Major Group-Specific Protein of Rat Type C Viruses

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The major internal protein of a rat type C virus pseudotype of murine sarcoma virus, MSV(RaLV), was purified by isoelectric focusing ($pI = 8.6$) and used to prepare antibody in guinea pigs. The protein was identified by its reaction with antisera reactive with the mammalian type C virus group-specific (gs) antigenic determinant, gs-3. The guinea pig antisera mainly contained species-specific (gs-1) antibody for reactions in gel diffusion with other type C viruses were limited to those of rat origin, whereas in complement fixation tests heterologous reactions could be eliminated by use of appropriate antiserum concentrations without affecting homologous reactions. Guinea pig antisera against mouse, hamster, or cat gs-1 determinants did not react with MSV(RaLV) purified gs protein or with any of several other rat type C viruses.

Tumors induced in both rats and hamsters by murine sarcoma viruses (MSV) have yielded sarcoma viruses of altered host range and antigenicity. Detailed studies of the viruses recovered from hamster tumors led to the hypothesis that these viruses were pseudotypes which were produced by rescue of the sarcoma genome from nonproducer tumor cells by an indigenous hamster type C virus (9, 11, 12). Evidence supporting this concept comes from recovery of non-sarcomagenic helper viruses from all of the hamster-specific sarcoma viruses investigated in detail (10) and the finding that the major virion structural polypeptide of these viruses can be distinguished by immunological and biophysical means from their structural homologue in murine type C viruses (16). A similar pattern of results has now been obtained with the virus recovered from a tumor induced in brown Norway rats by the Moloney strain (M) of MSV (20). This virus, originally termed M-MSV(O) (20), is more accurately termed M-MSV rat leukemia virus (RaLV), a term based on antigenic differentiation of envelope (20) and group-specific (gs) antigens (5) from mouse viruses and the recovery of a rat cell-specific helper virus from the sarcoma virus preparation (1).

Recent work has indicated that the well-characterized mammalian viruses (mouse, hamster, cat) contain at least two antigenic determinants associated with their major structural polypeptide (4, 6, 17). Following the original nomenclature of Old et al. (3, 7), gs-1 designates species-specific determinants and gs-3 designates interspecies cross-reactive determinants. This gs-3

designation differs from the polypeptide labeled p3 in the recent report of Nowinski et al. (13), which is clearly separable from the major virion polypeptide (p1 or gs-1). The previous finding that M-MSV(O) lacked mouse virus gs-1 determinants but did contain gs-3 (5) seemed sufficient to justify regarding the virus as representative of the rat; thus, RaLV for rat leukemia virus. The gs protein of this virus has now been purified and used to prepare anti-RaLV gs-1 antibody in guinea pigs.

MATERIALS AND METHODS

Viruses. The AKR strain of mouse leukemia virus (MuLV) was obtained from a rat lymphosarcoma cell line initiated from a transplant tumor induced by the AKR virus. The cell line has been maintained *in vitro* for several years and continuously sheds large amounts of virus.

The hamster-specific helper virus (HaLV) was obtained from chronically infected hamster embryo fibroblasts (9).

The feline lymphoma virus (FeLV) was obtained from suspension cultures of the FL-74 cell line derived from a lymphoma in the cat (19).

M-MSV(O), now M-MSV(RaLV), was obtained from the MSB-1 cell line (20) derived from a tumor induced by M-MSV in a female rat of the brown Norway strain.

Virus concentration. Large volumes of tissue culture fluid (20 to 25 liters) from the chronically infected cultures were clarified at $10,000 \times g$ by using a Sorvall RC2-B centrifuge modified for continuous-flow operation. The flow rate was 4 to 5 liters/hr. Clarified fluids were then centrifuged through 15 to 60% sucrose gradient by using a CF35 continuous-flow rotor in an L3-50 preparative ultracentrifuge (Spinco). Flow rates of

5.5 liters per hr at $122,000 \times g$ were employed. Virus obtained by this procedure was generally rebanded in a swinging-bucket rotor. Preparations were monitored by electron microscopy and serological tests to insure freedom from extraneous viruses and also that each virus maintained its distinctive reaction pattern in repetitive purifications.

Radioactive virus. Small volumes (~ 50 to 100 ml) of MSB-1 suspension cultures were incubated with ^3H -leucine ($10 \mu\text{Ci/ml}$, 38 Ci/mmol , New England Nuclear Corp.) for 24 to 48 hr. Clarified supernatant fluids were pooled with large-volume concentrates for the final purification step in sucrose gradients (16).

Virus disruption. Viral bands were extracted exhaustively with 0.2% Tween-80 and five volumes of diethyl ether at room temperature with continuous agitation. Extraction was considered complete when turbidity in the water phase monitored at 550 nm became minimal. This procedure was followed to prepare virus for isoelectric focusing or sucrose-gradient sedimentation.

Prior to analysis by polyacrylamide gel electrophoresis, virus was disrupted by sodium dodecyl sulfate (SDS), urea, and 2-mercaptoethanol, as previously described (16), with slight modifications (legend to Fig. 1).

Isoelectric focusing and polyacrylamide gel electrophoresis. The procedures described in detail for MuLV, HaLV, and FeLV (15–17) were followed without modification for M-MSV(RaLV).

Immunological analysis. Complement fixation and gel diffusion analyses were made as described previously (15). Antisera prepared in guinea pigs against the isolated gs protein of MuLV, HaLV, and FeLV were available from previous work. These sera, when used unconcentrated, detect only gs-1 determinants in gel diffusion assays and, at 4 units, react only with homologous viruses in complement fixation tests. Sera which detect the gs-3 determinants were prepared in Fisher rats by hyperimmunization with MSV-induced, MSV-shedding rat tumors (6) and also from a goat immunized with Tween-ether-disrupted FeLV. The latter antiserum was obtained from Roger Wilsnack (Huntingdon Research Laboratory, Baltimore, Md.). The reaction pattern of this serum with FeLV and several other mammalian type C viruses has recently been described (14).

After purification of MSV(RaLV) gs protein as described herein, antisera were prepared in two guinea pigs. The animals were inoculated four times at weekly intervals, each injection consisting of approximately $50 \mu\text{g}$ of protein from the peak fraction obtained by isoelectric focusing.

RESULTS

Isoelectric focusing. Initial separations of Tween 80-ether-disrupted MSV(RaLV) were monitored both by radioactivity (amino acid label) and by gel diffusion by using antiserum from rats immunized with MSV tumor homogenates. The latter serum detects the mammalian type C virus cross-reactive determinants (gs-3). In such separations, a major radioactive zone with a

peak at pH 8.6 coincided with the sole area of immunological reactivity (Table 1). By analogy with mouse, hamster, and cat type C viruses, this zone was considered to contain the major gs antigen of MSV(RaLV) and thus was used to prepare antisera in guinea pigs.

Polyacrylamide gel electrophoresis. Amino acid-labeled virus disrupted by SDS, urea, and mercaptoethanol revealed a pattern characteristic of other type C viruses when analyzed on 10% SDS-acrylamide gels. Several low-molecular-weight zones were seen, and the predominant feature was a protein with a molecular weight of approximately 27,000. The pH 8.6 fraction from isoelectric focusing migrated with a relative migration similar to that of the 27,000-molecular-weight component. As shown in Fig. 1, when gels

TABLE 1. Identification of rat type C virus group-specific (gs) antigen by isoelectric focusing^a

Fraction no.	pH	³ H amino acid counts/min	gs-3 Reactivity (gel diffusion)
30		660	—
31		1,000	—
32	7.10	1,120	—
33		1,180	—
34		1,240	—
35		820	—
36	7.58	920	—
37		1,680	—
38		1,480	—
39		1,160	—
40	8.18	700	—
41	8.35	820	—
42	8.48	1,600	+
43	8.64	4,420	+
44	8.80	2,480	+
45	8.96	800	—
46	9.10	800	—
47	9.26	700	—
48	9.44	320	—
49		410	—
50		480	—
51		240	—
52	10.02	180	—
53		60	—

^a ^3H -leucine-labeled virus was purified and disrupted as described in the text. Isoelectric focusing was made on a 110-ml column in 1% ampholine having a pH range of 3 to 10. The pH gradient was stabilized by a 0 to 40% sucrose gradient. Focusing was made at 5 C for 48 hr with a constant potential of 300 v. The pH was recorded at 5 C. Fifty-five 2-ml fractions were obtained, and only those in the 7 to 10 range are shown above. Serological activity for the common mammalian gs antigenic determinant was detected only in the three fractions shown, with maximal activity in fraction 43.

FIG. 1. Polyacrylamide gel electrophoresis of MSV(RaLV) and its major group-specific protein purified by isoelectric focusing. Prior to electrophoresis, virus and purified protein were treated with 1% sodium dodecyl sulfate (SDS), 6 M urea, and 0.1% 2-mercaptoethanol (2-ME) at 37 C for 2 hr. The samples were then dialyzed against 0.1 M phosphate buffer, pH 7.0, containing 0.1% SDS, 0.5 M urea and 0.1% 2-ME. Electrophoresis was for 5 hr at 8 ma per gel, and the gel composition was 10% acrylamide, 0.1% SDS, and 0.1 M sodium phosphate buffer, pH 7.0. Electrophoresis was from left to right (arrow). Gels were stained with Coomassie blue. Gel adjacent to arrow contains the pH 8.6 fraction.

were stained with Coomassie blue, the 8.6 fraction appeared homogeneous. Thus, this fraction is free of the multiple higher-molecular-weight proteins revealed by staining of unfractionated virus.

Guinea pig antisera. The pH 8.6 fraction from the above isoelectric focus separation was mixed with Freund complete adjuvant and administered subcutaneously at weekly intervals for a 1-month period. The serum, obtained 10 days after the last injection, was tested against various virus preparations in both complement fixation and gel diffusion assays. Both sera showed a high degree of species specificity when tested at 1.5 to 2.0 units (Table 2); however, at higher concentrations cross-reactions with heterologous gs antigens were observed. In gel diffusion assays carried out with the strongest complement-fixing reactive serum, single precipitin lines were obtained with purified, disrupted MSV(RaLV). Under similar

conditions, by using heated serum (56 C, 30 min), this serum did not react with MuLV, HaLV, or FeLV purified, gs antigens or disrupted viruses (Fig. 2).

Serological reactions of purified MSV(RaLV) gs antigen. The gs antigen purified by isoelectric focusing reacted in complement fixation (Table 3) and gel diffusion assays (4) with the anti-MSV(RaLV) gs sera and not with species-specific sera for mouse, hamster, and cat type C

TABLE 2. Serological analysis of guinea pig anti-RaLV group-specific serum^a

Test antigen	Guinea pig antiserum	
	No. 1 (1:60)	No. 2 (1:200)
MSV (RaLV)	128	256
AKR	< 2	< 2
HaLV	< 2	< 2
FeLV	< 2	< 2

^a Sera diluted to 1.5 units (serum no. 1) and two units (serum no. 2), based on homologous titrations, were tested with homologous and heterologous antigens of known titer (all at least 1:32). When compared with four-unit tests, the homologous reactions were unchanged and no heterologous cross-reactions were detected.

FIG. 2. Gel diffusion analysis of guinea pig anti-RaLV group-specific (gs) serum. Center well contains guinea pig antiserum; outer wells contain disrupted viruses, purified gs antigen, and buffer (B). As shown, a single precipitin line of identity was developed between RaLV and purified RaLV gs antigen.

viruses. Reactions of identity were obtained with the RaLV-specific sera and MSV I-7, a serum which detects MuLV gs-1 and interspecific gs-3 determinants (6). Similar reactions were obtained by using the goat anti-FeLV serum in place of MSV I-7. The gs-3 reactions were eliminated by absorption of the heterologous sera with purified MuLV gs antigen (Fig. 3). Similar inhibition tests with anti-MSV(RaLV) and MuLV gs antigen were without effect.

Assay of other rat type C viruses. Type C particles have been observed in tumors and cell cultures derived from a variety of rat strains, both inbred and random bred. To date we have tested

TABLE 3. *Distinction of MSV(RaLV) group-specific (gs) antigen from viral antigens of other species*

Test serum	Antigen prepn	
	MSV(RaLV) ^a	Purified MSV(RaLV) ^b gs antigen
Anti-MSV(RaLV) gs	256	64
Anti-MuLV gs	< 2	< 2
Anti-FeLV gs	< 2	< 2
Anti-HaLV gs	< 2	< 2

^a Virus disrupted by Tween-80-ether (300 μ g of protein/ml). Numbers are reciprocals of complement fixation titers. Guinea pig antisera was used at four units, based on homologous titrations.

^b 25 μ g protein/ml.

six separate isolates (Table 4), including the original reference virus in gel diffusion. These viruses all contain the gs-1 antigenicity characterized for MSV(RaLV). These viruses also have not reacted with sera specific for MuLV and FeLV major gs protein. Two of these virus preparations were from rat cells in which type C virus was activated by treatment with bromodeoxyuridine.

DISCUSSION

The lack of MuLV gs-1 determinants in the virus originally termed MSV(O) (20), led us to propose (5) that it represented a pseudotype sarcoma virus resulting from the *in vivo* rescue of the MSV genome by an indigenous, rat type C virus. The critical data presented here indicate that the major internal protein of this virus possesses specific determinants which are shared by a number of rat-derived type C viruses which originated in different laboratories and in diverse rat strains. This justifies renaming MSV(O) "MSV(RaLV)" to indicate that its structural antigens derive from a rat type C virus. The presumptive rat helper virus has been isolated by endpoint dilution methods from MSV(O) preparations (1). Beyond this, it is interesting to note that the major internal protein of MSV(RaLV) was identified on the basis of the mammalian interspecies determinant (gs-3) and yet, upon immunization, it induced mainly species-specific (gs-1) antibodies in guinea pigs. This pattern has

FIG. 3. *Species-specific and interspecific determinants on RaLV group-specific (gs) protein as revealed by absorption analysis. Species-specific antirat gs serum (RgsS) and goat anti-FeLV serum containing gs-3 antibodies (IS-8) were diffused against Tween-ether-disrupted rat-specific virus (RaLV). A single precipitin line of identity resulted (left); in contrast, when both serum wells were first filled with mouse gs antigen (Mgs) in order to absorb gs-3 antibodies (right), only the reaction developed with the rat-specific serum remained, which was quantitatively undiminished. Thus, the identity reaction (left) results from antibodies of two distinct specificities interacting with a single molecule carrying both determinants.*

TABLE 4. *Virus preparations showing rat-specific group-specific protein in gel diffusion assays*

Virus or cell line	Strain of origin	Reference
MSV(RaLV) R-35	Brown Norway Sprague Dawley	R. C. Ting (20) H. C. Chopra et al. (2)
Rat thymus XC-BUdR ^a	Osborne-Mendel Wistar-Random	Y. Teitz et al. (18) V. Klement et al. (in press)
Ki-MSV(NP)- BUdR ^b	Osborne-Mendel	V. Klement et al. (in press)
Wu-Fu	Wistar-Furth	P. S. Sarma et al. (manuscript in preparation)

^a Rous sarcoma virus-transformed rat cells (ordinarily virus free) after activation by bromodeoxyuridine (BUdR).

^b Rat cells transformed by the Kirsten strain of murine sarcoma virus (ordinarily virus free) after activation by BUdR.

repeated itself with other mammalian type C viruses, suggesting that the gs-1 determinants are the stronger immunogens and may perhaps inhibit or delay the response to gs-3 determinants. The gel diffusion-absorption experiments support our findings with other gs proteins (17), namely, that single lines of identity can result from the interaction of antibodies with two distinct specificities placed in adjacent wells with an antigen carrying both specificities.

The availability of antibody specific for the gs-1 determinant of rat type C viruses should allow the development of tissue culture isolation procedures based on antigen induction, as have proven useful for other species. It will not be surprising to find a widespread distribution of the virus or viral genome, as has been found for chickens, mice, cats, and hamsters (8).

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