Preparation of Syngeneic Tumor Regressor Serum Reactive with the Unique Determinants of the Abelson Murine Leukemia Virus-Encoded P120 Protein at the Cell Surface

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Antisera reactive with the Abelson murine leukemia virus (A-MuLV)-specified P120 (anti-AbT sera) were produced in C57L/J mice. Of many strains tested, only C57L/J reproducibly rejected syngeneic A-MuLV-induced tumor cells; after multiple immunizations their sera would immunoprecipitate both P120 and Moloney-MuLV (M-MuLV) proteins. Using labeled A-MuLV-induced nonproducer cells, only P120 could be detected by anti-AbT sera, suggesting that it may be the only A-MuLV-specified protein. Reactivity of anti-AbT sera with P120 was not blocked by M-MuLV virion proteins, implying that the sera recognize a portion of P120 that is not homologous to any M-MuLV product. Anti-AbT sera stained the surface of live, A-MuLV-transformed nonproducer cells in a two-stage immunofluorescence assay, and such staining was not blocked by M-MuLV protein. Also, intact A-MuLV-transformed cells absorbed much of the reactivity of certain anti-AbT sera for P120. Thus a portion of P120 appears to be exposed on the surface of transformed cells. P120 lacks detectable carbohydrate, is not affected by endoglycosidase H, and cannot be labeled by lactoperoxidase-catalyzed iodination. Thus P120 is an unusual surface protein.

Abelson murine leukemia virus (A-MuLV) is a defective, transforming retrovirus isolated from a corticosteroid-treated BALB/c mouse that had been inoculated with the replication-competent virus, Moloney MuLV (M-MuLV) (1). A-MuLV has unique biological and biochemical properties that distinguish it from its parent virus. Most importantly, A-MuLV transforms a different class of hematopoietic target cells from M-MuLV (9–11, 13, 17, 18, 21–23), and A-MuLV is capable of transforming fibroblastic cells (19). A-MuLV, like other defective, transforming retroviruses, requires a helper virus (like M-MuLV) to provide its structural proteins and its enzymatic functions for replication (5, 27).

A-MuLV RNA is 5,600 bases long and shares sequence homology with M-MuLV at its 5′ and 3′ ends (A. Shields, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1979). A single stretch of 3,600 nucleotides of sequence not derived from M-MuLV fills the center of the A-MuLV genome.

The only known protein encoded by the A-MuLV genome is one of 120,000 molecular weight called P120 (14, 29). This protein contains serological determinants of the M-MuLV amino-terminal gag gene products—p15, p12, and a portion of p30—fused to a region of approximately 90,000 molecular weight derived from the A-MuLV unique sequences.

In an attempt to make an antiserum that might recognize the unique (non-M-MuLV) determinants on the A-MuLV P120, we have used the one case we could find in which mice would reproducibly reject a syngeneic A-MuLV-transformed cell line. Because xenogeneic sera from animals that have rejected Rous sarcoma virus-induced tumors have been so useful in defining the transforming protein of that virus (3, 12), it seemed possible that syngeneic immunization with A-MuLV-induced tumors would be similarly useful. Risser et al. (16) have already shown the utility of this approach in their description of an A-MuLV-related cell surface antigen detected by antibody-mediated cytotoxity. We have prepared sera that precipitate P120 via its unique determinants and have used the sera in a two-stage immunofluorescence assay to detect an A-MuLV-specific cell surface antigen.

MATERIALS AND METHODS

Cell lines. A-MuLV-transformed BALB/c lymphoid cell lines 2M3 (nonproducer) and 2M3/M (producer of A-MuLV and M-MuLV), C57L/J lymphoid

cell line L1 and subclone L1-2, nonproducer NIH mouse fibroblast line A2, and rat cell line Ab-NRK have been previously described (17, 29).

Cell labeling and immunoprecipitation. All radioactive chemicals were purchased from New England Nuclear Corp., Boston, Mass. Cells were labeled with [^{35}S]methionine (50 μCi/ml) or [^{3}H]leucine (200 μCi/ml) in medium containing 1/100 the normal methionine or 1/50 the normal leucine concentration, respectively. Cells were pulse-labeled (30 min) with ^{14}C-mixed amino acids (25 μCi/ml) in balanced salt solution with glucose or were labeled in complete medium with D-[^{3}H]glucosamine at 100 μCi/ml.

Cells were iodinated by two techniques. Lactoperoxidase-catalyzed iodination at room temperature was performed as previously described (30) employing 500 μCi of Na^{125}I per 10^7 cells, with 50 μg of lactoperoxidase (Calbiochem, grade B). Specific activities were 10^7 to 3×10^7 cpm/10^7 cells. Iodination by ^{125}I-labeled Bolton Hunter reagent was performed at 0°C. Cells (10^5 to 2 $\times 10^7$) were washed with phosphate-buffered saline (pH 7.5), then resuspended in 1 ml of phosphate-buffered saline with 50 to 75 μCi of Bolton-Hunter reagent for 30 min. Cells were washed with phosphate-buffered saline, and excess reagent was quenched with glycine. Specific activities were 3×10^7 to 7×10^7 cpm/ 10^7 cells.

Labeled cells were extracted and clarified for immunoprecipitation as previously described (28, 29). Routinely, 10^7 labeled cells were extracted into 5 ml of lysis buffer (10 mM NaH$_2$PO$_4$-Na$_2$HPO$_4$, pH 7.5; 100 mM NaCl; 1% Triton X-100; 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulfate) at 0°C and clarified at 150,000 $\times$ g for 3 h. One-milliliter fractions were precipitated with normal or immune serum. Samples were analyzed by electrophoresis through 10% polyacrylamide-sodium dodecyl sulfate gels developed by fluorography (2, 7, 8). Films were exposed for 2 to 3 days.

Antisera used included rabbit anti-M-MuLV reverse transcriptase/p15, rabbit anti-M-MuLV gp70, and goat anti-M-MuLV virions, which have been previously described (28, 29).

Membrane immunofluorescence. All sera and media employed were filtered through 0.2-μm filters or clarified at 100,000 $\times$ g for 2 h before use to remove aggregates. Viable cells (2 $\times$ 10^6) were washed twice in RPMI-1640 with 10% heat-inactivated fetal calf serum and suspended at 0°C in 50 μl of normal serum or antiserum for 45 min. Cells were washed by sedimentation through heat-inactivated fetal calf serum, resuspended, and washed with RPMI-10% heat-inactivated fetal calf serum. Pretitrated dilutions of fluorescein-conjugated second-stage antibody (goat anti-mouse immunoglobulin G or goat anti-rabbit immunoglobulin G; Meloy Labs, Springfield, Va.) were mixed with cells for 45 min at 0°C. The cells were washed as above and suspended in RPMI-10% heat-inactivated fetal calf serum to view as wet mounts or, alternatively, in phosphate-buffered saline-50% heat-inactivated fetal calf serum to prepare air-dried, methanol-fixed smears. Slides were viewed with a Leitz Ortholux microscope, and photographs were taken on Kodak Tri-X film. All cell counts represented averages of two separate experiments counting 100 cells each.

Sepharose-coupled M-MuLV virion proteins. A 40-mg sample of unlabeled M-MuLV protein was derived from virions that were purified from culture supernatant fluids by two cycles of sucrose gradient centrifugation in the MIT Cell Culture Center. The virions were lysed with 5% Triton X-100-0.5 M NaCl, dialyzed to pH 8.5 (100 mM NaHCO$_3$), and coupled with 4 g of freeze-dried CNBr-activated Sepharose 4B (Pharmacia Fine Chemical Corp.) overnight at 4°C. [^{35}S]methionine-labeled M-MuLV (10^6 cpm) was added as a tracer. After washing, the final coupled protein was estimated at 2.5 mg/ml of swollen resin.

RESULTS

Production of an antiserum reactive with A-MuLV-specified P120 (anti-AbT). To develop a syngeneic regressor system for production of anti-A-MuLV sera, various cell line and mouse strain combinations were examined. The approach used involved injecting mice with live, in vitro-derived transformed lymphoid cells, monitoring the mice for tumor formation and subsequent rejection, and then challenging them with increasing cell doses. Activity against A-MuLV-specified proteins was determined by direct immunoprecipitation (see below).

Initially, 15 independently derived A-MuLV-transformed lymphoid cell lines of BALB/c origin were injected into syngeneic mice. Progressive, disseminated tumors developed in the majority of the mice, a finding consistent with earlier reports (10, 17, 21). Use of low doses (10^3 cells per mouse) of nonproducer cells given intraperitoneally resulted in 100% mortality in 3 weeks. When low doses of live or mitomycin C-treated cells (injected subcutaneously) did not kill all of the animals, the survivors failed to reject increased doses of cells and did not produce a demonstrable response to A-MuLV proteins. BALB/c-derived H-2^k congenic mice rejected high doses (>10^6 cells per mouse) of BALB/c-derived nonproducer cells but did not respond to A-MuLV proteins.

Because the BALB/c system proved unsuccessful, lymphoid cell lines derived from SWR/ J, C57BRcd/J, C57BL6/J, and C57L/J mice were examined. Groups of 20 mice were injected with two SWR/J and one C57BRcd/J-derived cell line, and all of the mice tested succumbed to the initial inoculum. Even doses of 10^3 cells of the C57BRcd/J nonproducer cell line BR48 killed most of the test animals in 3 to 4 weeks. C57BL6 mice, used to produce anti-Abelson sera by another laboratory (16), failed to reject a dose of 10^5 live syngeneic cells given subcutaneously. Forty percent (2/5) of the animals injected intraperitoneally with 10^5 cells survived this dose and two subsequent challenges. The serum from these mice, however, reacted predominantly with helper virus-specified struc-

tural proteins (data not shown).

With C57L/J mice we were finally successful. C57L/J mice were injected subcutaneously with either a focus-derived syngeneic cell line designated L1 or a subclone of this cell line, L1-2, at doses of 10^5 or 5×10^5 live cells per animal. Eighty-five percent (12/14) of the L1-injected mice survived the initial dose, and 100% (5/5) of the initial L1-2 group and 90% (27/30) of a larger second group survived. The initial inoculum grew in the mice as evidenced by the development of small (1- to 3-cm) nodules at the injection site about 1 week postinoculation; these growths disappeared by 2 to 2.5 weeks postinjection. Animals surviving the initial cell dose rejected subsequent intraperitoneal challenges of up to 10^7 cells per mouse.

Specificity of anti-Abelson tumor sera (anti-AbT): reactivity with P120. Serum samples collected from C57L/J animals 10 to 14 days after the second and subsequent tumor challenge doses were tested for their immunoprecipitation specificity. Extracts of 2M3/M cells (a BALB/c, A-MuLV-transformed, producer cell line) pulse labeled for 30 min with [^{35}S]methionine were used as the test antigen. This cell line expresses both the A-MuLV-encoded P120 and the major structural protein precursors of the helper virus (M-MuLV). Screening of serum samples of individual mice taken after the second tumor challenge showed that all animals had a response to the P120 protein of A-MuLV, but the intensity varied widely (Fig. 1). Animals numbered 1 to 12 were injected and boosted with cell line L1, and numbers 13 to 17 were injected with subclone L1-2. The presence of detectable antibody reactive to the M-MuLV core precursor (Pr65^{gag}) and envelope precursor (Pr80^{env}) varied but was more pronounced in the group injected with L1. During repeated cycles of immunization, these differences in precipitating antibody patterns were minimized and a common pattern of prominent response to P120 and Pr80^{env} and weak or absent response to Pr65^{gag} was found. Only antibody that could bind to the *Staphylococcus aureus* immune adsorbent was monitored in the above screenings, but subsequent testing of serum pools employing goat anti-mouse immunoglobulin in a two-stage precipitation assay did not reveal any additional specificities (data not shown). Tests with [^{35}S]methionine-labeled extracts of the L1 or L1-2 cell lines also did not reveal any additional specificities or unusual quantitative differences between these A-MuLV-transformed cell lines and those of other strains (data not shown).
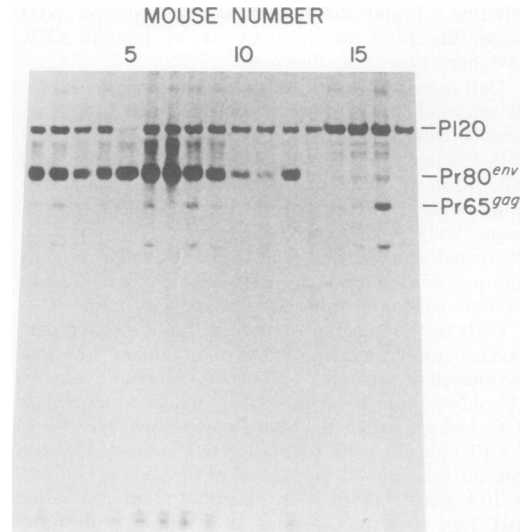
Lack of other A-MuLV-related products.

FIG. 1. *Screening of anti-AbT sera from individual mice. Mice of the C57L/J strain were inoculated with viable cells of the in vitro-transformed clonal lymphoid cell line L1 (mice no. 1 to 12) or a subclone, L1-2 (mice no. 13 to 17). Serum was harvested 12 days after the second inoculation, and 2.5-μl portions were tested by immunoprecipitation of extracts of 2M3/M cells labeled for 30 min with [^{35}S]methionine. Precipitates were collected on S. aureus immunoadsorbent (7) and analyzed by electrophoresis through 10% polyacrylamide-sodium dodecyl sulfate gels (8) developed by fluorography (2).*

To search for additional A-MuLV-specified proteins that might lack methionine, we labeled A-MuLV-transformed cells with a variety of protein precursors, or by chemical iodination techniques, and used extracts of these labeled cells in direct immunoprecipitation assays with pools of anti-AbT serum. To simplify the analysis, we used A-MuLV-transformed, nonproducer cell lines, like 2M3, to direct attention to A-MuLV genome expression rather than that of the M-MuLV helper virus. When 2M3 cells were pulse-labeled (1 h) with [^{3}H]leucine (Fig. 2A) or ^{14}C-mixed amino acids (Fig. 2C), or labeled for 18 h with [^{3}H]leucine (Fig. 2B), the only reproducibly immunoprecipitable protein was the P120 protein. Similar tests were done using mouse and rat A-MuLV-transformed nonproducer fibroblast cultures with the same results.

To further evaluate the specificities of the anti-AbT serum, and to search for putative A-MuLV-related proteins that could have extremely low synthetic rates but be stable enough to accumulate within the cells, we used chemical iodination techniques. Lactoperoxidase-catalyzed iodination labels available tyrosine and
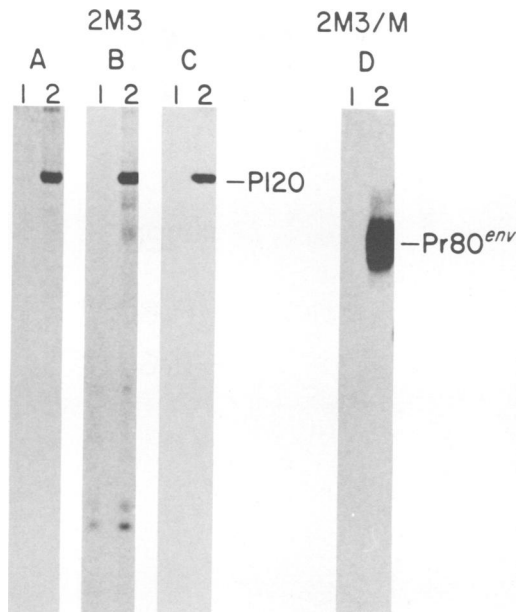
FIG. 2. Specificities of anti-AbT serum tested on metabolically labeled cells. Normal mouse serum (lane 1) was compared to pooled anti-AbT serum (lane 2) for immunoprecipitation of proteins from (A) 2M3 cells labeled for 1 h with [³H]leucine; (B) 2M3 cells labeled for 18 h with [³H]leucine; (C) 2M3 cells labeled for 1 h with ¹⁴C-mixed amino acids; or (D) 2M3/M cells labeled for 5 h with D-[³H]glucosamine. Samples were analyzed as described in Fig. 1.

FIG. 3. Specificities of anti-AbT serum tested on chemically iodinated cells. Viable 2M3 cells (A) or 2M3/M cells (B) were labeled with Na¹²⁵I by the lactoperoxidase technique, or, alternatively, 2M3 cells (C) and 2M3/M cells (D) were labeled with the ¹²⁵I-labeled Bolton-Hunter reagent. Extracts were immunoprecipitated with anti-AbT serum, and precipitates were analyzed as described in Fig. 1.

histidine residues on peripheral membrane proteins. It has been used to label the MuLV env gene product, gp70, on the virion and cell surface (6, 30, 31). A-MuLV-transformed nonproducer line 2M3 (Fig. 3, lane A) and its M-MuLV-superinfected derivative 2M3/M (Fig. 3, lane B) were labeled to high specific activity, and extracts were immunoprecipitated with anti-AbT serum. Only gp70 on the producer cell line 2M3/M was detected. Neither P120 or other potential A-MuLV proteins were labeled.

We extended our studies to a reagent that could penetrate into cells and label proteins—the Bolton-Hunter reagent. It reacts rapidly with free amino groups and will function at pH 7.5 and low temperature. Intact 2M3 (Fig. 3C) and 2M3/M (Fig. 3D) cells were labeled at 0°C with ¹²⁵I-labeled Bolton-Hunter reagent, washed, and extracted for immunoprecipitation with anti-AbT serum. P120 was now labeled in both cell lines, as were the Pr80^{env} and Pr65^{gag} precursors in the 2M3/M cells. No additional A-MuLV-related proteins were detected.

These studies do not rule out the possibility of an additional protein encoded by A-MuLV;
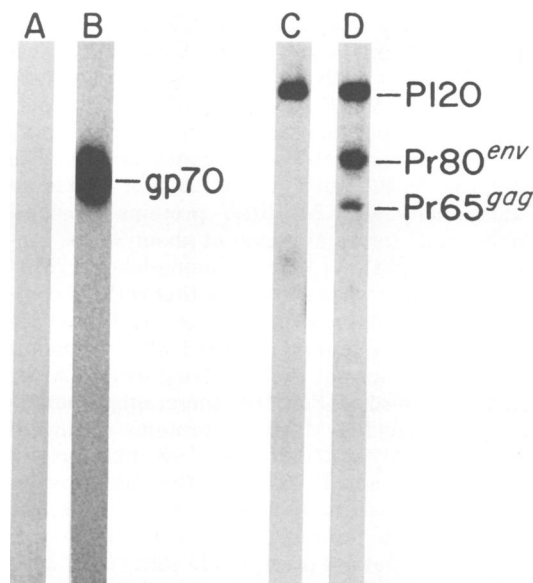
they show, however, that the anti-tumor antiserum we have prepared strongly precipitates P120 and that P120 is the only A-MuLV-specific protein detected even when various metabolic and chemical labels are used.

Lack of carbohydrate on P120. In several attempts, we have been unable to label P120 or any additional A-MuLV-related protein with sugar precursors of glycoproteins. When 2M3/M cells (the nonproducer line superinfected with M-MuLV) were labeled with D-[³H]glucosamine for 5 h (Fig. 2D), the Pr80^{env} precursor was heavily labeled but P120 was not labeled. The faint band(s) at 90,000 to 95,000 molecular weight is the glycosylated form of the M-MuLV gag gene (gp90^{gag}) similar to that described for other ecotropic MuLV strains (26). In other experiments, [³H]mannose did not label P120. Furthermore, P120 pulse-labeled with [³⁵S]methionine and isolated by immunoprecipitation was not sensitive to the action of endo-β-N-acetylglucosaminidase H, an enzyme that cleaves the high mannose structures from recently made glycoproteins and thus markedly changes their electrophoretic mobility (25, 32). Hence, no detectable fraction of P120 undergoes carbohydrate addition.

Reactivity with the A-MuLV-specific portion of P120. Because P120 shares serological determinants with the N-terminal *gag* gene products (p15, p12, and part of p30), we performed competition experiments to determine whether the anti-AbT serum contains antibodies reactive to the portion of P120 that shares no antigenicity with M-MuLV proteins (presumably the C-terminal region of about 90,000 molecular weight). A [^{35}S]methionine-labeled 2M3/ M cell extract was incubated either without competing protein, or with 50 or 150 μg of lysed M-MuLV virions, and precipitated with a constant amount of normal mouse serum (Fig. 4A) or anti-AbT serum (Fig. 4B). Increasing amounts of the unlabeled M-MuLV proteins competed out the ability of the anti-AbT serum to precipitate Pr80^{env} and Pr65^{gag}, but the P120 precipitation was essentially unaffected. We have also been able to proteolytically cleave P120 into fragments that are precipitable with either anti-*gag* gene product reagents or anti-AbT serum but not both (unpublished data).

A-MuLV cell surface antigen. To test whether anti-AbT serum could react with a cell surface antigen on A-MuLV-transformed cells, we have used a two-stage immunofluorescence procedure with intact viable cells as our test antigen. Sera with specificity for the helper virus M-MuLV gene products gp70, p30, and p15 as well as anti-AbT serum have been tested on several A-MuLV-transformed nonproducer cell lines as well as on producer cells containing both the A-MuLV and M-MuLV gene products (summarized in Table 1).

Only the anti-AbT serum was able to stain the cell membrane of A-MuLV-transformed nonproducer lines such as 2M3 (Fig. 5). Sera with specificity to the *gag* gene-related portion of P120, like anti-p15 and anti-p30, although able to precipitate P120 from lysed cells (29), could not stain the surface of viable cells. Absorption of anti-AbT serum with M-MuLV proteins coupled to Sepharose did not diminish the staining on A-MuLV-transformed nonproducer cells.

Absorption of anti-AbT serum with one A-MuLV-transformed nonproducer cell line, however, removed the ability to stain a second A-MuLV nonproducer cell line. A 1:20 dilution of the pool of anti-AbT serum used in Fig. 5 was absorbed with intact, viable A2 cells (a transformed NIH/3T3 fibroblast nonproducer). The absorbed serum and a control dilution were clarified and assayed for their ability to stain the surface of 2M3 cells (a BALB/c lymphoid nonproducer cell) and 2M3/M (the M-MuLV-superinfected counterpart; Fig. 6A and B). The ab-
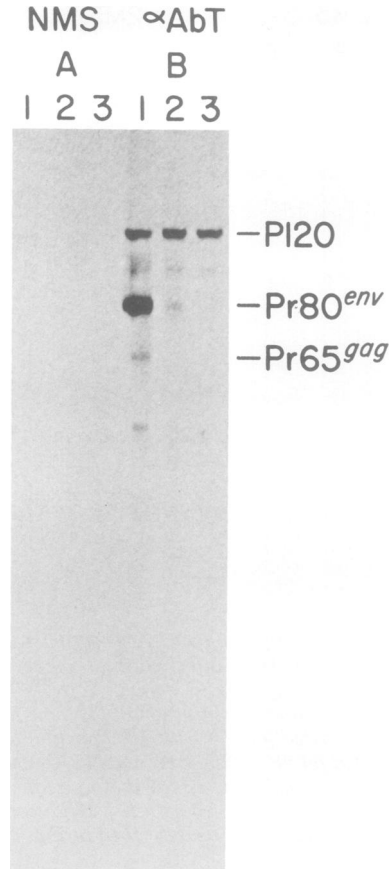

FIG. 4. *Demonstration of activity for the unique portion of P120 in anti-AbT serum. Extracts of 2M3/ M cells labeled for 30 min with [^{35}S]methionine; cells were immunoprecipitated with 2.5 μl of (A) normal mouse serum (NMS) or (B) anti-Abelson tumor serum (αAbT). Lane 1 had no competing protein; lane 2 had 50 μg and lane 3 had 150 μg of M-MuLV virion proteins included in the lysate. Samples were collected and analyzed as described in Fig. 1.*

sorbed serum was markedly reduced in its ability to stain the nonproducer cell line 2M3, which expresses only P120, but retained the ability to stain the M-MuLV-superinfected 2M3/M line, which also expresses at the cell membrane the M-MuLV *env* gene products gp70 and p15E. This difference was reflected in residual immunoprecipitation capability of the control and absorbed anti-AbT serum. Because A2 cells do not express the M-MuLV *env* gene, after absorption with these cells there was no detectable difference in the ability of anti-AbT serum to precipitate the Pr80^{env} precursor from 2M3/M cells (Fig. 6C). Absorption with A2 cells, however, caused a marked decrease in precipitating antibody for P120; after absorption only 10 to 20%

TABLE 1. *Cell surface fluorescence on A-MuLV-transformed cells*[a]

Serum	Percent of fluorescent cells in cell line[b]			
	A2	AbNRK	2M3	2M3/M
Normal mouse serum	<1	<1	<1	<1
Anti-AbT	>95 (++)	>95 (+++)	>95 (+++)	>95 (++++)
Anti-AbT absorbed with M-MuLV proteins coupled to Sepharose[c]	>95 (++)	>95 (++)	>95 (+++)	>95 (+++)
Normal rabbit serum	<1	<1	<1	<1
Rabbit anti-M-MuLV p15/ reverse transcriptase	<1	NT[d]	2 (+)	75 (+)
Rabbit anti-M-MuLV p30	<1	NT	<1	>95 (++)
Rabbit anti-M-MuLV gp70	<1	<1	<1	>95 (++++)

[a] Viable cells (10^6 to 2×10^6) were stained for cell surface antigens in suspension at 4°C with 50 μl of normal or immune serum at a final 1:20 dilution. Fluoresceinated goat anti-mouse or goat anti-rabbit immunoglobulin G (Meloy Labs) at 1:15 and 1:40 dilutions, respectively, were used for the second stage. Wet mounts and air-dried, methanol-fixed smears were counted (minimum of 100 cells each) to determine a percentage of positive staining. A subjective quantitation of + (dull) to ++++ (very bright) is shown.

[b] Cell lines: A2, NIH/3T3 nonproducer; AbNRK, normal rat kidney nonproducer; 2M3, BALB/c lymphoid nonproducer; 2M3/M, 2M3 superinfected with M-MuLV.

[c] A 1-ml sample of 1:10-diluted anti-AbT was mixed with 2 ml of wet packed Sepharose 2B coupled with lysed M-MuLV proteins (2.5 mg of final protein bound per ml of Sepharose) for 6 h at 4°C. The serum was recovered by centrifugation and reclarified before use.

of the precipitating activity for P120 was left in different pools (the residual P120 in lane 3, Fig. 6C, is not evident in the print but was detectable on the autoradiogram). Therefore, a variable portion of the antibody to P120 may be reactive with determinants that are not accessible at the cell surface, but the majority could be absorbed by live cells. Similar absorption experiments using M-MuLV-infected NIH/3T3 cells or M-MuLV-infected Moloney sarcoma virus-transformed cells did not remove anti-P120 activity from the anti-AbT serum.

In separate experiments (data not shown), we found that P120 in intact cells was resistant to digestion with the proteases trypsin and chymotrypsin under conditions that previously were shown to remove peripheral proteins like gp70 (30). Such protease treatment did not diminish the ability of anti-AbT serum to stain A-MuLV-transformed cells.

Thus, it is likely that P120, or perhaps a fraction of the P120 molecules, exposes a portion of its 90,000-molecular weight unique region above the cell membrane but that other regions of the molecule, including the *gag* gene-related amino terminus, are buried in or beneath the lipid bilayer and not accessible to antibody molecules. The external region is not, however, detectably labeled by lactoperoxidase-catalyzed iodination and is not as protease sensitive as a peripheral membrane protein like gp70.

DISCUSSION

In the process of rejecting syngeneic tumors caused by A-MuLV-transformed cells, C57L/J mice make a humoral antibody response to the A-MuLV-specified P120 protein. No other A-MuLV-specific protein is detected by such sera, and much of the antibody is directed to the A-MuLV-specific region of P120. The serum detects cell surface antigen by live-cell staining in a two-stage immunofluorescence test. Because P120 is the only A-MuLV protein reactive with the serum, the results imply that at least part of the A-MuLV-specific region of P120 is exposed on the cell surface. The ability of intact cells to absorb much of the reactivity to P120 also argues that the protein is partly exposed on the cell surface.

P120 is not, however, a classic cell surface protein. It does not detectably label with carbohydrate precursors, it is not labeled by lactoperoxidase-catalyzed iodination, it is not cleaved by mild external treatment of cells with protease, and its mobility is not affected by treatment with endoglycosidase H. By contrast, gp70 of MuLV has readily detectable carbohydrate, is iodinated, and is cleaved by protease, and the electrophoretic mobility of its intracellular form is increased by endoglycosidase H treatment (6, 30–32). Many other peripheral membrane proteins also have this set of characteristics. No good model exists for a non-glycosylated protein that is exposed on the cell surface. It is significant, however, that the feline oncornavirus-associated cell membrane antigen (FOCMA) of feline sarcoma virus has many properties in common with the A-MuLV P120 (20, 24) and is not
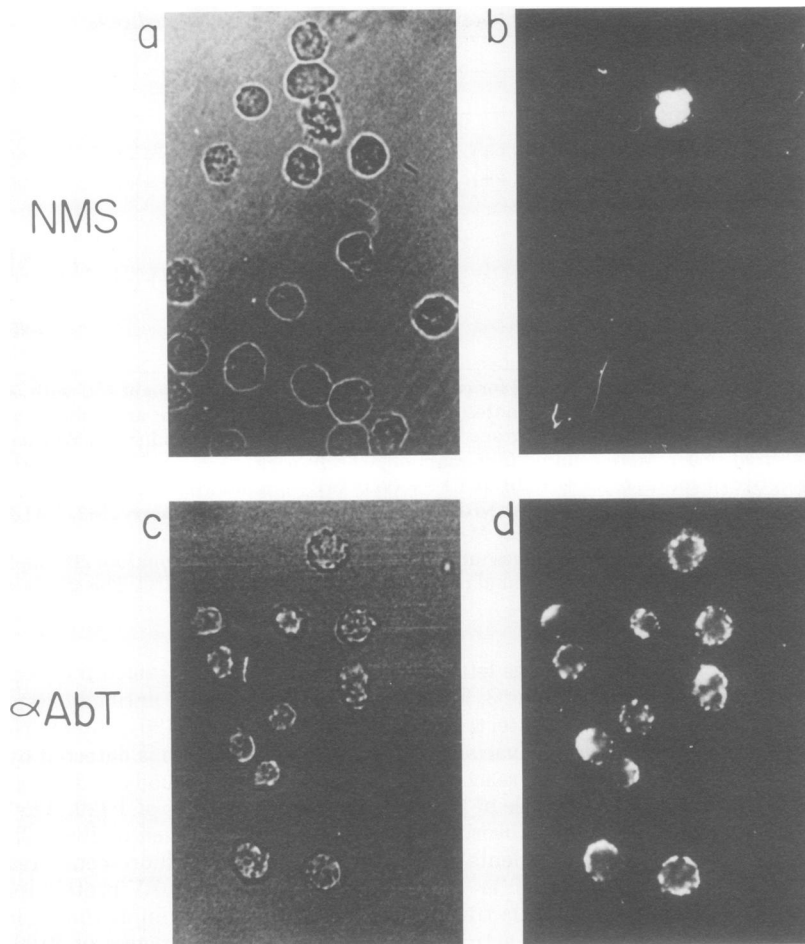
FIG. 5. *Detection of an A-MuLV cell surface antigen by immunofluorescence. Intact, viable 2M3 cells, a clonally derived BALB/c A-MuLV-transformed lymphoid cell line, were tested in a two-stage immunofluorescence assay (see the text) employing normal mouse serum (NMS) or anti-AbT serum (αAbT) at a final dilution of 1:20. After unbound antibody was washed away, fluoresceinated goat anti-mouse immunoglobulin G at 1:15 final dilution was used and wet mounts were photographed. (a) Cells treated with normal mouse serum (NMS), phase contrast; (b) cells treated with normal mouse serum, fluorescence; (c) cells treated with anti-AbT serum, phase contrast; (d) cells treated with anti-AbT serum, fluorescence. Specific fluorescent staining in (d) is seen as small clusters or caps on the outer cell surface. Nonspecific homogeneous staining of a single dead cell is seen in (b).*

glycosylated (Worley and Essex, personal communication). FOCMA is a surface antigen that is synthesized as part of a protein containing both *gag* determinants and a unique region. Also, a non-glycosylated adenovirus-simian virus 40 fusion protein is thought to be expressed at the cell surface (4) and may be important as a transplantation antigen.

It is curious that only C57L/J mice efficiently reject syngeneic A-MuLV-transformed cells. It is not known whether their ability to make anti-AbT antibody is related to their rejection ability. To date we have found no unique feature of the

C57L/J strain or their A-MuLV-transformed cells that can explain the efficient tumor regression we have observed. Risser et al. (15) have described two genetic loci that control the relative resistance to A-MuLV tumor induction. C57L/J is among the resistant strains, but so are C57BR/cdJ and C57BL6/J, which we found much less efficient at rejecting syngeneic tumor challenge. C57L/J transformed cell lines showed no qualitative or quantitative difference in the level or pattern of expression of either the A-MuLV P120 or M-MuLV proteins that we have yet discerned.
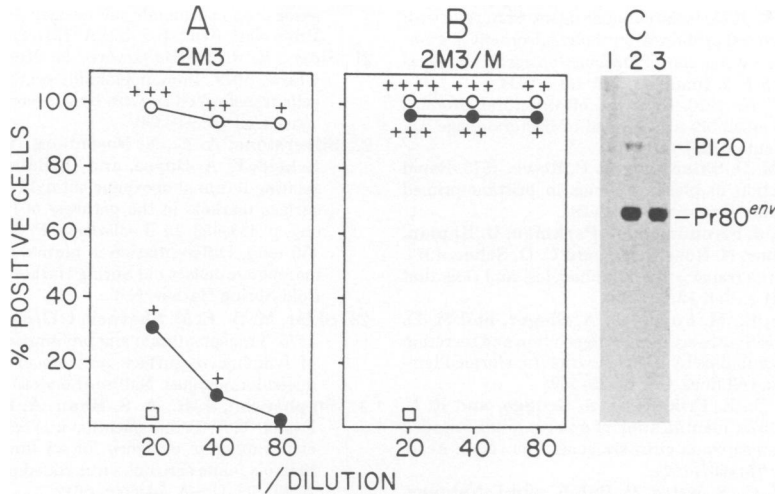
FIG. 6. *Absorption of anti-AbT cell surface reactivity with transformed nonproducer cells. Dilutions of anti-AbT serum, unabsorbed control (O) or absorbed with viable, intact A2 cells (A-MuLV-transformed NIH/3T3 nonproducer cell line) (●) were used to surface stain viable 2M3 cells (A) and 2M3/M cells (B). Absorption was carried out in three sequential cycles using 1 ml of 1:20-diluted serum, 3 × 10⁸ cells per cycle, and a 45-min exposure at 0°C. The percentage of positive cells and a visual estimate of average fluorescence intensity (+ = dull; ++++ = very bright) were plotted and compared to staining with normal mouse serum (□). (C) Immunoprecipitation of 2M3/M cells labeled with [³⁵S]methionine for 30 min. Equivalent amounts of normal mouse serum (lane 1), control unabsorbed anti-AbT serum (lane 2), and absorbed anti-AbT serum (lane 3). Sample analysis was as described in Fig. 1.*

The A-MuLV genome consists of a 5,600-base RNA of which about 3,600 bases are A-MuLV specific and 2,000 bases are derived from M-MuLV (Shields, Ph.D. thesis). P120 is encoded by about 2,700 bases of the A-MuLV-specific region, leaving 900 bases plus some of the M-MuLV sequences as regions that could potentially encode more A-MuLV-related proteins. Use of a second reading frame could make more coding potential available. It is not possible to exclude the existence of a second A-MuLV-specified protein, but no antiserum, whether anti-AbT or anti-M-MuLV, has consistently revealed a second protein. Thus, it appears on present evidence that the A-MuLV genome encodes a single polypeptide and, assuming that A-MuLV transforms through the action of a protein, P120 must be responsible for transforming cells. Whether the presence of part of P120 exposed on the cell surface is an aspect of its transforming activity remains to be demonstrated.

Risser et al. (16) have reported an Abelson tumor cell surface antigen detected by a cytotoxicity-absorption assay and have shown the presence of a cross-reacting antigen in normal lymphoid tissues (particularly bone marrow) of BALB/c mice and closely related strains. Whether the antigenic specificity(ies) monitored in those studies is identical to the anti-P120 activity defined in the present analysis is not clear.

LITERATURE CITED

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