Analysis of Proteins of Mouse Sarcoma Pseudotype Viruses: Type-Specific Radioimmunoassays for Ecotropic Virus p30's

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Received for publication 24 October 1979

Murine sarcoma virus pseudotypes were prepared by infection of nonproducer cells (A1-2), which were transformed by the Gazdar strain of mouse sarcoma virus, with Gross (N-tropic), WN1802B (B-tropic), or Moloney (NB-tropic) viruses. The respective host range pseudotype sarcoma viruses were defined by the titration characteristics on cells with the appropriate *Fv-1* genotype. Proteins from virus progeny were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Bands present in both the 65,000- and the 10,000- to 20,000-molecular-weight regions of the gel distinguished the pseudotype viruses from their respective helpers. Furthermore, two protein bands were noted in the p30 region of murine sarcoma virus (Gross), one corresponding to Gross virus p30, and another of slightly slower mobility. However, since the mobility of the putative sarcoma p30 is nearly identical to that of WN1802B, its presence could not be established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Type-specific radioimmunoassays for Gross virus p30 and for WN1802B p30 were applied for analysis of pseudotype preparations, and among several ecotropic viruses tested, only the homologous virus scored in the respective assay. By use of these assays, pseudotype viruses were found to contain only 8 to 48% helper-specific p30's; the remainder is presumably derived from the sarcoma virus.

Cells transformed by defective mammalian sarcoma viruses have varied contents of viral protein and viral RNA expression (11). Although all have a rescuable transformation gene, only some contain information for certain *gag* gene proteins (presumably from the "original helper" or progenitor virus), whereas others do not (18). The presence of the viral *gag* gene is necessary but not sufficient for expression of these proteins in nonproducer cells (14); murine sarcoma virus (MuSV) pseudotype viruses, produced by rescue from nonproducer cells, contain *gag* proteins of sarcoma origin only if the proteins themselves were expressed in the nonproducer cells (11). Passage of the virus pseudotypes to fresh cells generates nonproducer cells with the same capacity to express *gag* proteins as the original nonproducer cell line (1). Hamster cells transformed by the Gazdar strain of mouse sarcoma virus release noninfectious particles containing mouse gs-1 antigen (p30). Rescue from such cells with feline leukemia virus results in pseudotype virus with feline leukemia virus gp70 but with mouse and feline gs-1 antigens, which indicates that the sarcoma virus genome carries at least a portion of the *gag* gene (4, 15). A type-specific radioimmune assay has been developed for two ecotropic mouse retrovirus p30 proteins. The Gross virus strain (N-tropic), derived from AKR

mice, and WN1802B (B-tropic) virus, derived from BALB/c mice, were used to rescue the Gazdar strain of mouse sarcoma virus from A1-2 cells which had been nonproductively transformed. We report here that these *Fv-1* host-range pseudotypes of the Gazdar strain of mouse sarcoma virus contain more sarcoma-specific p30 than helper virus p30, although infectious helper virus is in excess by biological assays.

MATERIALS AND METHODS

Viruses and assays. Gross, WN1802B, and Moloney viruses were originally obtained from W. P. Rowe and Janet Hartley (Bethesda, Md.). Pseudotype viruses were prepared from nonproducer cells which were transformed with Gazdar sarcoma virus (4, 15). Propagation of viruses, XC syncytial plaque assay, and focus-forming assays were done as previously described (9). The sources of other viruses are identified in the text.

Protein analysis. Viruses for protein analysis were grown on SC-1 cells (9), collected at 24-h intervals, and purified by sucrose gradient centrifugation as described elsewhere (Tennant et al., manuscript in preparation). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done on 4 to 17% acrylamide linear gradient slab gels (8). Pelleted virus extracted with trichloroacetic acid-ether was dissolved in 1% SDS, 8 M urea, and 1% 2-mercaptoethanol before it was applied to the gel.

Radioimmune assays. Phosphocellulose chromatography and gel filtration on Ultragel AcA 44 (LKB) were used for purification of p30's from Gross and WN1802B viruses solubilized in Triton X-100 (17). These preparations were >90% pure as judged by stained SDS-PAGE profiles and interspecies radioimmunoassay. Rabbit antisera to Gross and WN1802B viruses were produced by multiple subcutaneous and intradermal injections of Tween-disrupted viruses in Freund adjuvant. Virus proteins disrupted by Nonidet P-40 were coupled to Sepharose 4B at 1 mg of protein per ml of beads for antiserum adsorption (8). Serum was absorbed four times by batchwise incubation of 1 ml of serum with 0.5 ml of virus protein beads overnight at 4°C on a tumbling apparatus. The final sera, diluted 10-fold by the absorption process, were clarified by centrifugation at $100,000 \times g$ for 1 h and stored at -20°C.

Antisera used for type-specific radioimmunoassays were 10 μ l of a final dilution of 1:120 of rabbit antiserum to Gross virus absorbed with WN1802B proteins (Gross assay) or 1:210 dilution of rabbit antiserum to WN1802B virus absorbed with Gross virus proteins (WN1802B assay). Interspecies assays of p30's were done with p30 purified from Scripps virus and goat antiserum to feline leukemia virus (OS 268) provided by R. Wilsnack through the Office of Resources and Logistics, National Cancer Institute. Target proteins were radioiodinated with chloramine-T to specific activities of 10 to 20 μ Ci/ μ g and antigen-binding capacities, and radioimmunoassays were performed as previously described (8, 16).

RESULTS

Type-specific p30 radioimmunoassays.

Rabbit antisera to Gross or WN1802B virus were absorbed with viral protein of the opposite type to remove cross-reactive antibody populations. Although the sera show some preference in antigen-binding capacity for the immunizing protein before absorption (Table 1), the sera, as finally absorbed, are specific for the p30 of the immunizing virus in that no binding capacity for the p30 of the absorbing virus could be detected. Homologous radioimmune assays for Gross and WN1802B virus p30's were established by use of these absorbed sera (Fig. 1). Competition of Gross virus p30 in the WN1802B assay was 200-fold less than that for the homologous p30 (Fig.

FIG. 1. Radioimmunoassays of ecotropic virus p30's. (A) Goat antiserum to feline leukemia virus reacting with 125 I-labeled p30 from Moloney leukemia virus. Competing virus proteins were Moloney ($\circ$), WN1802B ($\bullet$), and Gross (Δ). (B) Absorbed rabbit antiserum to WN1802B virus reacting with 125 I-labeled p30 from WN1802B virus. (C) Absorbed rabbit antiserum to Gross virus reacting with 125 I-labeled p30 from Gross virus. (B and C) Competing pure p30's from WN1802B ($\bullet$) or Gross (Δ).

1B), even though these proteins scored equally well in the interspecies assay (Fig. 1A). Competition of WN1802B p30 in the Gross assay was 1,000-fold less than that for Gross p30 (Fig. 1C).

Since p30's have been implicated as targets of *Fu-1* restriction (3, 6), we surveyed several ecotropic viruses of different host ranges to see whether the type-specific determinant(s) measured by these assays were related to virus tropism (i.e., do all N-tropic viruses score in the Gross assay and do all B-tropic viruses score in the WN1802B assay?). The results in Table 2 show that this is not the case. All viruses scoring in the WN1802B assay do so nearly quantitatively. Differences in quantitation between p30 detected by the interspecies and WN1802B assays were small ($\pm 20\%$) unless assays were performed with labeled p30 preparations that had been stored for long times. Virus preparations

TABLE 1. p30 antigen-binding capacities of rabbit antiserum

Antiserum to	Absorbed with	Binding capacity (μ g/ml)	
		Gross p30	WN1802B p30
WN1802B		50	80
WN1802B	Gross	<0.1	10.5
Gross		72	27
Gross	WN1802B	6.1	<0.1

TABLE 2. *p30 competition radioimmunoassays of ecotropic virus isolates*

Virus ^a	Apparent p30 (ng/ml) ^b			<i>Fv-1</i> tropism
	Interspecies	Gross	WN1802B	
Gross	1,213	1,568	18	N
WN1802B	709	<6	768	B
Moloney	177	8	10	NB
TOR-N	1,488	32	15	N
TOR-B	498	10	10	B
Scripps	62	<6	17	NB
BETH-N no. 1	1,644	9	15	N
BETH-B no. 1	1,184	6	1,088	B
Rauscher	245	21	<8	NB
BTL	365	<6	16	B
ETC18	1,122	<6	1,184	B
M138	2,112	<3	1,600	B
WN1802N	108	<3	2	N
MON-B	1,920	<3	1,184	B
MON-N	1,088	<3	2	N
CCL64-Xeno	370	<3	<1	
AKR	1,440	54	23	N
BETH-N no. 2	1,184	9	5	N
BETH-B no. 2	1,120	<5	1,760	B

^a Viruses tested as infected SC-1 cell supernates. Values in interspecies assay correlate roughly with virus titers.

^b Values expressed as nanograms per milliliter relative to standard purified p30's of the appropriate type. Interspecies assay detects total p30, whereas Gross and WN1802B assays detect only p30's carrying type-specific determinants.

whose p30 could be detected by the WN1802B assay were subsequently identified as progeny of WN1802B stocks. One exception, M138, was isolated from a lymphoma of aged BALB/c mice (5). It is possible that this virus isolate is identical to WN1802B, because it contains a p30 which is immunologically indistinguishable from that of WN1802B and is of similar origin. Virus ETC18, which is cloned from WN1802B virus (12), has a p30 that migrates faster in SDS-PAGE than does the p30 from its parent stock. Since it competes completely and quantitatively in the WN1802B assay, the type-specific determinant(s) measured by this assay are not related to the large size of stock WN1802B p30. The only virus that scored significantly in the Gross assay was Gross virus. Even the closely related AKR virus showed little cross-reactivity. Thus, both assays measure determinants that are unique to the individual virus and not related to *Fv-1* tropism.

Pseudotype virus characterization. MuSV pseudotype viruses from A1-2 were prop-

agated and titrated for focus formation or XC plaque formation on SC-1 cells (Table 3). Each of the pseudotype preparations has a higher XC titer than focus titer. Proteins from viruses purified from infected SC-1 cell supernatants were compared on SDS-PAGE. Figure 2 shows that protein profiles of pseudotype viruses MuSV (Gross), slot 3; MuSV (Moloney), slot 6; and MuSV (WN1802B), slot 8 differ from those of their respective helper viruses Gross, slots 2 and 7; Moloney, slot 5; and WN1802B, slots 4 and 9. Differences are apparent in the 60,000- to 70,000-molecular-weight region of the gel, where pronounced bands are present in the profiles of pseudotype preparations [especially MuSV (Gross), slot 3] but absent from those of the helper viruses. Furthermore, protein banding patterns of the MuSV pseudotypes differ from those of their helper viruses in the low-molecular-weight region of the gel (10,000 to 20,000), again indicating that sarcoma genome-coded proteins are present. Finally, a double band of p30 is noted in MuSV (Gross), slot 3. The faster-migrating band appears identical to Gross virus p30 and represents 10 to 30% of the total stain intensity, whereas the slower band has no counterpart in the helper preparation (slot 2) and is presumably derived from sarcoma. No p30 heterogeneity can be seen in the protein profiles of the other two MuSV pseudotypes, since the helper virus p30's have mobilities very similar to that of the sarcoma virus. To establish that MuSV (WN1802B) contains two p30's and to quantitate the relative amounts of each, we performed interspecies and type-specific assays on the same virus preparation. Table 4 shows that helper p30 comprises 8 to 18% of the total p30 in MuSV (Gross), which is in good agreement with estimates from SDS-PAGE analysis. Helper p30 represents 17 to 48% of MuSV (WN1802B). Assays on the first experiment represent low levels of p30, and the two subsequent experiments indicate that helper virus p30 contents are 17 to 18% for MuSV (Gross) and 38 to 48% for MuSV (WN1802B). Since the helper virus p30's give nearly the same or slightly higher values in the interspecies as in the type-specific assay (Table 2), the differences in values obtained in the two assays for the pseudotype p30 contents are reasonable estimates of the amount of sarcoma virus p30 present.

DISCUSSION

The mechanism of helper virus rescue of the sarcoma function is largely unknown. Since some sarcoma preparations have no associated *gag* gene (18), it is clear that *src* genes are not obligatorily linked to structural protein genes for

TABLE 3. *Biological assays of pseudotypes on SC-1 cells^a*

Virus prepn	Assay	Focus-forming units or XC plaques at log ₁₀ dilution														Titer
		1.5	1.8	2.1	2.4	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7
MuSV (Gross)	Focus forming	23	10	6	2	1	0									3.0
	XC plaques			38	19	12	6	3	1	0						4.0
MuSV (WN1802B)	Focus forming				TN ^b	TN	TN	75	28	12	6	3	1			5.31
	XC plaques							146	86	37	18	7	3	2	1	5.7
MuSV (Moloney)	Focus forming							57	23	14	8	4				5.3
	XC plaques				TN						TN	60	29	10	5	6.4

^a All work done at 10⁶ cells/well at time of infection with 1 ml of stock virus grown on SC-1 cells. Counts are average of two wells in a 4-day assay.

^b Too numerous to count.

FIG. 2. Acrylamide gradient (4 to 17%) SDS-PAGE analysis of viral proteins stained with Coomassie brilliant blue. Marker proteins slots 1 and 10: phosphorylase A, 96,000; bovine serum albumin, 68,000; ovalbumin, 45,000; myoglobin, 17,000; and horse heart cytochrome c, 12,000. Gross helper virus, slots 2 and 7; WN1802B helper virus, slots 4 and 9; Moloney helper virus, slot 5; MuSV-Gross, slot 3; MuSV-Moloney leukemia virus, slot 6; and MuSV-WN1802B, slot 8.

activity. Helper virus genes must be able to supply the protein functions necessary for sarcoma gene transmission. However, since the kinds and amounts of *gag* gene information present in a particular sarcoma are passed to progeny viruses, it follows that the *src* gene must remain linked to "original" helper virus genes and recombination with rescuing helper virus is not necessary for transmission.

Protein profiles of pseudotype preparations studied here are significantly different in the 65,000- as well as 10,000- to 20,000-molecular-weight regions of gels. The MuSV (Gross) preparation also has two distinct bands in the p30 region of the gel, one of which has been identified with Gross virus p30, and the other of which is presumably of sarcoma virus origin.

Correlation of interspecies and type-specific radioimmune assays for p30's of the pseudotype

preparations indicate that less than 50% of the p30 present is the helper virus type. The majority of the p30 reactivity must be sarcoma virus specified. This reactivity may be present as p30 determinants on a *gag* precursor molecule as well as on sarcoma p30 molecules. It is clear from SDS-PAGE, however, that at least some sarcoma p30 is present in the MuSV (Gross) preparation.

We cannot determine which p30 helper or sarcoma is associated with *src* gene virions, although much indirect evidence indicates that virus proteins are packaged in a mixed fashion during maturation (10, 13). It is possible that association of viral RNA with sarcoma p30 generates a defective virus and that the only active virus seen is that small fraction which is randomly associated with a majority of helper p30. The large amount of defective particles in all C-

TABLE 4. Quantitation of helper virus p30 in pseudotype virus preparations

Pseudotype ^a	Apparent p30 (ng/ml) ^b			% Helper p30
	Interspecies	Gross	WN1802B	
Gross				
no. 1	57	4.4	<1	8
no. 2	1120	194	7	17
no. 3	549	97	<5	18
WN1802B				
no. 1	57	<2	9.9	17
no. 2	1280	16	613	18
no. 3	552	5	211	38
Moloney				
no. 1	79	<2	<1	NA ^c
no. 2	1440	23	4	NA
no. 3	1472	10	<5	NA

^a Tested as dilutions of purified virus stocks.^b See footnote b, Table 2.^c Not applicable since no type-specific assay for Moloney virus p30 is available, and, thus, helper virus p30 cannot be quantitated.

type virus preparations could easily accommodate this model. Since there is as yet no known function for any of the *gag* gene products, independent tests of activity of the helper-versus-sarcoma virus p30's are not possible.

It is interesting to note that although sarcoma p30 is present in larger amounts than helper p30 in both MuSV (Gross) and MuSV (WN1802B) preparations, the plaque titer of each is greater than the focus-forming titer. If p30 *src* determined focus-forming properties and p30 helper determined XC plaque titer, one should find a correlation of these titers with protein content of the pseudotype preparations. However, if ratios of p30 *src*/p30 helper are compared with focus-forming titer/XC titer for both pseudotypes, no quantitative correlation is apparent. Thus, p30 probably does not determine XC plaque or focus formation properties. The mechanism by which pseudotypes can acquire *Fv-1* determinants is not known but involves a determinant other than gp70 since this virion component does not determine *Fv-1* host range (7). Although p30 has been implicated as an *Fv-1* determinant, confirmation cannot be made from our studies.

Although type-specific determinants are known to exist on p30's (2, 12), the extreme specificity of these assays was not expected. The Gross assay does not detect AKR p30, although Gross virus was presumably derived from the AKR mouse. The WN1802B assay is completely negative for WN1802N virus isolated also from BALB/c mice; furthermore, several other B-

tropic viruses do not score in the assay. This in itself does not preclude p30 as the *Fv-1* gene target, since a particular rabbit antiserum may not recognize putative *Fv-1*-specific sites on the molecule. Furthermore, since WN1802B virus p30 is somewhat larger than p30's from other viruses, it is possible that type-specific determinants reside in this area of the protein. However, the fact that ETC18 virus, cloned from WN1802B (12) but with a smaller p30 (Tennant et al., manuscript in preparation), scores in this assay indicates that type specificity of *Fv-1* host range is not necessarily associated with the larger-sized p30.

If xenotropic viruses have xenotropic coats with B-virus *gag* proteins, as suggested by Gautsch et al. (3), they should contain B-virus p30. Two xenotropic viruses of BALB/c origin did not compete in the WN1802B assay, and if the theory is correct, these viruses must have obtained their p30 from a different B-tropic BALB/c virus.

M138 virus from a BALB/c lymphoma has a p30 that scores in the WN1802B assay, and since WN1802B virus is an endogenous virus of BALB/c mice, this indicates that genetic information coding for this protein is activated from the endogenous state. The synthesis of this protein, monitored with the type-specific radioimmunoassay throughout the BALB/c life-span, may correlate with leukemogenesis.

Finally, type-specific assays should be useful in identifying recombinant viruses. Virus progeny from a mixed infection can easily be screened for tropism and p30 type. These data, correlated with type-specific assays for other *gag* gene products, should help pinpoint the site of intragenic recombination. Experiments of this nature to determine the relationship of p30 to *Fv-1* restriction are in progress.

ACKNOWLEDGMENTS

We thank Linda Foote, Frank Tsakeres, and Fred Myer for excellent technical assistance, and Norma Kwaak for manuscript preparations. We appreciate helpful criticisms of W. Yang and A. Brown.

This research was sponsored jointly by Public Health Service contract 1 CP-6-0500 within the Virus Cancer Program of the National Cancer Institute under Interagency Agreement 40-365-72 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

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