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Isolation and Comparison of Murine Leukemia Virus-Related Glycoproteins from AKR and New Zealand Mice

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The major glycoprotein (gp70) of murine leukemia virus occurs free of virus in the serum and body fluids of certain strains of mice. These glycoproteins were isolated from New Zealand Black mouse (NZB) ascites fluid and from AKR and New Zealand White mouse (NZW) serum by immunoaffinity chromatography and were compared by immunological tests and peptide mapping. Glycoproteins gp70-NZB and gp70-NZW were indistinguishable by all criteria tested and were more closely related to gp70 from Moloney leukemia virus than was gp70-AKR.

The major glycoprotein of murine leukemia virus (gp70-MuLV) is present on the surfaces of mature virions (6, 11, 26, 27). Since MuLV'S are budding viruses, gp70 is also present on the surface of cells that produce virus (9). Recently, gp70 has been found on the surface of thymocytes (5, 12, 18, 25) and in the genital tract (2, 14) of certain strains of unmanipulated mice. Competition radioimmunoassay has shown that material cross-reactive with gp70 is also present in spleens (23, 28) and in serum (4) of mice that are not viremic.

The expression of gp70 in normal mice complicates its role as an immunological target on leukemia cells; however, small differences in antigenic determinants residing on these molecules (25) may be important as targets for immunotherapy, just as anti-idiotype antibody has been used in immunotherapy of myelomas (1). These studies were undertaken to show that serum protein cross-reactive with gp70 was indeed a 70,000-molecular-weight glycoprotein, but that there are significant antigenic and structural differences between gp70 from serum of unmanipulated mice and gp70 from exogenous Moloney leukemia virus (MLV).

To identify the relationship among gp70 molecules found in serum and exogenous virus (MLV), purified molecules from these sources were compared by direct antigen-binding curves and peptide mapping. The comparison showed gp70's from New Zealand Black mouse (NZB) ascites fluid (gp70-NZB) and from New Zealand White mouse (NZW) serum (gp70-NZW) to be indistinguishable from each other, whereas gp70 from AKR serum (gp70-AKR) is different. The gp70-NZB and gp70-NZW are more closely related to gp70 from MLV than is gp70-AKR, but endogenous proteins from all strains tested are unique and distinct from the gp70 of laboratory virus, MLV.

MATERIALS AND METHODS

Source of gp70's. Three-month-old AKR mice (Jackson Laboratory, Bar Harbor, Me.) and 6-month-old NZW mice (Scripps Clinic and Research Foundation Colony, La Jolla, Calif.) were exsanguinated under ether anesthesia, and serum was separated from pooled blood samples. Interspecies competition radioimmunoassay performed in the laboratory of F. J. Dixon (Scripps Clinic) (2, 22) showed that AKR serum contained 7 to 9 μg of gp70 and <0.5 μg of p30 per ml, whereas NZW serum contained 50 μg of gp70 and <0.5 μg of p30 per ml.

gp70 was purified from 20 ml of NZW serum and from 40 ml of AKR serum by immunoaffinity chromatography as previously described (10). Briefly, 2.5 mg of enriched antibody to gp70-MuLV was coupled to 0.5 ml of cross-linked Sepharose 4B, and the resultant conjugate was used as a specific immunosorbent for gp70. KSCN (3 M) was used to elute bound gp70 from the antibody resin, and eluates were dialyzed against 0.1 M Tris-hydrochloride (pH 7.6) and 1 mM EDTA (Tris-EDTA) before concentration by vacuum dialysis and storage at -20°C in Tris-EDTA containing 10 μg of phenylmethylsulfonyl fluoride and 50 μg of Polybrene per ml (10).

gp70-NZB was purified by immunoaffinity chromatography of adjuvant-induced ascites fluid (10). gp70-MLV was purified from MLV by KBr extraction and phosphocellulose chromatography (10, 21). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done on 10% acrylamide slab gels (13) that were stained for carbohydrate (24) and, subsequently, for protein.

Peptide mapping. The labeling and mapping methods are discussed in detail elsewhere (10). Briefly, 10 μg of protein (15) from each gp70 preparation was radioiodinated to high specific activities with 1 mCi of ^{125}I (New England Nuclear Corp., Boston, Mass.) and 5 μg of chloramine-T (16). La-

beled proteins, further purified by tube gels, were digested with trypsin, and the digests were subjected to two-dimensional peptide mapping (3, 17).

Antiserum binding of gp70. (i) **Antisera.** Goat antiserum to gp70 from Scripps leukemia virus (G145) was made as described (10). To accentuate differences in antigen binding of the various gp70 preparations, absorbed antisera were prepared as follows: 100 μl of G145 serum was treated with 4 ml of (i) normal rabbit serum, (ii) NZB ascites fluid, (iii) NZW serum, or (iv) AKR serum plus 50 μg of purified gp70-AKR; samples were incubated for 16 h at 4°C, and then cleared by centrifugation at 100,000 × g for 2 h and stored at −20°C until needed.

Rabbit antiserum to gp70 from AKR virus (rabbit anti-AKR gp70) was a gift from J. Ihle, Frederick Cancer Research Center, Frederick, Md. Goat antiserum to rabbit immunoglobulin G (IgG) and rabbit antiserum to goat IgG were prepared as described (10).

(ii) **Antigens.** Labeled gp70 preparations were freed of contaminating mouse immunoglobulin by absorption of the immunoglobulin with immobilized antibody. Five micrograms of radioiodinated gp70, in 0.5 ml of Tris-EDTA containing 5 mg of bovine serum albumin per ml was mixed at 4°C for 4 h with 0.5 ml of Sepharose 4B that had been coupled with 5 mg of IgG from goat antiserum to mouse IgG. Labeled gp70, free of mouse IgG, was separated from beads by filtration through glass wool and stored at −20°C until used. No preparation was stored longer than 5 days.

(iii) **Antigen binding.** Details of the antigen-binding test are published elsewhere (10). Briefly, 5 μl of serial twofold dilutions of antisera was incubated overnight with 5 ng of radioiodinated gp70 preparation in a final volume of 100 μl. Immune complexes were precipitated by a second or "sandwich" antibody technique. The amount of background antigen precipitation (usually 1 to 2%), determined by use of normal serum instead of primary antibody, was subtracted from each experimental sample, and maximum precipitation of ^{125}I from each gp70 preparation was normalized to 100% (4, 10).

RESULTS

Protein purification. Purifications of gp70-AKR and gp70-NZW by immunoaffinity chromatography were monitored by protein determinations (15) and interspecies radioimmunoassay (2). Nearly all of the protein and about one-half of the gp70 were recovered in the unabsorbed fraction (Table 1). Elution of retained gp70 was about 50% efficient, with final yields of 18% for gp70-NZW and 22% for gp70-AKR. Since gp70-AKR was in lower concentration in the starting material, a larger increase in purity index was accomplished (Table 1). SDS-PAGE profiles of these samples are shown in Fig. 1. Starting material (mouse serum) slots 2 and 4 show similar patterns of carbohydrate stain (panel A) and protein bands (panel B). Slot 3 shows the gel pattern of the gp70-NZW preparation. A major band that stained for carbohydrate and protein was present in the gp70 region of the gel. This band was not detected among the carbohydrate-staining bands of the starting material (slot 2) and is obscured by albumin in the protein-staining pattern. The major impurities in gp70-NZW were γ and κ, λ chains of mouse IgG (10). Slot 5 shows the staining pattern for gp70-AKR. No carbohydrate stain was detected in the gp70 region of the gel due to either low gp70 concentration or low sialic acid content. The protein stain (panel B) shows one band at the position of gp70, one band slightly below gp70 in the position of serum albumin, and bands corresponding to the mobilities of IgG heavy and light chains. A very faint band midway between the γ and p30 marker proteins reacts with antibody to gp70 and has been ascribed to a breakdown product of the parent gp70 molecule (10).

The facts that the gp70-AKR preparation (i) represents a 2,000-fold increase in purity index (Table 1), (ii) contains a protein band in the 70,000-molecular-weight region of SDS-PAGE gels (Fig. 1 and 2), and (iii) reacts with at least two antisera to viral gp70's indicate a significant enrichment of gp70 cross-reacting material from this source. Since staining for carbohydrate is negative (Fig. 1), the glycoprotein nature of this protein has not been established in this work.

TABLE 1. *Recovery of protein and gp70*[a]

Fraction from:	NZW serum			AKR serum		
	Total protein (mg)	gp70 recovered (%)	Purity index	Total protein (mg)	gp70 recovered (%)	Purity index
Starting material	740	100	1.0	1,400	100	1.0
Bypass		52	~0.5		45	~0.5
Eluate	0.150	18	890	0.130	22	2,370

[a] Fractions from the immunoaffinity chromatography purification procedure were analyzed for protein content by the Lowry method (15) and for gp70 interspecies reactivity in radioimmunoassay (2). Purity index is a ratio of total protein to gp70, normalized to 1.0 for each starting material.

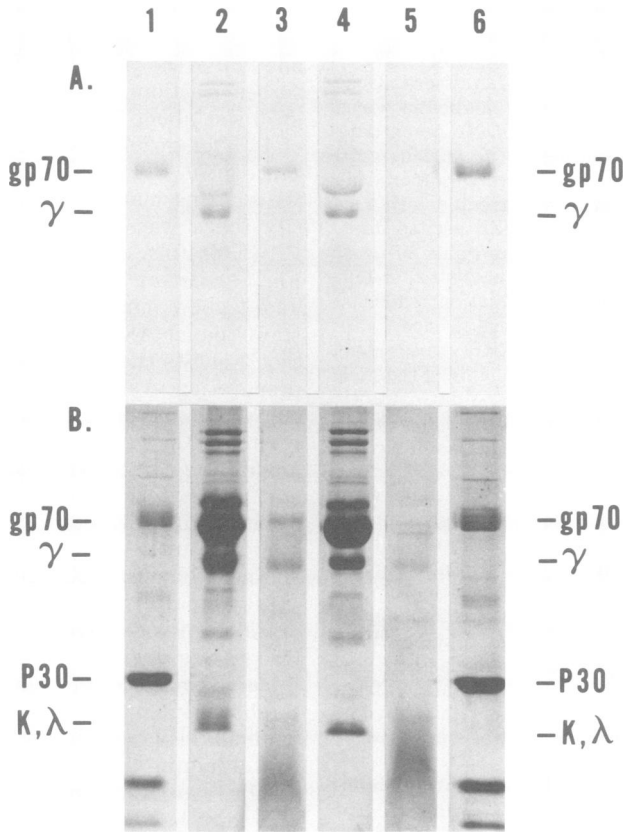

FIG. 1. *SDS-PAGE of mouse serum and gp70 preparations. (A) Stained for carbohydrate; (B) stained for protein. Slot 1, Total MLV proteins (20 μg); slot 2, NZW serum proteins (45 μg); slot 3, gp70-NZW (7 μg); slot 4, AKR serum protein (45 μg); slot 5, gp70-AKR (4 μg); and slot 6, total MLV proteins (20 μg). Known protein bands gp70, γ, p30, and κ, λ are marked as references.*

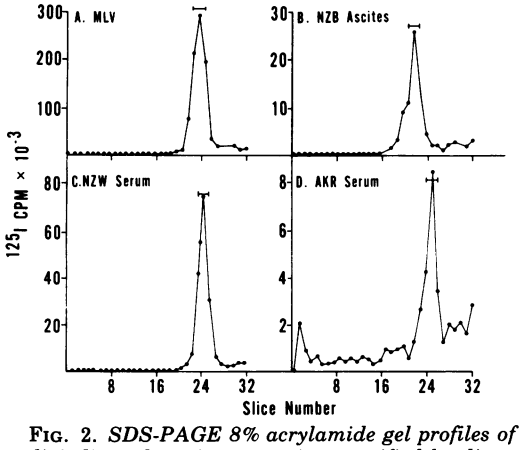

FIG. 2. *SDS-PAGE 8% acrylamide gel profiles of radioiodinated gp70 preparations purified by direct immunoprecipitation. Migration is from left to right. Bars represent slices of tube gels pooled for peptide mapping. (A) gp70-MLV; (B) gp70-NZB; (C) gp70-NZW; (D) gp70-AKR.*

Peptide mapping. Comparison of tryptic peptides of the purified proteins was done by peptide mapping. gp70 preparations were further purified by direct immunoprecipitates of radioiodinated gp70 preparations. The fractions of radioactivity precipitated were similar to the values for maximum precipitation of each gp70 preparation as determined in the antigen-binding tests (Table 2). Immunoprecipitated proteins were further purified on SDS-PAGE tube gels (Fig. 2). Internal marker proteins for molecular weight determinations were not included, because they could contaminate subsequent peptide map procedures. Mobilities, therefore, cannot be correlated with molecular weights. It is likely that differences in band mobilities (i.e., panels D and B) are due to different amounts of stacker gel included in the initial gel fractions. Slices containing iodinated, purified gp70 (indicated by bars) were pooled for tryptic digestion and mapping. A

TABLE 2. *Physical constants of gp70 preparations*[a]

gp70 prepn	gp70/test (ng)	Sp act (μCi/μg)	Precipitation (%)	
			Maximum	Background
gp70-MLV	4.4	32.5	80	1
gp70-NZB	5.0	11.5	33	2
gp70-NZW	6.4	37.3	40	2
gp70-AKR	3.8	23.2	11	3

[a] Preparations of gp70 from mouse serum and ascites fluid were standardized by interspecies radioimmunoassay (2). A 10-μg amount of each protein sample was radioiodinated and freed of mouse IgG as described in the text. Calculations of the amount of gp70 and specific activity assume there were no losses of antigen during the labeling procedure. Maximum and background precipitation are, respectively, the amount of total radioactivity bound by excess G145 antiserum and normal goat serum.

composite photograph of autoradiographic analyses of the maps is shown in Fig. 3. The arrow in all panels indicates the position of [^{125}I]iodotyrosine relative to the marker dyes (10) as determined on parallel runs. It is not known why all gp70 maps have a spot in this position.

Analysis of the major spots indicates virtually identical patterns for gp70-NZW (panel C) and gp70-NZB (panel D), whereas the other gp70's share some spots but have relatively unique patterns. Peptide maps of iodinated proteins are subject to quantitative differences in iodination due to protein conformation; thus, the map of gp70-MLV appears different when iodination is conducted in denaturing solvents (J. Elder and S. J. Kennel, unpublished observation). The near identity of the two maps for gp70-NZB and gp70-NZW is thus highly signifi-

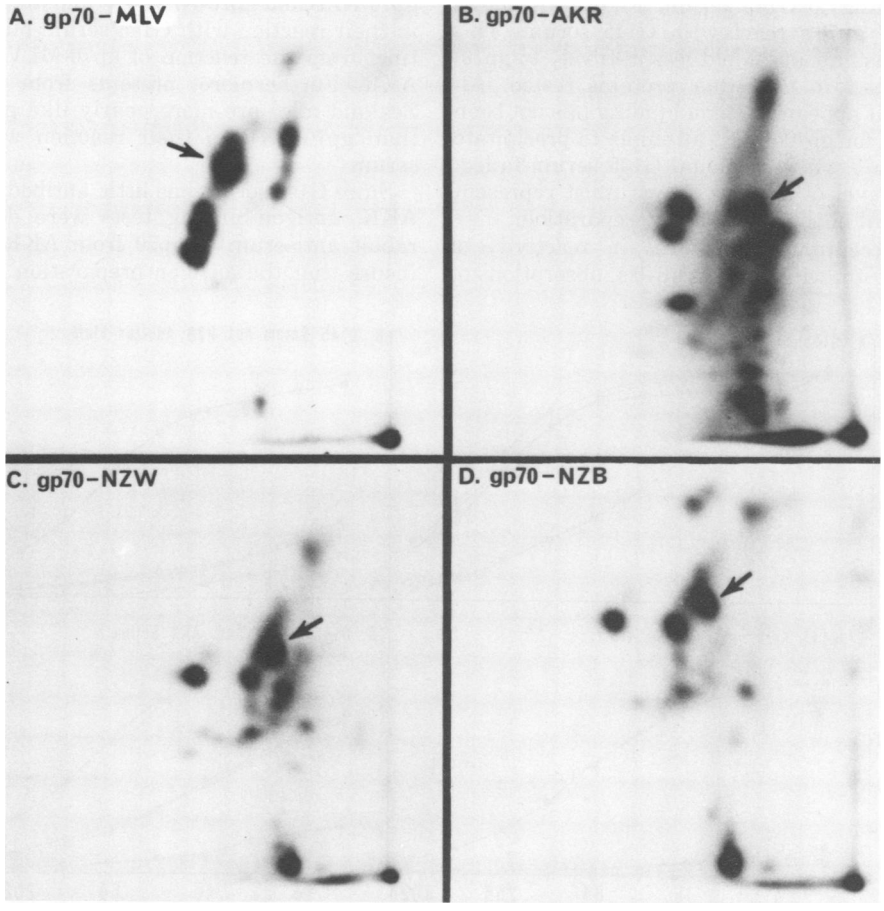

FIG. 3. *Autoradiograms of two-dimensional maps of trypic peptides of gp70's. The arrows indicate the position of tyrosine iodinated by chloramine-T as determined by mobility relative to visible dyes on parallel experiment.*

cant, whereas differences in patterns for gp70-MLV (panel A) and gp70-AKR (panel B) are less significant.

Antigen-binding studies. To investigate the immunological relatedness of gp70 preparations, radioiodinated proteins were tested for binding to G145 serum. Since mouse IgG is a major contaminant of gp70's isolated from mouse serum, labeled samples were treated with solid-phase antibody to remove the mouse IgG and thus lower background due to precipitation of mouse IgG by the second antiserum. Physical constants of the iodinated preparations for one experiment are shown in Table 2. The fraction precipitated by antiserum reflects purity of the samples and is not due to limiting antiserum. The gp70-AKR preparation is the least pure (see Fig. 1 also) due to the small amounts of gp70 in the starting material.

Radiolabeled gp70 preparations were tested for binding to G145 serum. Figure 4A shows that all gp70's react with G145 serum. This serum has about 20-fold less antibody to gp70-AKR than to the other proteins tested. Although it appears plateau binding has not been reached for gp70-AKR, attempts to precipitate more gp70 with additional G145 serum failed, so the level of binding shown must represent nearly all of the gp70 in the preparation.

To accentuate differences in reactivity of G145 serum with various gp70's, absorptions of G145 serum were done to remove antibodies to particular gp70 molecules, and the resultant serum was tested for antigen binding of the gp70 preparations. Figure 4 shows that absorption of G145 serum with NZB ascites fluid reduced the amount of antibody to gp70-MLV from that with G145 serum alone (cf. panels A and B) and eliminated all significant antigen-binding capacity for the other gp70's tested. These data indicate that all antigenic determinants on gp70-NZW and gp70-AKR that react with G145 serum are also on gp70-NZB. Similarly, panel C indicates that all determinants on gp70-NZB and gp70-AKR that react with G145 serum are also on gp70-NZW. Finally, panel D shows that absorption of G145 with AKR serum removes all antigen-binding capacities for gp70-AKR but leaves about the same amount of antibody to gp70's NZW and NZB.

The data presented in Fig. 4 thus show that gp70-NZB and gp70-NZW are indistinguishable in their reaction with G145 serum but are distinct from the reaction of gp70-MLV or gp70-AKR. Furthermore, proteins from the New Zealand mice are more nearly like gp70-MLV than gp70-AKR in their reaction with G145 serum.

Since G145 serum has little antibody to gp70-AKR, antigen-binding tests were done with rabbit antiserum to gp70 from AKR virus to insure that the antigen preparation was good

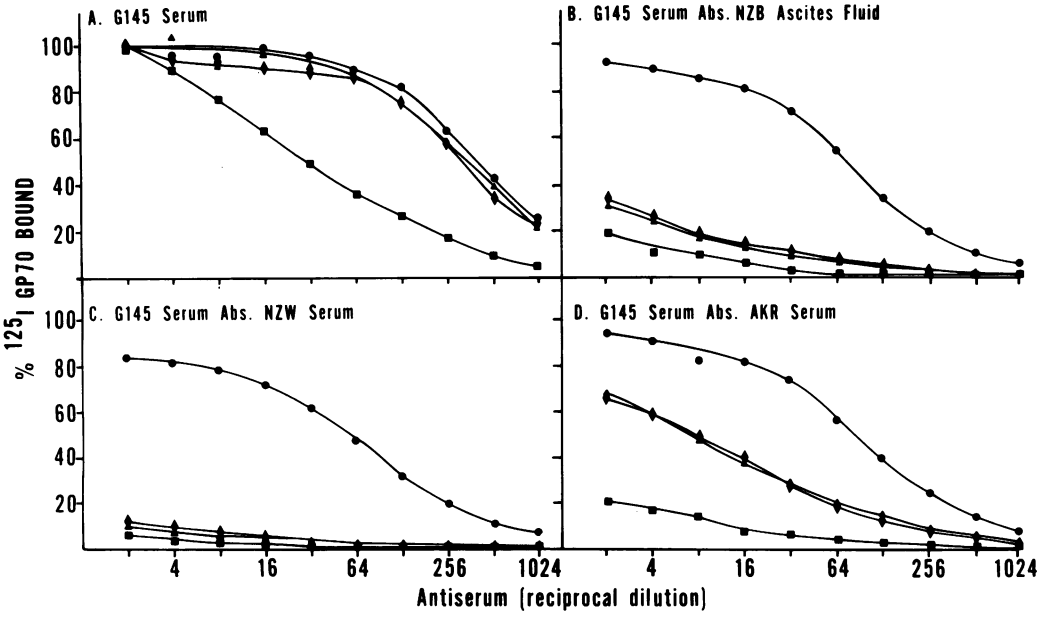

FIG. 4. *Antigen-binding curves of G145 serum for gp70-MLV (●), gp70-NZW (▲), gp70-NZB (◆), and gp70-AKR (■). (A) G145 serum; (B) G145 serum absorbed with NZB ascites fluid; (C) G145 serum absorbed with NZW serum; (D) G145 serum absorbed with AKR serum and purified gp70-AKR (see text for details).*

and that gp70-AKR was closely related to the AKR virus protein. Antigen-binding tests shown in Fig. 5 demonstrate that gp70-AKR reacts better with rabbit anti-AKR-gp70 than does gp70-MLV or gp70-NZW. Thus, gp70-AKR must retain at least some of its type-specific determinants throughout the purification procedure. Rabbit anti-AKR-gp70 and G145 serum used in excess precipitate nearly equivalent amounts of radioactivity from the gp70-AKR preparation.

Finally, all gp70's isolated by immunoaffinity chromatography were eluted with 3 M KSCN. To determine if this treatment affects antigenicity; antigen-binding tests were done with G145 serum and gp70-MLV with and without treatment with 3 M KSCN. Figure 6 shows that the antigen binding is not altered by this treatment. This experiment does not rule out possible denaturation of unique antigenic determinants found on the serum gp70's.

DISCUSSION

At least three classes of endogenous viruses have been defined by their host range and reactivity in radioimmunoassays for viral proteins p12, p30 (19), and gp70 (4). The three strains of mice tested here contain genetic information to produce all three types: AKR mice spontaneously produce N-tropic AKR virus (type I) (20), NZW mouse embryo cells spontaneously produce an N-tropic virus (type I) and can be induced to produce a xenotropic virus closely related to the BALB virus-2 (type II) (19), and NZB mouse embryo cells spontaneously produce a xenotropic virus closely related to virus induced from NIH Swiss mouse cells (type III) (19).

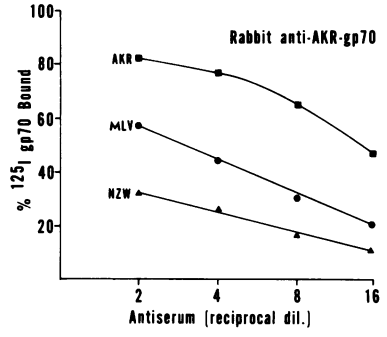

FIG. 5. *Antigen-binding curves for rabbit anti-AKR virus gp70 for gp70-AKR* (■), *gp70-MLV* (●), *and gp70-NZW* (▲).

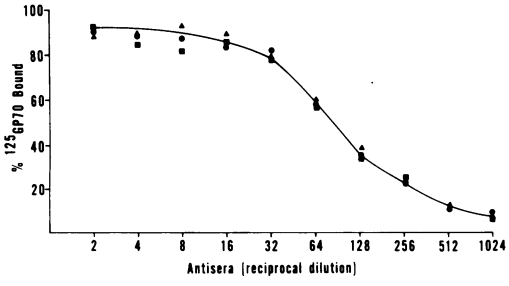

FIG. 6. *Antigen binding of G145 serum for gp70-MLV untreated* (●), *treated with bovine serum albumin in phosphate-buffered saline and dialyzed* (■), *or treated with KSCN and dialyzed* (▲).

Data from competition radioimmunoassays have shown that gp70 from AKR serum is indistinguishable from type I viral gp70 and that gp70 from NZB serum is closely related to the gp70 from type III virus (4). Studies reported here on purified proteins from these sources indicate that not only is gp70-AKR different from gp70-NZB, but also that gp70-NZW and gp70-NZB are indistinguishable. Thus, the NZW mouse, which can produce type I and type II viruses, apparently produces a gp70 related to the type III gp70. Since no virus is detected, this must represent selected expression of virus-related genetic information. A similar circumstance occurs in the AKR mouse, where gp70 isolated from the genital tract is different from the gp70 of AKR virus (2).

The expression of various kinds of viral proteins during the natural life cycle of the mouse could complicate the immunosurveillance of virus-induced tumors. For example, expression of the AKR type of viral information early in life could render the animal's immune mechanisms hyporesponsive to AKR leukemia virus and thus more sensitive to AKR virus-induced leukemia. Furthermore, an immune response to a different type of superinfecting virus could reactivate or restore the response to AKR-type determinants and result in immune-complex disease (9, 28) or other complications related to autoimmune reactions.

Antibody to viral proteins is present in normal mouse serum (8). The type-specific nature of this antibody (7) could be due to the animal's tolerance for more common portions of the viral protein or for in vivo absorption of common antibody to determinants of endogenous and exogenous viral proteins.

This paper demonstrates the differences in immunological and structural composition of gp70-MLV and endogenous gp70's. The data show that an antibody that reacts specifically with gp70-MLV can be selected. Since viral gp70 is displayed on the surface of tumor cells producing virus (5, 9, 12, 18), the unique determinants of this molecule should be useful as targets for tumor elimination by passive antibody transfer.

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