: the major structural protein of Rauscher murine leukemia virus (MuLV) with a molecular weight of about 30,000 (p30), the analogous protein of Rickard feline leukemia virus (FeLV) with a molecular weight of about 27,000 (p27), and the membrane glycopeptides of Rauscher virus with apparent molecular weights of 69,000 and 71,000 (gp69-71). By use of these purified proteins as ^{125}I-labeled antigens in the competition radioimmunoassay, and with the appropriate choice of antiserum and competing protein, it has been possible to analyze the proteins for each of the three different classes of antigenic determinants. The major internal protein contained interspecies and group-specific antigens as previously reported by Gilden et al. (17) and Oroszlan et al. (35); moreover, it was found that this protein demonstrated additional antigens not shared by other murine viruses, which were thus classified as type specific. The envelope glycopeptides

were also found to contain multiple antigenic determinants, with strongly reactive type- and group-specific antigens, as well as the interspecies determinants previously reported (45).

MATERIALS AND METHODS

Purification of virus proteins. The major structural proteins of Rauscher MuLV (p30) (23), Rickard FeLV (p27), and the membrane glycopeptides of Rauscher MuLV (gp69-71) were purified by phosphocellulose column chromatography and Sephadex gel filtration, as previously described (45).

Antisera. Anti-Rauscher MuLV and anti-Rickard FeLV sera were obtained from rabbits injected with purified virus particles that had been degraded by incubation at 45 C for 1 min with 0.2% sodium dodecyl sulfate and mixed with an equal volume of Freund complete adjuvant. In some experiments, as indicated, the immunoglobulin was partially purified by Na_2SO_4 precipitation as described by Kekwick (24). Rabbit and goat antisera against the purified MuLV p30 and MuLV gp69-71 proteins were prepared by multiple injections of purified proteins in Freund complete adjuvant. Horse anti-rabbit immunoglobulin IgG serum was a generous gift from R. Porter, Oxford University. Goat anti-Moloney virus serum (IS-166) and pig anti-goat IgG serum (2S-533) were provided by R. Wilsnack, Huntingdon Research Center, Baltimore, Md.

Radioimmunoassay. Quantitative analysis of viral antigens was carried out by radioimmunoassay as described by Hunter (22). The reaction mixture contained the following: normal rabbit or goat serum (0.005 ml) (corresponding to the antiserum used); ^{125}I-labeled virus protein (0.01 ml) (1 to 5 ng of protein containing 10^4 to 10^5 counts per min per ng); and diluted rabbit or goat antiserum (0.01 ml), 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 3 h, after which 0.03 ml of horse anti-rabbit or pig anti-goat IgG serum (corresponding to the antiserum used) was added to precipitate the antigen-antibody complex. The mixture was incubated for 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 same reaction mixture was used for the competition radioimmunoassay. For this procedure, a limiting amount of antiserum sufficient to precipitate approximately 50% of the labeled antigen was added, and the competitive inhibition of binding of the labeled 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. Control studies have shown that antigen release from virus and cells was maximal under these conditions and that the Triton X-100 added to the reaction mixture had no effect on antigen precipitation (M. Strand and J. T. August, unpublished observations).

Viruses. Rauscher MuLV was kindly provided by S. Mayyasi and D. Larson of the John L. Smith Memorial for Cancer Research. The virus was propagated in a BALB/c mouse bone marrow culture (JLS-V9) continuously infected with Rauscher virus as described by Wright et al. (48), harvested and purified as described previously (46), and suspended in 50 mM sodium citrate. The Rickard strain of FeLV propagated in the F-422 suspended cell culture derived from the thymus gland of a leukemic cat (37) was obtained from Electro-Nucleonic Laboratories, Inc. This virus was also received as a gift from E. Scolnick, Meloy Laboratories, Springfield, Va., and J. Hoekstra, Rush-Presbyterian-St. Luke's Medical Center, Chicago. Moloney MuLV (104-6-5) and Gross MuLV (270-50-10) were obtained from Electro-Nucleonic Laboratories, Inc. Friend MuLV, "N-tropic", propagated in mouse embryo cell cultures as described by Stewart et al. (43), was kindly provided by M. Sveda of this institution. Gardner feline sarcoma virus, received from E. Scolnick, was propagated by Electro-Nucleonic Laboratories, Inc., in a cat embryo cell line (13). The Gardner-Arnstein strain of FeLV propagated in a human rhabdomyosarcoma cell line (27) and a rat-tropic Kirsten murine sarcoma virus (MuSV) propagated in a clone of productively infected, transformed normal rat kidney cells (25) were a generous gift of M. Nicolson and R. M. McAllister, Children's Hospital of Los Angeles.

RESULTS

Polyacrylamide gel electrophoresis of C-type viruses and purified viral proteins. Each of the purified viral proteins and murine viruses utilized in this study was examined by high-resolution, gradient polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The p30 and p27 components can be clearly distinguished as a component of the different viruses present in approximately equal concentration; these proteins were purified to apparent homogeneity from Rauscher MuLV and Rickard FeLV (Fig. 1). Two glycopeptides purified from Rauscher MuLV migrate with apparent molecular weights of 69,000 and 71,000 (MuLV gp69-71); these are not distinctively identified as components of the different viruses, since there are other polypeptides of approximately the same molecular weight and possibly contaminating bovine serum albumin as well.

Although not a subject of this study, it should be noted that there are several obvious differ-

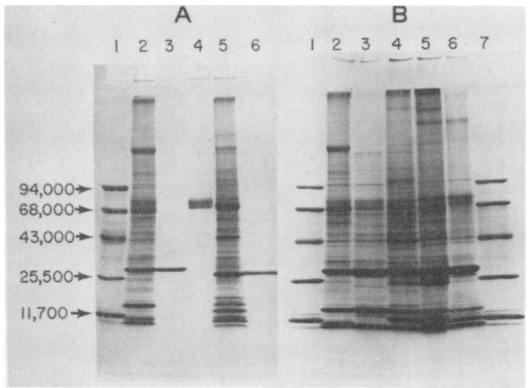

FIG. 1. *Polyacrylamide gel electrophoresis of C-type viruses and purified viral proteins. Electrophoresis in a high-resolution, 5 to 20% gradient polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate was carried out as described by Baum et al. (5). Gel A: (1) standard proteins: cytochrome c (3.3 μg; mol wt 11,700), chymotrypsinogen (3.3 μg; mol wt 25,500), ovalbumin (4.2 μg; mol wt 43,000), bovine serum albumin (2.5 μg; mol wt 68,000), phosphorylase A (6.6 μg; mol wt 94,000); (2) Rauscher MuLV, 60 μg of protein; (3) purified Rauscher MuLV p30, 3.4 μg of protein; (4) purified Rauscher MuLV gp69-71, 7.6 μg of protein; (5) Rickard FeLV, 55 μg of protein; (6) purified Rickard FeLV p27, 4 μg of protein. Gel B: (1) standard proteins as above; (2) Rauscher MuLV, 60 μg of protein; (3) Friend MuLV, 51 μg of protein; (4) Moloney MuLV, 63 μg of protein; (5) Gross MuLV, 70 μg of protein; (6) Kirsten MuSV, 60 μg of protein; (7) standard proteins as above.*

ences in the polypeptides associated with the different murine viruses. For example: (i) there appear to be slight differences in the molecular weights of the approximately 30,000-dalton major proteins of these viruses; (ii) Moloney and Gross viruses contain a dominant protein of about 23,000 daltons that is not evident in the other viruses; (iii) Moloney, Gross, and Kirsten viruses contain a polypeptide of about 12,000 daltons that is not evident in the Rauscher and Friend viruses; (iv) there are many differences in the minor polypeptides associated with the virions. Further investigation is required to determine whether these differences reflect specific variations in the virion protein composition, differences in proteins contributed by the host cell, or changes associated with virus growth or purification.

Interspecies, group-, and type-specific antigenic determinants of the MuLV p30 and FeLV p27 proteins. The antigenic determinants of the purified Rauscher MuLV p30 and Rickard FeLV p27 proteins were examined by radioimmunoassay. All of the acid-precipitable radioactivity of the ^{125}I-labeled proteins was precipitated by either anti-MuLV or anti-FeLV serum, thus indicating the presence of the cross-reactive interspecies antigens (Fig. 2). It also was found that each antiserum contained a greater concentration of antibodies directed against the homologous protein than against the heterologous protein. This could be explained if each of the purified proteins contained antigenic determinants specific to that protein in addition to the shared interspecies determinants. Evidence provided by immunodiffusion tests for the presence of more than one class of antigenic determinant on these proteins has previously been reported by Gilden et al. (17) and Oroszlan et al. (35).

Further characterization of the multiple antigenic determinants of these proteins was carried out by competition radioimmunoassay as described in Materials and Methods. Group- and type-specific determinants were measured by use of a homologous system of labeled antigen and antiserum, that is, murine virus protein and anti-MuLV serum or feline virus protein and anti-FeLV serum. With such a homologous

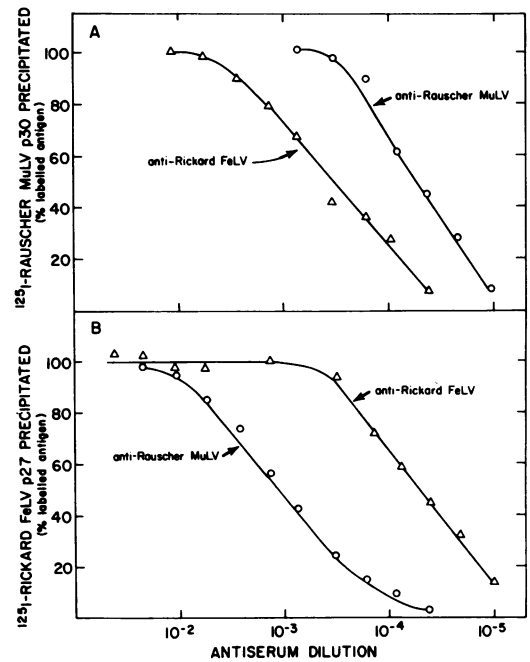

FIG. 2. *Radioimmunoassay of antiserum precipitation of Rauscher MuLV p30 and Rickard FeLV p27. The assay was performed as described under Materials and Methods. (A) 2.5 ng of ^{125}I-labeled MuLV p30 (12,400 counts per min per ng) and a final dilution of rabbit anti-FeLV or rabbit anti-MuLV serum as indicated. (B) 2.4 ng of ^{125}I-labeled Rickard FeLV p27 (50,000 counts per min per ng) and a final dilution of rabbit anti-FeLV or rabbit anti-MuLV serum as indicated.*

system, all of the reactive antigenic determinants of the protein for which there were antibodies would be bound, whether interspecies or group, or type specific. These different varieties of antigens were then measured by the extent to which proteins of different viruses competed with the labeled antigen. For example, if there was binding of group- or type-specific as well as the interspecies determinants of the MuLV protein, then the addition of competing FeLV would bind only the antibodies directed against the interspecies determinants and only partially block precipitation of MuLV antigen.

Such an experiment was carried out by using ^{125}I-labeled Rauscher MuLV p30 and anti-MuLV serum, comparing the effect of the purified FeLV p27 with the standard competition by unlabeled MuLV p30. Two parameters of the competition assay are shown by the data: (i) the amount of FeLV p27 required for 50% of maximal competition for the interspecies reactive antibodies was approximately 20-fold greater than the amount of MuLV p30 required for 50% competition of all antibodies, and (ii) the extent of the competition by the FeLV p27 was only 30% (Fig. 3A). This indicates that (i) the interspecies antigens common to MuLV p30 and FeLV p27 proteins comprise only a portion of the total determinants of the MuLV p30, and (ii) as expected in this case, the majority of the antibodies of the anti-MuLV serum were directed at antigens other than interspecies determinants. A trivial explanation of these results is that the reduced competition by the FeLV p27 interspecies antigens was due to degradation or impurity of the FeLV p27 protein. To exclude this possibility, the competing proteins were analyzed in a heterologous assay system of the same labeled MuLV antigen and anti-FeLV serum in place of the anti-MuLV serum. In this system, only the interspecies determinants of the murine antigen were bound. Both MuLV and FeLV competing proteins gave equal and complete competition, demonstrating that the preparations contained comparable amounts of the interspecies antigens (Fig. 3B).

In order to extend these findings and to compare the Rickard FeLV protein with the murine virus protein, the reciprocal experiments were also carried out, with ^{125}I-labeled FeLV p27 as antigen and the MuLV p30 as competitor (Fig. 4). The results were completely analogous to those of the previous experiment. Only a fraction of the anti-FeLV p27 antibodies were bound by the competing MuLV p30, and approximately threefold more murine virus than feline virus protein was required for 50% of the maximal observed competition. The heterologous assay, measuring the interspecies determinants, again showed that both proteins contained comparable amounts of the interspecies antigens. In this experiment, the difference in the slopes of the inhibition curves may be attributed to the properties of the anti-MuLV serum, and possibly to a greater affinity of the antibodies for the MuLV p30 than for the FeLV p27 (22).

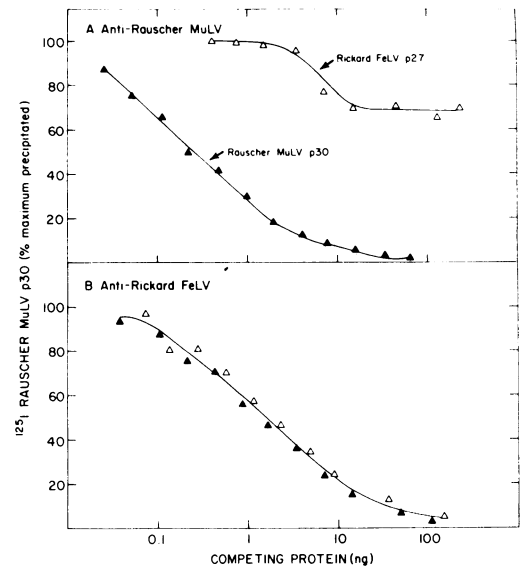

FIG. 3. *Analysis of the interspecies antigenic determinants of Rauscher MuLV p30 by competition radioimmunoassay. The assays were performed as described under Materials and Methods with 5 ng of ^{125}I-labeled MuLV p30 (20,000 counts per min per ng) and (A) rabbit anti-Rauscher MuLV serum, 1:2,400 final dilution, or (B) rabbit anti-Rickard FeLV serum, 1:2,400 final dilution. The competing proteins, purified Rauscher MuLV p30 and Rickard FeLV p27, were added as indicated.*

All of these experiments were repeated by adding whole virus degraded by Triton X-100 as competitor instead of the purified proteins. The results were the same except that the concentration of total competing viral protein required was 10- to 20-fold higher, appropriate to the estimated concentration of the p30 or p27 proteins in the viruses (data not shown).

These results suggest that, with the particular antisera used in these experiments, the proportion of interspecies antigens among all of the reactive determinants was 5% for the Rauscher MuLV p30 and 30% for the Rickard FeLV p27. These calculations assume that differences in the amounts of competing protein required for 50% maximal competition are proportional to differences in the concentrations of antigenic determinants rather than to differ-

ences in the affinities of antibodies for the proteins. Therefore, in the case of the murine protein, since the anti-MuLV serum may have a higher affinity for the MuLV p30 than for the FeLV p27, the 5% value is possibly an underestimate. Nevertheless, it is clear that the interspecies antigens do not constitute the total antigenic determinants of the murine p30 and feline p27 proteins bound in these experiments and that these proteins must therefore contain group- or type-specific determinants, or both, in addition to the shared interspecies antigens.

Further characterization of the antigens of these proteins was achieved by testing the competition of different types of murine viruses in the homologous assay system containing ^{125}I-labeled Rauscher MuLV p30 and anti-Rauscher MuLV serum. In this way, group- and type-specific antigens could be distinguished. Inhibition greater than that of feline virus would indicate group determinants common to the different types of murine viruses. Failure to compete for all the antibodies would indicate the existence of type-specific determinants. Friend, Moloney, and Gross viruses all showed strong competition, thus confirming the presence of group-specific determinants of MuLV

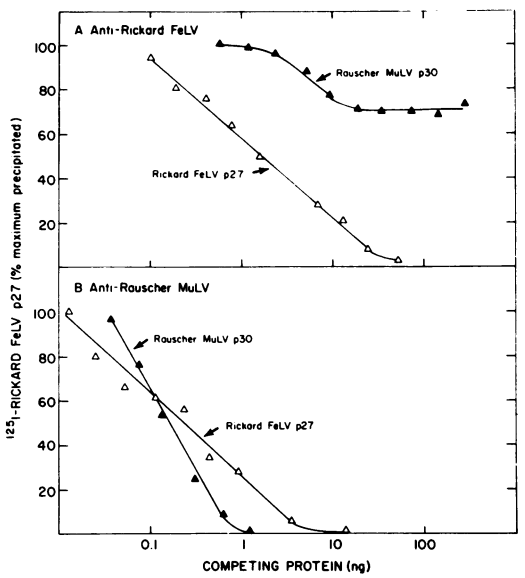

FIG. 4. *Analysis of the interspecies antigenic determinants of Rickard FeLV p27 by competition radioimmunoassay. The assays were performed as described under Materials and Methods with 2.5 ng of ^{125}I-labeled Rickard FeLV p27 (50,000 counts per min per ng) and (A) rabbit anti-Rickard FeLV serum, 1:3,000 final dilution, or (B) rabbit anti-Rauscher MuLV serum, 1:3,000 final dilution. The competing proteins, purified Rauscher MuLV p30 and Rickard FeLV p27, were added as indicated.*

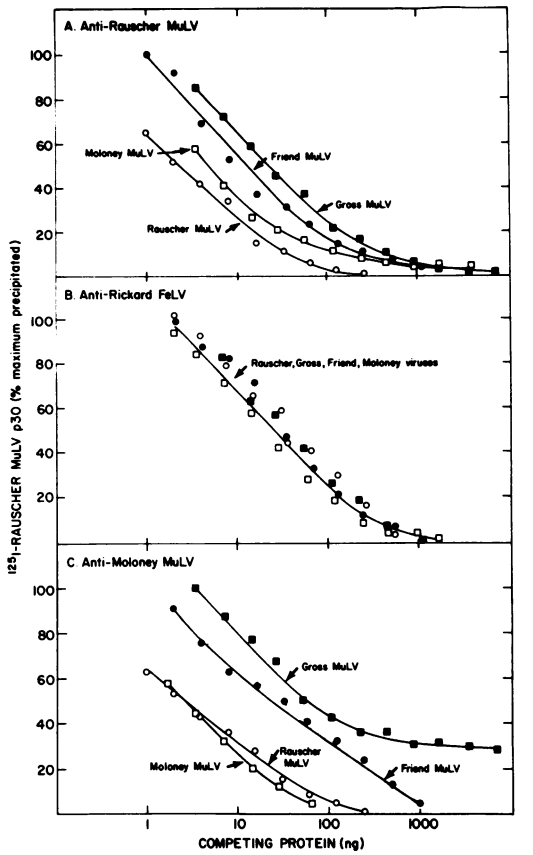

FIG. 5. *Analysis of species- and type-specific antigenic determinants of Rauscher MuLV p30 by competition radioimmunoassay. The assays were performed as described under Materials and Methods with 2.0 ng of ^{125}I-labeled Rauscher MuLV p30 (32,000 counts per min per ng) and (A) rabbit anti-Rauscher MuLV serum, 1:12,000 final dilution, (B) rabbit anti-Rickard FeLV serum, 1:2,400 final dilution, or (C) goat anti-Moloney MuLV serum, 1:96,000 final dilution. The competing purified Triton X-100-disrupted virus was added as indicated.*

p30 shared by different types of murine viruses (Fig. 5A). Since there was almost complete competition when a sufficiently high concentration of the competing virus protein was added, it appeared that the majority of the antibodies of the anti-MuLV serum were directed at these group-specific antigens. It also was evident, however, that among the different viruses the concentration of group determinants or the affinity of these determinants for antibodies of the anti-MuLV serum was not identical, since increased amounts of protein were required for competition in the order or Rauscher < Moloney < Friend < Gross. Assuming that the concentration of p30 interspecies determinants of these viruses was the same, the differences

were not attributable to the level of p30 protein in the viruses, since each was equally effective in the heterologous assay measuring the apparent concentration of the interspecies determinants of this protein (Fig. 5B). Moreover, analysis of these viruses by polyacrylamide gel electrophoresis also suggested that they contained a similar concentration of the p30 protein (Fig. 1).

Analogous results were obtained from an experiment making use of anti-Moloney virus serum and the ^{125}I-labeled Rauscher MuLV p30. In this case, since the assay measured determinants common to the two viruses, Moloney virus and Rauscher virus would be expected to give similar competition curves, as was observed (Fig. 5C). Here again, however, the determinants common to Moloney and Rauscher viruses were not shared equally by Friend and Gross viruses since greater concentrations of these latter viruses were required for competition. Moreover, even at high concentrations of Gross virus the inhibition was incomplete, indicating that the anti-Moloney virus serum contained antibodies which bound Rauscher virus determinants not shared by Gross virus. It thus appeared that there were distinctive differences among the group-specific determinants of the murine virus p30 proteins, either in the number of determinants per molecule of protein or in their affinity for anti-Rauscher MuLV p30 antibodies. In addition, there also appeared to be Rauscher p30 determinants which were not shared by all of the other murine viruses, which then would be classified as type-specific antigens.

The detection of type-specific differences was greatly augmented when the reaction was carried out with a monospecific antiserum obtained from goats immunized with the purified Rauscher MuLV p30 (Fig. 6). As compared with the standard competition curve obtained with Rauscher MuLV, a greater amount of competing protein of Gross, Friend, and Moloney viruses was required for 50% maximal competition. Even at high concentrations, the competing antigen failed to bind all of the anti-Rauscher p30 antibodies. The results show that there are Rauscher p30 determinants not present in other murine viruses. Moreover, the reduced slope of the inhibition curves of the test viruses suggests that the affinity of the antibodies for the group-specific determinants of the Gross, Friend, and Moloney p30 proteins was less than the affinity for the corresponding Rauscher p30 determinants.

Interspecies, group-, and type-specific antigenic determinants of the MuLV gp69-71 protein. Studies similar to those carried out with the p30 protein have also been performed

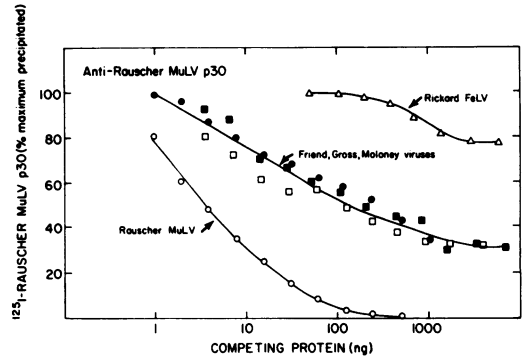

FIG. 6. *Analysis of species- and type-specific determinants of Rauscher MuLV p30 with monospecific anti-Rauscher MuLV p30 serum. The competition radioimmunoassay was performed as described under Materials and Methods with 2 ng of ^{125}I-labeled Rauscher MuLV p30 (91,000 counts per min per ng) and goat anti-Rauscher MuLV p30 serum, 1:48,000 final dilution. The competing Triton X-100-disrupted viruses were added as indicated: Friend MuLV, ●; Gross MuLV, ■; Moloney MuLV, □.*

with the Rauscher virus envelope glycopeptides gp69-71. The identification and characterization of the interspecies antigenic determinants of these components has been reported elsewhere (35). The results given here show the presence of group- and type-specific determinants as well.

In the experiments carried out with the homologous assay system, ^{125}I-labeled Rauscher MuLV gp69-71, and anti-Rauscher MuLV serum, the effects of several different feline viruses and Kirsten MuSV were compared with the standard competition given by Rauscher MuLV and purified Rauscher MuLV gp69-71 (Fig. 7). Compared with the purified gp69-71, competition by Triton X-100-treated Rauscher MuLV required 12-fold more protein, appropriate to the concentration of glycopeptides in the virion (35). By comparison, a 25-fold greater concentration of feline virus than of Rauscher MuLV was required to achieve 50% of maximal competition, and with an excess of competing protein less than half of the antibodies were competitively bound. The same results were obtained with four different preparations of feline viruses, including virus grown in the human RD cell. It thus appeared that a minor component of the antigens of the labeled gp69-71 antigen bound by the anti-MuLV antibodies was interspecies determinants present in feline virus.

Group- and type-specific determinants of the Rauscher gp69-71 were also revealed in this experiment by the use of Kirsten MuSV (Fig. 7A). The more effective competition by Kirsten

virus than by the feline viruses clearly indicated the presence of group-specific determinants. The same competition curve gives evidence for gp69-71 type-specific antigens as well; greater amounts of Kirsten virus than Rauscher virus protein were required for competition, and even high concentrations of Kirsten virus failed to compete for all of the antibodies binding the Rauscher protein.

As a control, all of the viruses used in this experiment were examined in the heterologous assay system for the concentration of the interspecies determinants of the gp69-71 proteins (Fig. 7B). By this criterion, there was no difference in the concentration of the protein in the different viruses.

The species and type-specific determinants of the gp69-71 protein were further analyzed by testing the competition of Friend, Moloney, and Gross viruses in an assay using a goat monospecific anti-Rauscher MuLV gp69-71 serum (Fig. 8A). Few of the antibodies of this serum reacted with the interspecies determinants, since there was only slight inhibition by FeLV. Friend,

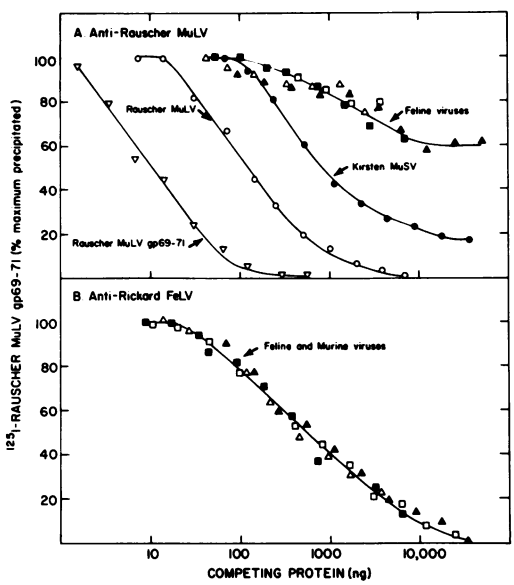

FIG. 7. *Analysis of the Rauscher MuLV gp69-71. The competition radioimmunoassay was performed as described under Materials and Methods with 5 ng of ^{125}I-labeled Rauscher MuLV gp69-71 (10,000 counts per min per ng) (A) Rabbit anti-Rauscher MuLV serum, 1:1,500 final dilution, and the competing purified protein or Triton X-100-disrupted viruses as indicated: Rickard FeLV, △: Rickard FeLV, ▲: Gardner-Arnstein FeLV, □; Gardner feline sarcoma virus, ■. (B) Rabbit anti-Rickard FeLV serum, 1:450 final dilution; the competing Triton X-100-disrupted viruses were added as indicated: Rauscher MuLV, □; Gardner-Arnstein FeLV, △; Kirsten MuSV, ▲; Rickard FeLV, ■.*

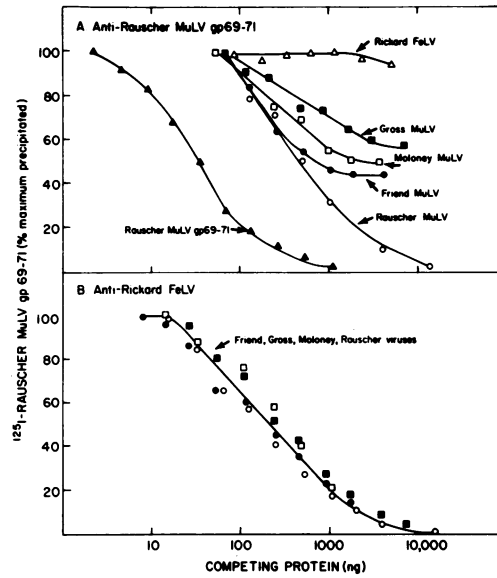

FIG. 8. *Analysis of the Rauscher MuLV gp69-71 with monospecific anti-Rauscher MuLV gp69-71 serum. The competition radioimmunoassay was performed as described under Materials and Methods with 1 ng of ^{125}I-labeled Rauscher MuLV gp69-71 (47,000 counts per min per ng) and (A) rabbit anti-Rauscher MuLV gp69-71 serum, 1:2,400 final dilution, or (B) rabbit anti-Rickard FeLV serum, 1:450 final dilution. The competing purified protein or Triton X-100-disrupted viruses were added as indicated.*

Moloney, and Gross viruses all showed a much stronger competition, clearly indicating the presence of group-specific determinants in the Rauscher protein. There also appeared to be significant differences in the concentrations and affinities of the competing group-specific antigens of these viruses. Since the extent of competition with the test viruses was only approximately 50% that of Rauscher virus, the remaining antibodies of this monospecific serum appeared to be directed solely against type-specific antigens. Again, the control experiment measuring the interspecies determinants of the gp69-71 protein indicated that the concentration of this protein in the different viruses was the same by this criterion (Fig. 8B).

DISCUSSION

The finding that both the p30 major internal protein and the gp69-71 glycopeptides of mammalian C-type RNA viruses contain each of the three types of antigenic determinants that have been formally classified (i.e., interspecies, group specific, and type specific) indicates that the antigenic properties of the viral proteins are more complex than previously had been recog-

nized. It may be predicted, however, that this is not an unusual property of the viral proteins and that others will be found to have such a spectrum of antigen determinants. Basically, any viral protein that is antigenic must express one or more of these determinants. Proteins of related viruses of a given species have common properties which may be reflected by group-specific determinants. Type-specific determinants could reflect variable regions in any protein, some of which, as with glycoproteins of the virus envelope, may be related to biologically significant differences among types of viruses. In addition, since all of the C-type viruses are structurally similar, interspecies determinants may exist in relation to a common functional role of some of the properties. For example, the RNA-dependent DNA polymerase has been found to contain both group-specific and interspecies determinants (36). The data also suggest that the three classes of antigens should not be thought of as defining unique structures. Rather, there appears to be a spectrum of determinants ranging in specificity from interspecies to group specific to type specific. Thus, even among closely related types of viruses, a given protein may differ in the concentration and affinity of the shared group or interspecies determinants as well as in the presence of type-specific antigens.

As other proteins of mammalian C-type viruses are isolated, the techniques described here provide a means to test these hypotheses. One of the advantages of the procedure is that it can be carried out with any purified protein. This is particularly useful for type-specific antigens which previously had been characterized chiefly by use of virus-neutralizing serum. Since the neutralization assay depends upon the biological properties of a protein, it is effective only with those proteins which induce such neutralizing antibodies, presumably the envelope glycoproteins. By use of the radioimmunoassay, the definition of type-specific determinants may now be broadened and not limited to those that induce neutralizing antibodies.

There is evidence that at least some of the determinants of the gp69-71 glycopeptides are viral envelope surface antigens. Studies in collaboration with R. Steeves of this institution (unpublished data) have shown that the monospecific anti-gp69-71 serum has strong virus neutralizing activity. Moreover, these glycopeptides are iodinated when intact virions are treated by the lactoperoxidase procedure (M. Strand and T. August, unpublished observations), results which appear analogous to those reported by Witte et al. (47). Taken with the fact that they are a major glycoprotein component of the virion (45; M. Strand and T. August, unpublished observations), it can be predicted that the properties of these glycopeptides will include many of those associated with the functions of the viral envelope.

The assembly of C-type RNA virus particles at the periphery of the host cell and the budding of the particle through the cellular outer membrane (8) suggest that some constituents of the viral envelope are of cellular origin. With respect to gp69-71, the antigens of these components were not found in uninfected cells by the radioimmunoassay under conditions in which 1% of that present in JLS-V9 cells productively infected with Rauscher virus would have been detected (45). Moreover, the complex assortment of virus-related antigenic determinants, as described in this report, is further evidence that synthesis of the antigens, if not the proteins, is in some manner directed by viral gene expression. This, however, is not proof of the viral genetic origin of gp69-71 as might be obtained by genetic studies or analysis of the transcription products of viral RNA. It is possible that some of the antigenic determinants are a property of the carbohydrate moiety of the molecule, the structure of which is controlled by protein(s) coded for or induced by the virus.

ACKNOWLEDGMENTS

We are indebted to Jack Gruber and the Office of Program Resources and Logistics, National Cancer Institute, through whose courtesy we were provided with the Rauscher MuLV and other of the C-type viruses used in this investigation. We also acknowledge the generosity of F. Deinhardt, J. Hoekstra, R. M. McAllister, M. D. Nicolson, E. Scolnick, and M. Sveda, who also provided purified samples of viruses, and to R. Wilsnack, for antiserum preparations.

This investigation was conducted under Public Health Service contract 71-2251 within the Special Virus Cancer Program of the National Cancer Institute, and was also supported in part by grant GM 11301-11 from the National Institute of General Medical Sciences.

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