

# THE ONCORNAVIRUS GLYCOPROTEIN gp69/71\*: A CONSTITUENT OF THE SURFACE OF NORMAL AND MALIGNANT THYMOCYTES‡

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Recently, we and others have described a glycoprotein complex which is present on the surface of oncornaviruses (1, 2). This complex can be resolved by electrophoresis in polyacrylamide gels into two major components which have molecular sizes of 69,000 and 71,000 daltons. The complex represents approximately 5% of the virion mass and approximately 350 molecules are present per virus particle. The gp69/71 glycoprotein has group, type, and interspecies antigenic determinants and is one of the important antigens involved in the neutralization of virus by antibody (3). Since the gp69/71 type-specific determinants of murine viruses are not changed by growth on different cell types, it appears that gp69/71 is a virus gene product (3) and may in fact be expressed in a number of transformed cells even in the absence of expression of other virus phenotypes (4). Of considerable significance is the fact that gp69/71 shares antigenic determinants with a similar molecule on the surface of at least some differentiated cells such as "normal thymocytes" (1). Indeed, recent studies suggest that the  $G_{IX}$  trait is a property of gp69/71 (E. Stockert, personal communication, cited in 3). In this report we extend our previous studies and show that gp69/71 is present on the surface of virus-induced lymphoma cells as well as virions and normal thymus cells and that some tumor-bearing mice make antibody to gp69/71. Furthermore, differences in molecular size of molecules which react with antibody to gp69/71 were observed in tumor cells isolated from different organs of the same mouse.

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\* The nomenclature of virus-related proteins is that proposed by August et al. (22). gp69/71 has also been referred to as OSA, Interspec II, M2, G, II, V; p30 has been referred to as ga-1, 3, P4, III.

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## Materials and Methods

*Cells and Viruses.* SCRF 60A is a continuously growing lymphocyte cell line which was obtained from a New Zealand Black (NZB) mouse, and which was grown as described by Lerner (5). SCRF 60A cells are positive for  $\theta$ -antigen and produce 3–5  $\mu\text{g}$  of an oncornavirus (Scripps leukemia virus [SLV])<sup>1</sup> per  $10^6$  cells per day as determined by radioimmune assay for p30 (5).

Several preparations of SLV were used in this study. All were obtained from supernatant fluids of SCRF 60A. SLV was concentrated from cell-free supernates by precipitation with 6% (wt/vol) polyethylene glycol 6,000, and purified by sedimentation to its isopycnic density in sucrose gradients. Material with a density of 1.16 gm/cm<sup>3</sup> was collected and stored at  $-195^\circ\text{C}$  until used. Strain TC460 of Moloney leukemia virus (MLV) was obtained from the National Cancer Institute and was propagated on BALB/c C11 fibroblast cultures (obtained from Dr. V. Klement, Children's Hospital, Los Angeles, Calif.). Supernatant fluids were filtered through 0.45  $\mu$  filters, then stored frozen until used. For some studies virus was inactivated by treatment with 10 mM  $\beta$ -propiolactone (BPL) at  $4^\circ\text{C}$  for 1 h, then  $37^\circ\text{C}$  for 2 h, and stored at  $-195^\circ\text{C}$  until used. The infectivity of each virus preparation was determined by the X-C test (6) using NIH Swiss or BALB/c mouse embryo fibroblast cultures as the substrate cell.

*Experimental Animals.* Mice were obtained from Strong Research Laboratories, San Diego, Calif., Jackson Laboratories, Bar Harbor, Maine, or the Scripps vivarium. The following mouse strains and hybrids were used: BALB/c, (BALB/c  $\times$  NZB)F<sub>1</sub>, C3H/St, C57BL/6J, Nu/nu, nu/nu, NZB, NZW, (NZB  $\times$  NZW)F<sub>1</sub>. All mice were injected within 24 h of birth with SLV, MLV, or BPL-inactivated SLV. Mice were weighed and bled from the retro-orbital plexus at monthly intervals. Moribund animals were sacrificed and autopsied. Diagnosis of lymphomas was made on the basis of histological examination of tissue sections.

*Radioiodination of Cell Surfaces.* Spleen, thymus, and in some cases lymph nodes were gently teased apart using a 50 mesh stainless steel screen into labeling medium (Earle's balanced salt solution without phenol red, pH 7.2, containing 10  $\mu\text{M}$  KI [1]). Dispersed cells were passed through a 200 mesh nylon filter to remove large clumps. Cells were sedimented by centrifugation at 800  $g$  for 10 min, resuspended in labeling medium, and washed two more times. Tumor cells were counted after making a 1:20 dilution in 3% acetic acid. In some experiments, viable tumor cells were isolated from tumor cell suspensions by sedimentation onto a cushion of Ficoll-Hypaque as described by Mendelsohn (7) and then washed with labeling medium. SCRF 60A cells were obtained from logarithmically growing suspension cultures (less than  $2 \times 10^6$  cells/ml), then washed with labeling medium as described above.

Washed cells were suspended at a concentration of  $2 \times 10^7$  cells per ml. 1 ml of this suspension was radioiodinated at room temperature by the addition of 3–5 mC of [<sup>125</sup>I]Na (NEN NEZ-033H), 20  $\mu\text{g}$  of lactoperoxidase, and two additions of H<sub>2</sub>O<sub>2</sub> 1 min apart so that the final concentration of H<sub>2</sub>O<sub>2</sub> was 45  $\mu\text{M}$  (1).

Radioiodinated cells were diluted with Eagle's minimal essential medium (MEM), centrifuged, then washed three times with MEM. The washed cell pellet was resuspended in 4 ml of reticulocyte standard buffer (RSB) containing 0.5% Nonidet P-40 (NP-40) (Shell Chemical Co., New York). Cells were then sonicated for two 15-sec periods at  $0^\circ\text{C}$  with a 20KC sonicator (Heat Systems-ultrasonics, Inc., Plainview, N.Y.) equipped with a microprobe. Particulate material was removed by sedimentation at 100,000  $g$  for 60 min. The resulting soluble fraction was then subjected to immune precipitation as described below.

*Radioiodination of Virus.* Purified SLV, which had been disrupted by the addition of 0.1% tween 80, was radioiodinated by the solid state lactoperoxidase method (8). 200  $\mu\text{g}$  of lactoperoxidase was coupled to 1 ml Sepharose 4B by the cyanogen bromide method (9). The suspension was stored at  $4^\circ\text{C}$  in 0.1% merthiolate and washed four times with 0.1 M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.4 (STE) before use. 10  $\mu\text{g}$  lactoperoxidase (50  $\mu\text{l}$  of suspension) was used to label 1 mg of tween-dis-

<sup>1</sup> Abbreviations used in this paper: BPL,  $\beta$ -propiolactone; MLV, Moloney leukemia virus; MuLV, murine leukemia virus; RSB, reticulocyte standard buffer; 10 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris, pH 7.4; Seph LP, Sepharose-bound lactoperoxidase; SLV, Scripps leukemia virus; STE, 0.1 M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.4.

rupted virus in 1 ml of STE. The reaction was initiated by addition of 3–5 mC  $^{125}\text{I}$ , 10  $\mu\text{M}$  KI, and 45  $\mu\text{m}$   $\text{H}_2\text{O}_2$ . After 10 min at 37°C, the Sepharose-bound lactoperoxidase (Seph-LP) was removed by centrifugation. Radioiodinated virus was sonicated before it was used for immune precipitation.

*Antisera.* Rat anti-MLV (1344) was obtained from Fisher rats immunized with syngeneic tumor cells induced by MLV and was given by Dr. Roger Wilsnack, Huntington Research Labs, Brooklandville, Md. Rabbit antirat IgG was provided by Dr. S. J. Kennel, Scripps Clinic. This serum is specific for rat IgG as shown by immunoelectrophoresis. Goat antimurine IgG was provided by Dr. R. L. Levy, Scripps Clinic. This serum is specific for mouse IgG as shown by immunoelectrophoresis. Normal sera were obtained from animals in the Scripps vivarium.

*Immune Precipitation.* Indirect immune precipitations were carried out as described by Kennel et al. (1). Test tubes were coated with 50  $\mu\text{l}$  of normal rabbit or goat serum and 50  $\mu\text{l}$  5% NP-40. (Normal rabbit serum was used for rabbit antirat IgG precipitates and normal goat serum was used for goat antimouse IgG precipitates.) 5  $\mu\text{l}$  of primary antibody or normal serum and 0.5 ml STE were added to the coated tubes and then  $^{125}\text{I}$ -labeled antigen was added. Primary binding was allowed to proceed for 15 min at 37°C. Sufficient rabbit antirat IgG or goat antimouse IgG was then added to precipitate all of the Ig from the primary antibody in the reaction mixture. Immune precipitates were collected by centrifugation at 2,000  $g$  for 15 min, washed with STE containing 0.1% NP-40, and finally with ice cold distilled water.

*Polyacrylamide Disc Gel Electrophoresis.* Immune precipitates were solubilized in 8 M urea, 1% sodium dodecyl sulfate (SDS), and either 2%  $\beta$ -mercaptoethanol or 0.01 M dithiothreitol, then  $^{131}\text{I}$ -labeled human IgG and IgM proteins were added as molecular size markers. This mixture was then heated to 56°C for 30 min, then to 100°C for 2 min before electrophoresis. Samples were analyzed by electrophoresis on 6% acrylamide gels prepared as described by Weber and Osborn (10). Electrophoresis was for 2½ h at a constant current of 15 mA per gel. Gels were frozen on dry ice, then cut into 1-mm slices with a Joyce Loebel gel slicer (Joyce, Loebel & Co., Inc., Burlington, Mass.) and counted in a Nuclear-Chicago auto gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Data were corrected for background,  $^{131}\text{I}$  crossover, and counting efficiency and were plotted with a Hewlett-Packard system (Hewlett-Packard Co., Palo Alto, Calif.).

*Polyacrylamide Gradient Slab Gel Electrophoresis.* High resolution polyacrylamide gradient slab gels were prepared by a modification of the method of Laemmli (11). Gels of 1.5 mm thickness were formed between glass plates 14.5  $\times$  16.5 cm. Linear gradients were prepared by mixing 5% and 24% acrylamide solutions with a mixing device, and were then overlaid with buffer. After polymerization of the same gel, 3% acrylamide stacker gel was prepared with a sample template. Samples were solubilized with 8 M urea, 1% SDS, 0.01 M dithiothreitol and heated to 100°C for 2 min, then loaded in the template wells. Electrophoresis was for 5–5½ h at a constant current of 20 mA. Gels were immersed in 50% trichloroacetic acid, 0.05% coomassie brilliant blue for 4–12 h, and then destained with 7% acetic acid, 10% methanol, then dried. Autoradiograms were made by placing the dried slab gel in direct contact with Kodak Royal X pan film (Eastman Kodak Co., Rochester, N.Y.) in a light-proof box for 1–7 days.

## Results

*Characterization of Rat Anti-MLV.* In order to have a reference standard for comparison of virus-related antigens on lymphomas to known virus and cell surface-associated antigens, the reactivity of rat anti-MLV was determined by indirect immune precipitation using four kinds of radioiodinated antigen preparations: proteins from disrupted SLV, and surface proteins from three kinds of cells—SCRF 60A cells, normal spleen cells, and normal thymus cells. Labeled proteins which reacted with rat anti-MLV were characterized by SDS gel electrophoresis. As shown in Fig. 1, this serum reacts with three classes of labeled proteins from disrupted SLV—gp69/71, p45,<sup>2</sup> and p30. This is similar to our

<sup>2</sup> In this work we refer to this molecule from SLV as p45 rather than gp45 because we have not yet been able to demonstrate the presence of carbohydrate.

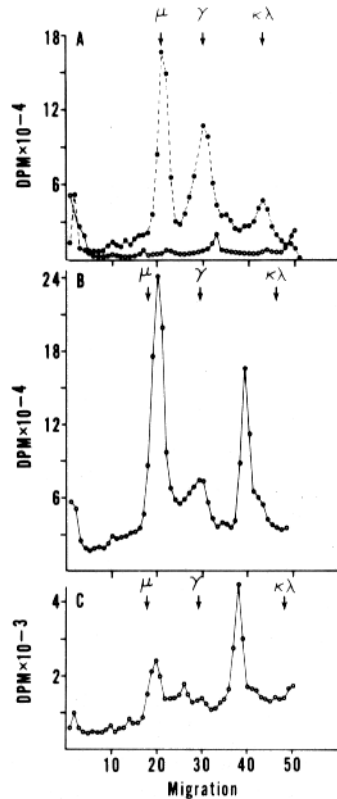


FIG. 1. Gel electrophoresis of immune precipitates of  $[^{125}\text{I}]$ SLV proteins. (A)  $[^{125}\text{I}]$ SLV proteins which had been reacted with normal rat serum. (B)  $[^{125}\text{I}]$ SLV proteins which had been reacted with rat anti-MLV proteins. (C) Total  $[^{125}\text{I}]$ SLV proteins (without immune precipitation). (O—O),  $^{125}\text{I}$ ; (●---●),  $[^{131}\text{I}]$   $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

earlier observations with other rat anti-MLV sera (1). As shown in Fig. 2, the principal antigen from the surface of SCRF 60A cells that reacts with this rat anti-MLV is gp69/71; very little labeled p45 and no p30 were observed. gp69/71 was also detected on thymocytes obtained from thymuses of NZB, (NZB  $\times$  NZW) $F_1$ , NZW, and NIH Swiss mice but not on thymocytes or spleen cells from BALB/c mice (Figs. 3 and 4).

*Virus-Related Surface Antigens of Lymphoma Cells.* Tumor cells from 34 involved thymuses, 31 involved spleens, and 3 involved lymph nodes of 35 different mice with murine leukemia virus (MuLV)-induced lymphomas were analyzed to characterize virus-related cell surface antigens. As controls, thymuses and spleens from 27 different normal mice were studied. Histological examination of each tissue from lymphomatous mice demonstrated that essentially all of the normal cells had been replaced with malignant cells. The tumors were classified as either lymphocytic lymphomas or reticulum cell sarcomas. Previous studies from our laboratory utilizing histologic and immunofluorescent studies of the tumor cells have shown that almost all lymphomas induced by SLV are of thymocytic origin. Cell suspensions were isolated from the spleens and

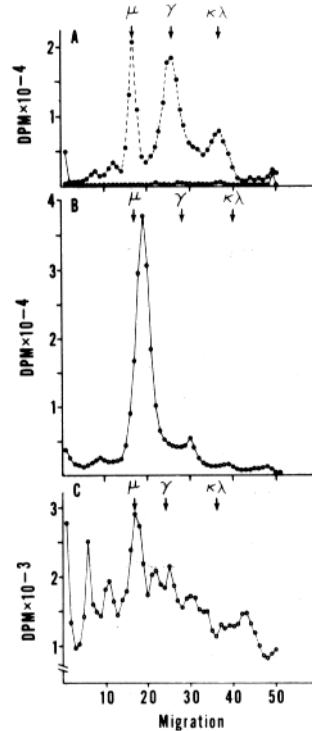


FIG. 2. Gel electrophoresis of immune precipitates of [ $^{125}\text{I}$ ]SCRF 60A surface proteins. (A) [ $^{125}\text{I}$ ]SCRF 60A surface proteins which had been reacted with normal rat serum. (B) [ $^{125}\text{I}$ ]SCRF 60A surface proteins which had been reacted with rat anti-MLV proteins. (C) Total [ $^{125}\text{I}$ ]SCRF 60A surface proteins (without immune precipitation). (O—O),  $^{125}\text{I}$ ; (●—●), [ $^{125}\text{I}$ ]  $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

thymuses of moribund mice and were radioiodinated as described in Methods. Virus-related proteins were identified by indirect immune precipitation with rat anti-MLV, followed by SDS polyacrylamide gel electrophoresis. In general, the three classes of the virus-related proteins described above (i.e., gp69/71, p45, p30) were detected associated with the surface of cells from lymphomas induced by SLV and MLV. Considerable variation in the relative amounts of the various proteins was observed (Figs. 5–7) in different tumors and different organs, but since the techniques used in this study are not quantitative the significance of this observation is not known. Labeled virus-related surface proteins were not detected in 22 of 68 tumor preparations. No antigenic difference between lymphomas induced by SLV and MLV was observed, nor was any difference between lymphocytic lymphomas and reticulum cell sarcomas observed.

*Variation in the Molecular Size of Virus-Associated Proteins Present in Different Tumors.* One of the most interesting observations made during our survey was that virus-related cell surface antigenic determinants of the 70,000 dalton molecular size class obtained from some tumors had different electrophoretic mobilities compared to the molecules present on the surface of SCRIF 60A

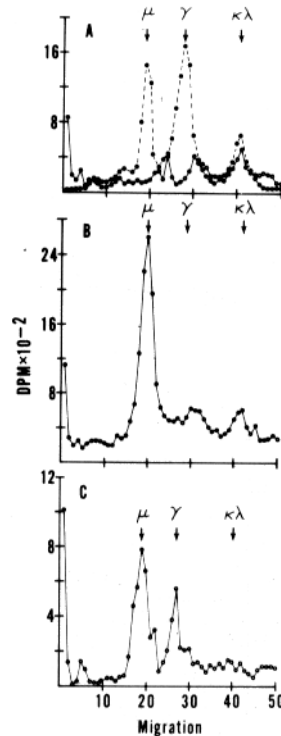


FIG. 3. Gel electrophoresis of immune precipitates of [ $^{125}$ I]surface proteins of cells from normal tissues of NZB mice. Pools were made from spleens or thymuses of four NZB mice, then radioiodinated with  $^{125}$ I. (A) [ $^{125}$ I]thymus cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}$ I]thymus cell surface proteins which had been reacted with rat anti-MLV serum. (C) [ $^{125}$ I]spleen cell surface proteins which had been reacted with rat anti-MLV serum. (O—O),  $^{125}$ I; (●---●), [ $^{131}$ I] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

cells and purified virus (see Fig. 7). This was suggested by a broadening of the gp69/71 peak, or by a shift in the mobility of the gp69/71 peak in reference to the [ $^{131}$ I] $\mu$ -chain marker protein. Since the observed differences were small, we wanted to confirm our finding in an analytical system of higher resolution and therefore the solubilized immune precipitates were studied by electrophoresis in polyacrylamide gradient slab gels (Fig. 8). Again, it is evident that antigenic molecules present on lymphoma cells from the thymus have molecular sizes larger than the molecules present on the surface of tumor cells in the spleen and the molecules from purified virions.

*Presence of Antibody to gp69/71 in Infected Mice.* Sera from 2-3-mo old mice which were injected as neonates with SLV, MLV, or BPL-inactivated SLV were tested by indirect immune precipitation followed by polyacrylamide gel electrophoresis for antibodies which reacted with labeled surface antigens of SCRF 60A cells, or with disrupted, labeled SLV. As shown in Fig. 9, we could detect antibodies against gp69/71 in some mouse sera by this method. Anti-gp69/71

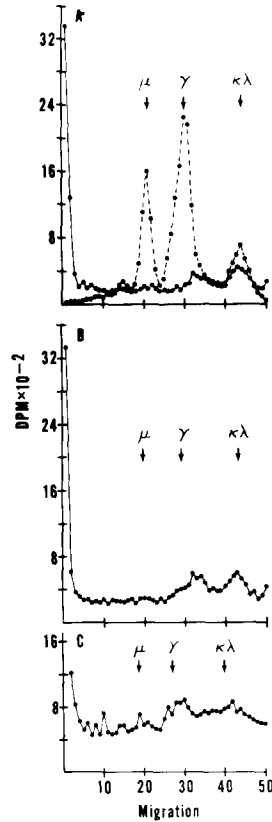


FIG. 4. Gel electrophoresis of immune precipitates of [ $^{125}$ I]surface proteins of cells from normal tissues of BALB/c mice. Pools were made from spleens or thymuses of four BALB/c mice, then radioiodinated with  $^{125}$ I. (A) [ $^{125}$ I]thymus cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}$ I]thymus cell surface proteins which had been reacted with rat anti MLV serum. (C) [ $^{125}$ I]spleen cell surface proteins which had been reacted with rat anti MLV serum. (O—O),  $^{125}$ I; (●—●), [ $^{131}$ I] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

antibodies were detected in the sera of 29% (37/128) of mice infected at birth. No antibodies to gp69/71 were found in 28 uninfected mice. There was, however, no correlation between the presence of antibody to gp69/71 and the incidence of lymphoma.

To determine whether virus replication was required for the induction of anti-gp69/71 antibodies, the sera from mice infected with BPL-inactivated SLV or with  $10^{-1}$  TCID $_{50}$ /mouse were tested for anti-gp69/71 by indirect immune precipitation of radioiodinated surface proteins of SCRF 60A cells. None of 28 mice injected with BPL-SLV had anti-gp69/71, while 4 of 19 BALB/c  $\times$  NZB)F $_1$  injected with  $10^{-1}$  TCID $_{50}$  of SLV had antibodies which precipitated labeled gp69/71. Sera were also tested for antibodies against disrupted labeled virus. The sera from those mice which precipitated gp69/71 from SCRF 60A also reacted with gp69/71 from SLV; no free anti-p30 has been detected in the sera of these

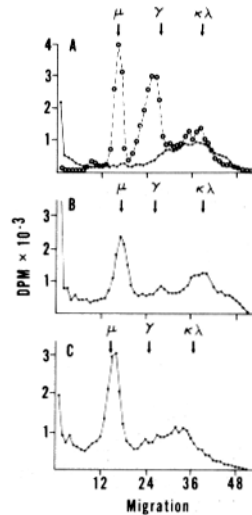


FIG. 5. Gel electrophoresis of immune precipitates of [ $^{125}\text{I}$ ]surface proteins of lymphomas from an MLV-infected NZW ( $\varnothing$  5) mouse. (A) [ $^{125}\text{I}$ ]spleen cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}\text{I}$ ]spleen cell surface proteins which had been reacted with rat anti-MLV serum. (C) [ $^{125}\text{I}$ ]thymus cell surface proteins which had been reacted with rat anti-MLV serum. Both spleen and thymus are gp69/71 $^{-}$ , p45 $^{-}$ , p30 $^{-}$ . ( $\bullet$ — $\bullet$ ),  $^{125}\text{I}$ ; ( $\circ$ — $\circ$ ), [ $^{131}\text{I}$ ] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

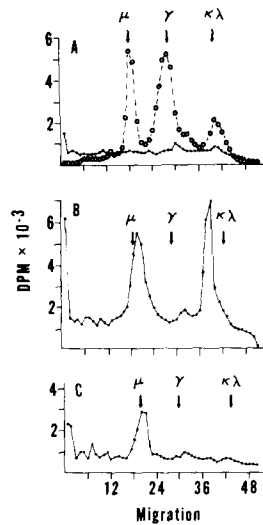


FIG. 6. Gel electrophoresis of immune precipitates of [ $^{125}\text{I}$ ]surface proteins of lymphomas from an MLV infected NZB ( $\delta$  26) mouse. (A) [ $^{125}\text{I}$ ]thymus cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}\text{I}$ ]thymus cell surface proteins which had been reacted with rat anti-MLV serum. (C) [ $^{125}\text{I}$ ]spleen cell surface proteins which had been reacted with rat anti-MLV serum. Thymus is gp69/71 $^{+}$ , p45 $^{+}$ , p30 $^{+}$ , but spleen is gp69/71 $^{-}$ , p45 $^{-}$ , p30 $^{-}$ . ( $\bullet$ — $\bullet$ ),  $^{125}\text{I}$ ; ( $\circ$ — $\circ$ ), [ $^{131}\text{I}$ ] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.



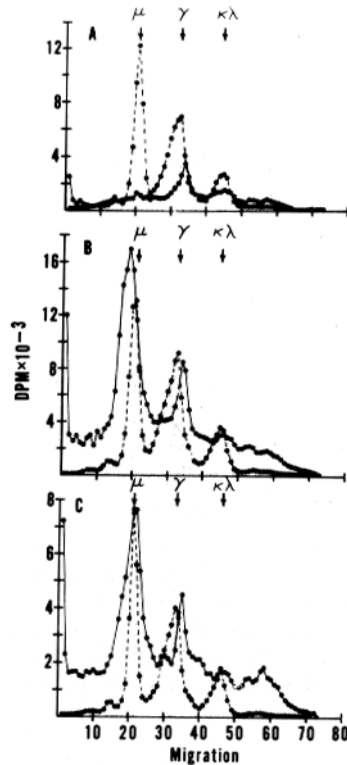


FIG. 7. Gel electrophoresis of immune precipitates of proteins of lymphomas from an SLV-infected BALB/c ( $\delta$  8) mouse. (A) [ $^{125}$ I]Thymus cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}$ I]Thymus cell surface proteins which had been reacted with rat anti-MLV serum. (C) [ $^{125}$ I]Spleen cell surface proteins which had been reacted with rat anti-MLV serum. Both spleen and thymus are gp69/71 $^{+}$ , p45 $^{+}$ , p30 $^{-}$ . However, the molecular size of gp69/71 is significantly greater in the thymus than in the spleen. See also Figs. 1, 2, and 8. (O—O),  $^{125}$ I; (●---●), [ $^{131}$ I] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers.

mice. By contrast, adult mice immunized with SLV or SCRF 60A produce antibodies which react with gp69/71, p45, p30, and other viral proteins (unpublished observations). In addition, several positive sera were studied for antibodies to gp69/71 by radioimmune assay. The results indicated that low levels of antibodies were indeed present (antigen-binding capacity of up to 700 ng gp69/71 per ml mouse serum, P. J. McConahey, unpublished results).

*Presence of Antibody Against Autologous Lymphoma Cell Surface Antigens.* Since we had determined that gp69/71 was present on the surface of lymphoma cells and since infected mice make antibody against gp69/71, it was of interest to determine whether mice with lymphoma had antibodies against their own tumors. Indirect immune precipitates were prepared using lactoperoxidase-labeled lymphoma cell surface antigen preparations and autologous sera obtained at sacrifice. Two MLV infected mice, an NZW and an (NZB  $\times$  NZW) $F_1$ , of 25 tested had evidence of antibody to their own lymphoma cell surface

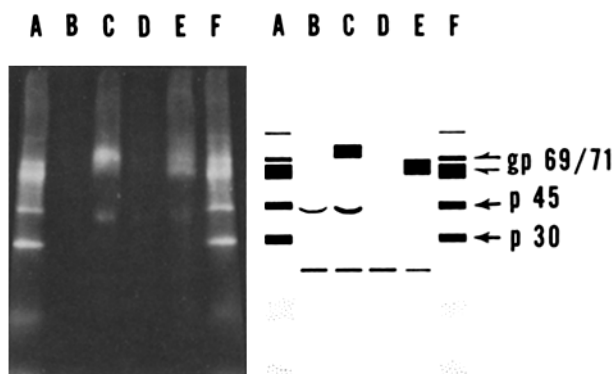


FIG. 8. Gradient slab gel electrophoresis of immune precipitates of [ $^{125}$ I]surface proteins of lymphoma cells from an SLV-infected BALB/c ( $\beta$ 8) mouse. Left side is an autoradiograph; right side is a representation of the autoradiograph. [ $^{125}$ I]SLV marker proteins (A and F). [ $^{125}$ I]Thymus cell surface proteins which had been reacted with normal rat serum (B) or with rat anti-MLV serum (C). [ $^{125}$ I]Spleen cell surface proteins which had been reacted with normal rat serum (D) or with rat anti-MLV serum (E).

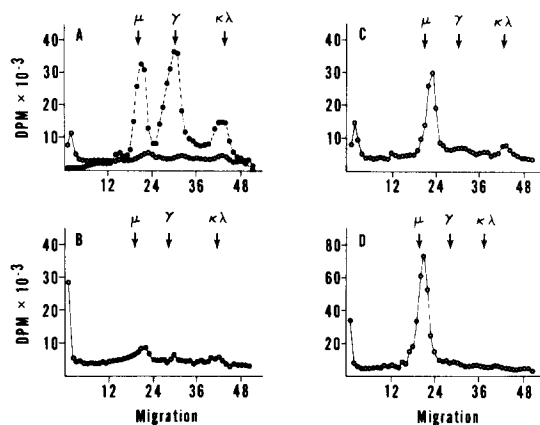


FIG. 9. Demonstration that sera of SLV-infected mice may have antibodies against gp69/71. [ $^{125}$ I]SCRf 60A surface proteins were subjected to indirect immune precipitation with sera obtained from SLV-infected mice and goat antimurine IgG. Immune precipitates were analyzed by SDS gel electrophoresis. (A) and (B) negative sera from (NZW  $\times$  BALB/c) $F_1$  mice, (C) and (D) positive sera from (NZW  $\times$  BALB/c) $F_1$  mice. (O—O), [ $^{125}$ I]; (●---●), [ $^{131}$ I] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

antigens. This is shown in Fig. 10 for the (NZB  $\times$  NZW) $F_1$  mouse. The major antigen class with which these sera reacted was gp69/71. To confirm these results, sera obtained from these mice at 1, 2, 3 mo and at sacrifice were used to precipitate labeled surface proteins from SCRf 60A cells. Again, sera from these mice contained antibodies against gp69/71. The relative amount of antibody in these sera reached a maximum at 3 mo and decreased at sacrifice. Sufficient quantities of these sera were not available to measure antibodies against gp69/71 by radioimmune assay.

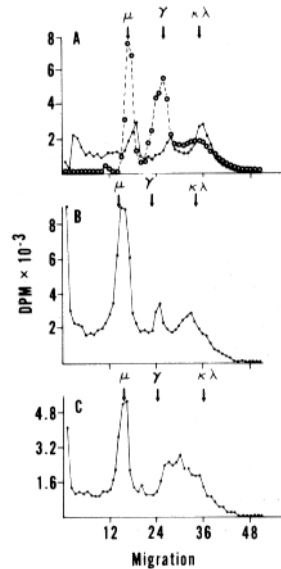


FIG. 10. Gel electrophoresis of immune precipitates of [ $^{125}\text{I}$ ]surface proteins of lymphoma cells from the spleen of an MLV-infected (NZB  $\times$  NZW) $F_1$  ( $\approx$  21) mouse. (A) [ $^{125}\text{I}$ ]Spleen cell surface proteins which had been reacted with normal rat serum. (B) [ $^{125}\text{I}$ ]Spleen cell surface proteins which had been reacted with rat anti-MLV serum. (C) [ $^{125}\text{I}$ ]Spleen cell surface proteins which had been reacted with autologous serum obtained at sacrifice. Spleen is gp69/71 $^+$ , p45 $^-$ , p30 $^-$ . Autologous serum has antibodies against gp69/71. ( $\bullet$ — $\bullet$ ),  $^{125}\text{I}$ ; ( $\circ$ — $\circ$ ), [ $^{131}\text{I}$ ] $\mu$ ,  $\gamma$ ,  $\kappa$ ,  $\lambda$  molecular size markers; also indicated by arrows.

## Discussion

The surface antigens of virus-induced lymphomas were studied by selective radioiodination with lactoperoxidase followed by immune precipitation with reference antisera and SDS polyacrylamide gel electrophoresis. Using these methods we have observed that 70% of lymphomas have detectable virus-related antigens associated with their cell surfaces. Three major classes of antigens, gp69/71, p45 and p30, were observed. An interesting variation in the electrophoretic mobility of the virus-related antigen(s) of the 70,000 dalton size class was detected depending on whether tumor cells from the thymus or spleen were studied. This variation may be the result of a somatic cell modification of a protein which is otherwise coded for by the virus genome.

There are two reservations concerning our conclusions which must be kept in mind. First, although the lactoperoxidase method is selective for radioiodination of cell surfaces, it is not possible to distinguish between molecules which are an integral part of the cell surface and those which are passively adsorbed. In this study this problem was controlled by the simultaneous measurement of the internal virion protein, p30. Even though up to 1,000  $\mu\text{g}$  of p30 per gram of lymphoma and 17  $\mu\text{g}$  of p30 per ml of serum are present in mice with tumors, p30 was detected on the surface of cells from only 4 of 68 tissues studied. By contrast, gp69/71 was associated with the surface of cells from 50 of the same 68 tissues. A similar finding for p30 has been reported by Yoshiki et al. (12). Further evidence

against the possibility of passive adsorption of gp69/71 was our finding that subtle differences in the size of this molecule were observed in tumor cells present in the spleen and thymus of the same mouse. In a strict sense, however, our results can only be taken as a demonstration of the association of gp69/71 with the surface of lymphoma cells and additional studies will be necessary to rule out the possibilities that some or all of the gp69/71 associated with tumor cells is in virus particles or that free gp69/71 has a unique affinity for cell surfaces. Nevertheless, even though one cannot be certain as to why gp69/71 and occasionally p30 are associated with the cell surface, their presence is operationally of extreme biological significance since antisera to either of these molecules is cytotoxic for cells which have either of these proteins associated with their plasma membranes (13). Second, although careful immunologic and biochemical analysis of radioiodinated cell surface proteins is an extremely sensitive approach which can be applied to the study of many surface antigen systems (14-16), it is limited by several factors: (a) the labeling efficiency of an antigen; (b) the ability to solubilize the antigen; (c) the availability and titer of antisera; and (d) the extent to which other labeled proteins are trapped in control precipitates formed with radioiodinated surface proteins, normal serum, and the anti-Ig serum. The net result of these limitations is that it is not possible to determine the specific activity and thus the absolute amount of cell surface antigens; and therefore lymphomas which we describe as "negative" could still have virus-related surface antigens. Nevertheless, these techniques, insofar as they outline surface-associated immunogenic systems, greatly complement and often precede quantitative techniques such as radioimmune assays.

Mice which had been infected as neonates with SLV or MLV are capable of producing antibodies against gp69/71. Although we did not detect antibody to gp69/71 in uninfected mice, antibody to this and other viral antigens has been found by several workers (17-20). Ihle et al. (20) reported that sera from normal (C57BL/6 × C3H/Anf)F<sub>1</sub> mice consistently react with viral antigens having mol wt of 68,000, 43,000 and 17,000 daltons and occasionally with viral antigens having mol wt of 19,000 and 15,000 daltons. The fact that surface antigen and antibody may be present simultaneously in an intact animal implies that either there is an excess of antibody or that certain antigenic determinants not available in vivo are exposed after disruption of virus or cells and are thus detected in vitro. It is of obvious importance both to determine the nature of such antibodies and to define the available immunogenic determinants in virions and cells in vivo. For example, it remains to be determined if the presence of gp69/71 on the surface of lymphomas and presence of antibody to this complex have a positive or negative selective pressure for tumor growth.

Virus-related surface antigens, especially gp69/71, and the immune response to these antigens could be an axis about which several factors interact to determine the fate of an infected mouse. In this study, we have shown that gp69/71 is found on the surface of MuLV-induced lymphomas as well as virus particles and the surface of "normal" thymus cells of some mice. Thus, antibody to gp69/71 can react with the surface of three independent structures with replicative potential (virus, "normal" thymocytes, and tumor cells). Such antibody may have different consequences for oncogenesis, depending on the localization of the

antigen. Antibody reacting with the antigen present on the surface of virus could delay the development of lymphomas by neutralizing the virus. Likewise, reaction with the antigen on the surface of tumor cells could either kill or protect the cells depending on whether the antibody was lytic or blocking. By contrast, reaction with the antigen on the surface of "normal" thymus cells might accelerate tumor growth by killing thymocytes and thus suppressing the immune response to the tumor. Since it is likely that virus, thymus cells, and tumor cells are present simultaneously in an infected mouse, the oncologic outcome probably depends on the balance between the amount of antibody and the relative amount of antigen on virus, tumor cells, and thymocytes.

Finally, with these concepts in mind it will be important to determine the relationship between virion, leukemia associated, and differentiation dependent markers such as gp69/71, TL,  $G_{1X}$ ,  $G_T$ ,  $G_L$ , and  $\theta$ . For example, if, as appears likely, the  $G_{1X}$  trait is a property of gp69/71 molecule, then this system will be of considerable importance for study of somatic cell controlled variation in expression of viral protein phenotypes. Probably the first order of business is to learn if the  $G_{1X}$  determinant is a constant feature of the gp69/71 molecule or if its presence is confined to virus expression in certain cell types or to virions derived from these cells. Regardless, the studies here show that the gp69/71 antigen shares one important similarity with antigens of the  $G_{1X}$  and TL systems in that the  $gp69/71^- \rightarrow gp69/71^+$  conversion accompanies leukemogenesis in mice such as BALB/c (21).

### Summary

The oncornavirus related proteins associated with the surface of normal and malignant thymocytes were studied. Three virion-associated proteins (gp69/71, p45, p30) were associated with lymphoma cells from about 70% of the tumors studied. Two virion-associated proteins (gp69/71 and p45) were associated with normal thymocytes from some but not all strains of mice. In  $gp69/71^-$  mice, conversion to the  $gp69/71^+$  phenotype accompanied leukemogenesis. An interesting difference in the apparent molecular size of virus related antigens of the 70,000 dalton size class was detected in lymphoma cells present in involved spleens as compared to involved thymuses. Mice infected as neonates with Scripps leukemia virus make antibody to gp69/71 and some make antibodies to molecules associated with the surface of their own tumors. The significance of the restricted presence of antigens coded for by the viral genome to the surface of some differentiated cells is discussed in reference to (a) the relationship between virion, leukemia associated, and differentiation dependent markers, and (b) the possible consequence to the host of having similar antigenic determinants on three independent structures with replicative potential (virus, normal thymocytes, and tumor cells).

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*Note added in proof:* Tung et al. and Obata et al. (this issue) have demonstrated that the gp 69/71 molecule carries MuLV-specific  $G_{1X}$  cell surface antigenic determinants.

The phenotypic conversion from gp 69/71<sup>-</sup> to gp 69/71<sup>+</sup> reported here for lymphoma cells is analogous to the conversion from G<sub>IX</sub><sup>-</sup> to G<sub>IX</sub><sup>+</sup> which is characteristic of many MuLV-induced lymphomas. In order to better define this conversion, we performed radioimmune assays for MuLV p30 and gp 69/71 on extracts of normal and lymphomatous tissues from BALB/c mice. The assay for p30 has been described (23); the gp 69/71 assay was a modification of that of Strand and August (2). In the latter assay, only interspecies determinants are measured. The results of these analyses are shown in Table I. The amounts of p30 and gp 69/71 in lymphomatous spleens and thymuses were dramatically increased compared to normal tissues. Interestingly, the ratio of gp 69/71 to p30 in the malignant tissues varies from 0.4 to 3.3. This variation suggests that the regulation of the expression of these two molecules, each of which is probably coded for by viral genes, is not coordinate and is in agreement with the results of strand et al. (24).

TABLE I  
Quantitation of viral antigens in BALB/c Tissues

		gp 69/71 ( $\mu\text{g/g}$ )	p30 ( $\mu\text{g/g}$ )	Ratio $\frac{(\text{gp } 69/71)}{(\text{p30})}$
Normal spleen*		NEG	0.12 $\pm$ 0.2	—
Normal thymus‡		NEG	2.6 $\pm$ 0.8	—
Lymphomatous spleen§	1	146	60.5	2.4
	2	172	144	1.2
	3	229	78.0	2.9
	4	235	71.5	3.3
	5	187	89.8	2.1
	6	154	140	1.1
Mean (SD)		187 $\pm$ 37	97.3 $\pm$ 36	
Lymphomatous thymuses§	6	106	271	0.4
	7	133	94.0	1.4
Mean (SD)		120 $\pm$ 19	183 $\pm$ 125	

\* Values obtained from 10 normal spleens.

‡ Values obtained from four normal thymuses.

§ Lymphomas induced by inoculation of SLV into newborn mice.

|| Numbers refer to individual mice.

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