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Abelson Murine Leukemia Virus-Induced Tumors Elicit Antibodies Against a Host Cell Protein, P50

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When BALB/c mice were injected with a syngeneic cell line transformed by Abelson murine leukemia virus (A-MuLV), the tumor was usually lethal. In sera from tumor-bearing mice, and at highest levels in sera from mice that reject their tumors, was an antibody that immunoprecipitates a specific protein from [^{35}S]-methionine-labeled A-MuLV-transformed BALB/c cells. This protein was not the previously characterized A-MuLV-specific protein (P120) but a 50,000-molecular-weight protein (P50). Such sera may also immunoprecipitate P120, but no other protein was reproducibly precipitated by them. A monoclonal antibody (RA3-2C2) that has been shown to stain normal B-lymphocytes also selectively immunoprecipitated P50. P50 was present in A-MuLV-transformed lymphoid and fibroblastic cells of a variety of mouse strains. One A-MuLV-transformed cell line had a very low P50 level, the L1-2 tumor of C57L origin. This tumor was previously shown to be rejected by C57L mice and is used to produce anti-P120 (anti-AbT) sera. P50 was not a Moloney MuLV protein and was found at low levels in normal cells or cells transformed by agents other than A-MuLV; thus, it was probably a host cell protein whose concentration was selectively accentuated by A-MuLV transformation. P50 was phosphorylated and, by using indirect immunofluorescence, anti-P50 serum stained live A-MuLV-transformed cells. The protein was not glycosylated and did not label by lactoperoxidase-catalyzed iodination. Thus, P50 was very like P120 in its cellular localization and properties, but it did not exhibit protein kinase activity in vitro. The selective accentuation of this protein in A-MuLV transformants and its strong antigenicity in syngeneic animals suggest that it is a unique and functionally important protein.

Abelson murine leukemia virus (A-MuLV) can transform certain murine bone marrow cells into continuous cell lines (18–20). When these lines are injected into syngeneic mice, the cells usually grow progressively and ultimately kill their host (18). Only one cell line, the L1-2 line from C57L mice, is reproducibly rejected by its host, and after multiple challenges animals make antisera that recognize the A-MuLV protein (28). In the prototype A-MuLV strain, the viral protein is called P120 and is the only known product of the viral genome (15, 29). A-MuLV is a defective virus and must be propagated with a helper virus, usually Moloney MuLV (M-MuLV); infected cells, therefore, often contain the helper virus proteins $Pr65^{gag}$ and $Pr80^{env}$.

We have now found that sera taken from mice with growing tumors occasionally contain antibodies against another protein. This protein, P50, is a cellular, not a viral, protein that is peculiarly antigenic.

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MATERIALS AND METHODS

Mice. BALB/cN female mice between 8 and 12 weeks of age from our colony were used in the present experiments.

Cell lines. The following A-MuLV-transformed cell lines of BALB/c origin were used: 2M3, a nonproducer; 2M3/M, a producer of A-MuLV and M-MuLV; 3-1, 1-8, 18-48, 18-81, and FL-E1 (18, 23); 70Z, a BALB/c chemically induced pre-B-lymphocyte line (13), Sp2/0, a nonproducer variant of the P3/X63Ag8 myeloma (7); and RL♂1, a BALB/c radiation-induced T-cell leukemia (21).

The following cell lines of C57L/J origin were used: L1-2, an A-MuLV-transformed lymphoid cell line (28); and L691, a radiation-induced leukemia (1).

The following cell lines of NIH/3T3 derivation were used: ANN-1, A-MuLV-transformed nonproducer (22); A2, A-MuLV-transformed nonproducer; and A2/M, A2 superinfected with M-MuLV.

Lymphoid cell lines were grown in RPMI-1640 enriched with 10% heat-inactivated fetal calf serum and 2×10^{-5} M β-mercaptoethanol. Fibroblastic cell lines were grown in Dulbecco-modified Eagle medium enriched with 10% calf serum.

Immune sera. Anti-P50 antibodies were obtained from BALB/c mice bearing syngeneic A-MuLV-in-

duced tumors. Anti-P120 antibodies were obtained from C57L/J mice that had rejected the syngeneic L1-2 A-MuLV-induced tumor cells and were further immunized with the same tumor cells (28). Goat anti-M-MuLV virion protein antibodies were provided by the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. The RA3-2C2 monoclonal anti-P50 antibody was obtained after fusion of S194/Bu-1 mouse myeloma cells with spleen cells obtained from rats hyperimmunized with the mouse cell line RAW112, induced by infection of BALB/c mice with A-MuLV (R. Coffman and I. Weissman, manuscript in preparation). RA3-2C2 was chosen for study because the antibodies showed surface binding to the A-MuLV-induced tumor cells.

Cell labeling and immunoprecipitation. Radioactive chemicals were purchased from New England Nuclear Corp., Boston, Mass. Cells were washed several times with phosphate-buffered saline and then suspended in the labeling medium at a concentration of 10^7 cells per ml. When labeled with [^{35}S]methionine, cells were resuspended in Dulbecco-modified Eagle medium without methionine and enriched with 10% dialyzed heat-inactivated fetal calf serum and 125 μCi of [^{35}S]methionine per ml. Cells were incubated for 1 h at 37°C. When labeled with [^{3}H]leucine, 200 to 400 μCi/ml was added to cells resuspended in Dulbecco-modified Eagle medium without leucine and incubated for 1 to 2 h at 37°C. When labeled with $^{32}PO_4$, the cells were resuspended in Dulbecco-modified Eagle medium without PO_4, 300 μCi of $^{32}PO_4$ per ml was added, and the cells were incubated for 5 h at 37°C.

Cells were iodinated by two techniques. Lactoperoxidase-catalyzed iodination at room temperature was performed by adding 500 μCi of Na^{125}I per 2×10^7 cells with 50 μg of lactoperoxidase (grade B, Calbiochem, La Jolla, Calif.). Iodination by ^{125}I-labeled Bolton-Hunter reagent was performed at 0°C. Cells (2×10^7) were mixed with 75 μCi of ^{125}I-labeled Bolton-Hunter reagent, suspended in phosphate-buffered saline, and incubated for 1 h on ice. For both of these techniques, cells were washed after labeling with phosphate-buffered saline containing bovine serum albumin (1 mg/ml) to quench excess reagent.

Labeled cells were washed in phosphate-buffered saline, and the pellet of cells was extracted into 5 ml of lysis buffer (10 mM NaH_2PO_4-Na_2HPO_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 × g for 2 to 3 h as previously described (29). Fractions of 0.5 to 1 ml of cell lysate were immunoprecipitated with normal or immune serum. Antigen-antibody complexes were collected by binding to *Staphylococcus aureus* (6). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on the discontinuous stacking system of Laemmli (9).

Membrane immunofluorescence. All sera and media employed were filtered through 0.2-μm filters. Viable cells were washed several times in phosphate-buffered saline with 10% heat-inactivated fetal calf serum and finally resuspended to 0.5×10^6 cells per 50 μl, and 5 μl of normal or immune serum was added. Cells were incubated for 30 min on ice, then washed twice with cold phosphate-buffered saline with 10% heat-inactivated fetal calf serum, and resuspended in 50 μl of pretitrated, fluorescein-conjugated second-stage antibody. The second-stage antibody was goat anti-mouse immunoglobulin G (Meloy Laboratories, Springfield, Va.) when mouse sera were used and rabbit anti-rat immunoglobulin G when the hybridoma reagent was used.

Partial protease digestion. Partial protease digestion was performed by the method of Cleveland et al. (3). Certain batches of sera and RA3-2C2 immunoprecipitate a single protein band; for these cases protein digestion was performed directly after immunoprecipitation of the proteins. *S. aureus* V-8 enzyme was added to the immunoprecipitated protein and incubated at 37°C for 1 h. The reaction was stopped by adding 2× sample buffer and boiling for 2 min. Samples were then analyzed by electrophoresis through 10% polyacrylamide-sodium dodecyl sulfate gels as described above.

RESULTS

Subcutaneous injection of 5×10^4 2M3/M cells (an A-MuLV-transformed, producer cell line) into syngeneic BALB/c mice produced a 95 to 100% tumor take within 10 to 12 days, which was usually lethal within another 20 to 30 days. Sera taken at a late stage of tumor development from some of these mice could immunoprecipitate a 50,000-molecular-weight protein (P50) from lysates of [^{35}S]methionine-labeled 2M3/M cells (results with sera from 21 individual mice are shown in Fig. 1). Because the P50 happens to migrate at the leading edge of a large band of mouse immunoglobulin, its migration rate often seems slightly variable. By examining stained gels, it is evident that the variability is a consequence of varying amounts of immunoglobulin.

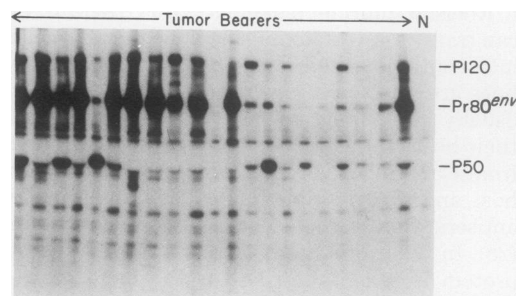

FIG. 1. *Detection of anti-P50 activity. BALB/c mice were inoculated subcutaneously with 5×10^4 viable 2M3/M syngeneic A-MuLV-transformed lymphoid cells, and individual serum samples obtained from 21 mice were screened. Mice were kept in groups of four to five. Serum was collected from tumor bearers 3 weeks after tumor appearance. Portions of 5 μl were used to immunoprecipitate extracts of [^{35}S]methionine-labeled 2M3/M cells. Normal serum (N) of BALB/c mice was also tested. Precipitates were collected on S. aureus and analyzed by electrophoresis through a 10% polyacrylamide-sodium dodecyl sulfate gel.*

The injected tumor cells contained both P120, the A-MuLV protein, and helper M-MuLV proteins ($Pr80^{env}$ and $Pr65^{gag}$); thus, some sera, besides precipitating P50, also precipitated P120, $Pr80^{env}$, or occasionally $Pr65^{gag}$. Sera collected from mice at early stages of tumor development failed to immunoprecipitate any specific proteins. We will call sera that precipitate P50 "TB sera" to denote their origin in tumor-bearing animals.

Is P50 related to the M-MuLV viral genome? To determine whether P50 was a viral or a cellular protein, we first examined the possibility that it was encoded by an M-MuLV genome in the transformed cells. Extracts of [^{35}S]methionine-labeled 2M3/M cells were, therefore, precipitated with a limiting amount of TB serum, and excess unlabeled M-MuLV proteins were added as competitors. The virion proteins failed to block precipitation of P50 by TB sera (Fig. 2, lanes a through d), but they did block the immunoprecipitation of M-MuLV-specific products ($Pr80^{env}$, $Pr65^{gag}$, and a 50K M-MuLV protein) (Fig. 2, lanes e through h) evident in cells infected with M-MuLV and immunoprecipitated with goat anti-M-MuLV sera.

In a separate test of the origin of P50, lysates of 2M3 cells (nonproducer cells transformed by A-MuLV) were immunoprecipitated with normal mouse serum, TB serum, or goat anti-M-MuLV serum. Normal serum precipitated no detectable proteins; TB serum precipitated a faint band of P120 and a strong one of P50, and goat anti-M-MuLV precipitated only P120 (Fig. 2, lanes i through k). In addition, sera of mice bearing a 2M3 tumor also immunoprecipitated the P50 protein from A-MuLV-transformed nonproducer cell lines (unpublished data). Thus, the P50 protein immunoprecipitated from A-MuLV-transformed cell lines does not appear to share immunological determinants with M-MuLV proteins.

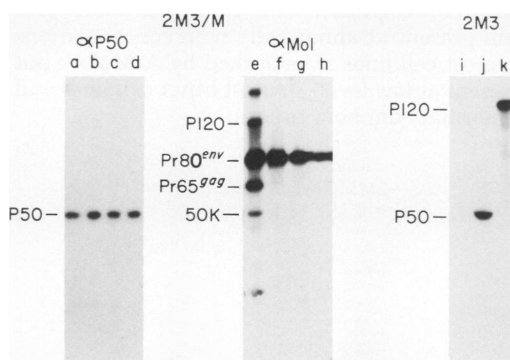

FIG. 2. *Lack of blocking of P50 by M-MuLV virion proteins. Extracts of [^{35}S]methionine-labeled 2M3/M cells were immunoprecipitated with TB serum (lanes a through d) or with goat sera reactive with M-MuLV virion proteins (lanes e through h) in the presence of the following concentrations of M-MuLV virion proteins: lanes a and e, 0; lanes b and f, 50 μg; lanes c and g, 75 μg; and lanes d and h, 100 μg. Extracts of [^{35}S]methionine-labeled 2M3 cells were also immunoprecipitated with (i) normal serum, (j) TB serum, or (k) goat anti-M-MuLV virion protein serum.*

Presence of P50 protein in other A-MuLV-transformed cell lines. To test whether P50 is generally present in A-MuLV-transformed cell lines, a variety of such lines was examined. The first group of cell lines tested were BALB/c lymphoid cell lines induced by an in vitro infection of bone marrow cells with A-MuLV. Most of these cell lines were previously characterized as pre-B-lymphocytes (14, 23, 24) and had been found to contain the P120 A-MuLV protein. Cell lines 18-48, 3-1, 1-8, 18-81, FL-E1, and 2M3/M contained the P50 protein, as demonstrated by immunoprecipitation with TB sera (Table 1). Cell line 37-2, an A-MuLV-induced lymphoid cell line of C57BL/6 origin, also had P50 (Fig. 3). This cell line contains a P160 A-MuLV protein rather than the P120 found in other lines; this is a consequence of the A-MuLV virus strain used to produce the cell line (N. Rosenberg, O. Witte, and D. Baltimore, Cold Spring Harbor Symp. Quant. Biol., in press). Another lymphoid cell line, L1-2 of C57L origin (28), had either no detectable P50 when lysates of [^{35}S]methionine-labeled cells were immunoprecipitated with two different batches of TB sera or a very faint band (Fig. 3). P50 was also undetectable when a [^{3}H]leucine-labeled L1-2 cell lysate was immunoprecipitated with TB sera. The L1-2 cell line demonstrates a clear band of P120 when it is immunoprecipitated with anti-AbT sera (the sera of C57L mice that have rejected the L1-2 tumor [28]). Anti-AbT sera were characterized previously as precipitating P120, but P50 was not detected. It appears from the present result that the lack of anti-P50

TABLE 1. *Presence of P50 in BALB/c A-MuLV-transformed lymphoid cell lines*

Cell line	Origin	P120[a]	P50[a]
2M3/M	Bone marrow	++	+++
18-48	Bone marrow	++	++
18-81	Bone marrow	++	++
3-1	Bone marrow	++	++
1-8	Bone marrow	++	++
FL-E1	Fetal liver	++	+

[a] A band of P120 or P50 was evident in extracts of cell lines and was estimated as: +++, equivalent to a radioactive protein band of 3,000 cpm; ++, equivalent to a radioactive band of 1,000 to 2,000 cpm; and +, equivalent to a radioactive protein band of less than 1,000 cpm.

antibodies in anti-AbT sera could be a consequence of the low amount of P50 on L1-2 cells. Occasional anti-AbT sera do precipitate P50, implying that L1-2 may have some P50 (see below). Whether the reproducible rejection of L1-2 tumors by C57L mice is a consequence of the lack of P50 on these cells is under investigation.

Another group of cell lines tested for P50 were A-MuLV-transformed fibroblasts of NIH/Swiss origin. TB sera immunoprecipitated a P50 protein from lysates of two [^{35}S]methionine-labeled nonproducer cell lines, ANN-1 (Fig. 3) and A2 (unpublished data). The amount of P50 detected in ANN-1 and A2 was significantly lower than that in A-MuLV-induced lymphoid lines tested, but was much higher than in uninfected NIH/3T3 fibroblasts, where at most a very faint band of P50 was present. After a 1-h pulse and chases for 1, 2, and 4 h, P50 was present at a greatly increased concentration in ANN-1 cells compared with NIH/3T3 cells. Thus, both fibroblastic and lymphoid A-MuLV-transformed cell lines contain a P50 protein that is specifically immunoprecipitated with TB sera.

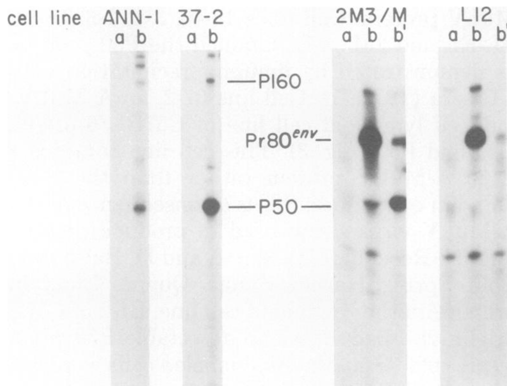

FIG. 3. *Presence of P50 in A-MuLV-transformed fibroblastic cells and cells of different mouse strains. Cells tested were: ANN-1, fibroblastic cells of NIH/3T3 origin; 37-2, lymphoid cells of C57BL/6 origin; and L1-2, lymphoid cells of C57L/J origin. Extracts were precipitated with (a) normal serum, (b) TB serum, and (b') a separate TB serum.*

Detection of the P50 in non-A-MuLV-transformed cell lines and normal lymphoid cells. The following group of cell lines, all produced by means other than A-MuLV transformation, were examined for the presence of P50. They were: RL♂1, a radiation-induced T-cell leukemia of BALB/c origin (1); 70Z, a chemically induced pre-B lymphoid cell line (13); Sp2/0, a nonproducer variant of the mouse myeloma P3/X63Ag8 (7); and L-691, a radiation-induced T-cell leukemia of C57L mice (1). All of these lines gave rise to a very faint band of P50 that was detected on autoradiograms after a very long exposure (Fig. 4). TB sera immunoprecipitated a heavy band of P50 protein from 2M3 cells under the same experimental conditions. A faint band of P50 was also detected in extracts of [^{35}S]methionine-labeled normal tissues (thymus, spleen, bone marrow, lymph nodes, or fetal liver cells) immunoprecipitated with TB sera. Figure 4 illustrates an example of labeled BALB/c thymus cells; for this experiment 10-fold more cells were used than for 2M3, showing how much less P50 is present in normal cells than in A-MuLV-transformed cells.

These results suggest that the P50 specifically immunoprecipitated with TB sera is a host protein present at abnormally high concentrations in most cell lines transformed by A-MuLV, but present at low levels in most other cell lines and in normal lymphoid tissues.

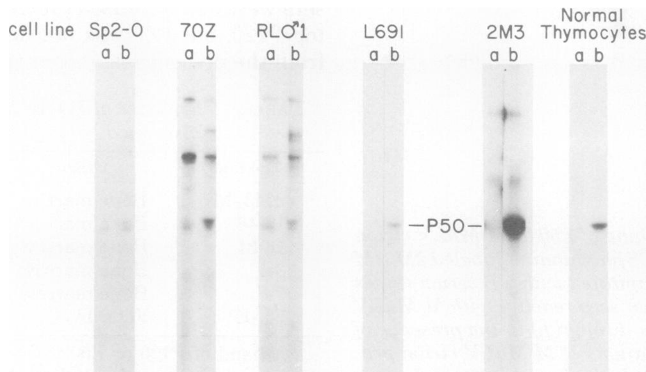

FIG. 4. *Presence of P50 in cells transformed by means other than A-MuLV and in normal thymocytes. Extracts of [^{35}S]methionine-labeled Sp2/0, 70Z, RL♂1, L-691, and 2M3 cells were immunoprecipitated with (a) normal serum or (b) TB serum. Thymus cells (10^8) of BALB/c mice were also labeled with [^{35}S]methionine, lysed, precleared with S. aureus, and immunoprecipitated with (a) normal serum or (b) TB serum.*

Characterization of P50. To test whether P50 is a phosphoprotein, 2M3 cells were labeled for 5 h with $^{32}PO_4$, and a cell lysate was prepared. TB serum immunoprecipitated a labeled 50,000-molecular-weight protein from the lysate as well as the A-MuLV P120 (Fig. 5, lane c). The P120 phosphorylated A-MuLV protein was also detected when this cell lysate was immunoprecipitated with anti-AbT serum (lane b). No phosphoproteins could be detected when normal serum was used (lane a). Labeling the same cells with [^{3}H]leucine and immunoprecipitation with the same sera showed marker bands of P120 and P50 (Fig. 5).

To determine whether determinants of P50 were expressed on the exterior surface of cells, live 2M3 (A-MuLV-transformed, nonproducer) cells were incubated on ice with TB or normal serum and then reacted with a fluoresceinated goat anti-mouse antiserum. Fluorescence was detected on the cell surface as a ring of staining (Fig. 6a). The TB serum used in this experiment contained some anti-P120 activity that could have contributed to the cell surface labeling. Thus, we repeated the experiment with the monoclonal anti-P50 antibody (RA3-2C2) described below and again found staining. This reagent also stained L1-2 cells, although weakly.

Because P50 appears to be a membrane protein, it was of interest to test whether it is also a glycoprotein. To this end, a [^{35}S]methionine-labeled 2M3/M cell lysate was immunoprecipitated with TB or goat anti-M-MuLV serum and then treated with endo-β-acetylglucosaminidase H, an enzyme that cleaves off the mannose-containing carbohydrate structures from recently made glycoproteins (26). If P50 were a glycoprotein, such treatment should change its electrophoretic mobility. No change, however, was evident in the mobility of P50 after such treatment (Fig. 7, lanes a and b). The experiment was internally controlled by the increased mobility of $Pr80^{env}$ of M-MuLV after treatment (Fig. 7, lanes a and b). Thus, although P50 is a membrane protein, it is apparently not glycosylated. In its surface localization, phosphorylation, and lack of glycosylation, P50 is like the A-MuLV P120 (28).

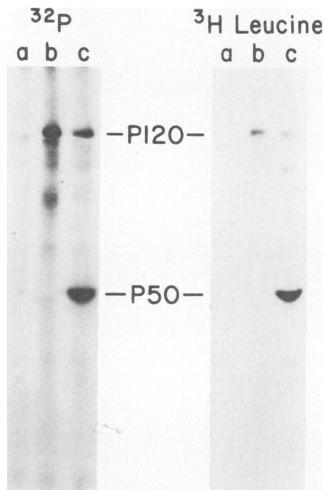

FIG. 5. *In vivo phosphorylation of P50. 2M3 cells were labeled for 5 h with $^{32}PO_4$, and a cell lysate was immunoprecipitated with (a) normal serum, (b) anti-AbT serum, and (c) TB serum. [^{3}H]leucine-labeled 2M3 cells were also immunoprecipitated with the same antibodies.*

Because P50 is expressed on the cell surface, it seemed possible that it could be labeled by lactoperoxidase-catalyzed iodination. When 2M3/M cells were labeled in this way, however, the only iodinated protein that could be detected was the viral gp70 (Fig. 7, lanes c and d). With 2M3 cells, labeling of P50 was also not detectable. P50 could be iodinated when 2M3/M cells were labeled with Bolton-Hunter reagent (Fig. 7, lanes e and f). Under these conditions P120 and $Pr80^{env}$ were also iodinated. This reagent penetrates into cells and reacts rapidly with free amino acid groups.

Antisera that precipitate P50. The TB sera used in the studies described above were not the only sera that precipitated P50. Some (2 of 80) BALB/c mice rejected the syngeneic A-MuLV-induced tumor, 2M3/M, and became immune to further challenge: their sera contained high titers of antibody that precipitated P50 from 2M3/M cells, but not L1-2 cells (Fig. 8, lanes c). Also, certain anti-P120 antibody preparations made in C57L mice (28) have anti-P50 activity.

More importantly, however, antibodies from quite different sources also precipitated P50. One highly active anti-P50 serum was the monoclonal antibody RA3-2C2 derived by immunizing rats with murine A-MuLV-induced tumor cells (Coffman and Weissman, in preparation). This monoclonal antibody was shown to stain all B-lymphocytes, but not thymocytes. The antibody precipitated a 50,000-molecular-weight protein from 2M3/M cells, but no detectable protein from L1-2 cells (Fig. 8, lanes d).

To examine whether RA3-2C2 actually reacted with the same protein as did TB sera, the P50 bands precipitated by the sera were analyzed by partial protease digestion and electrophoresis (Fig. 9). There was complete identity of partial cleavage products (lanes b and d), and the protein precipitated by serum from BALB/c mice that had rejected tumors also gave the

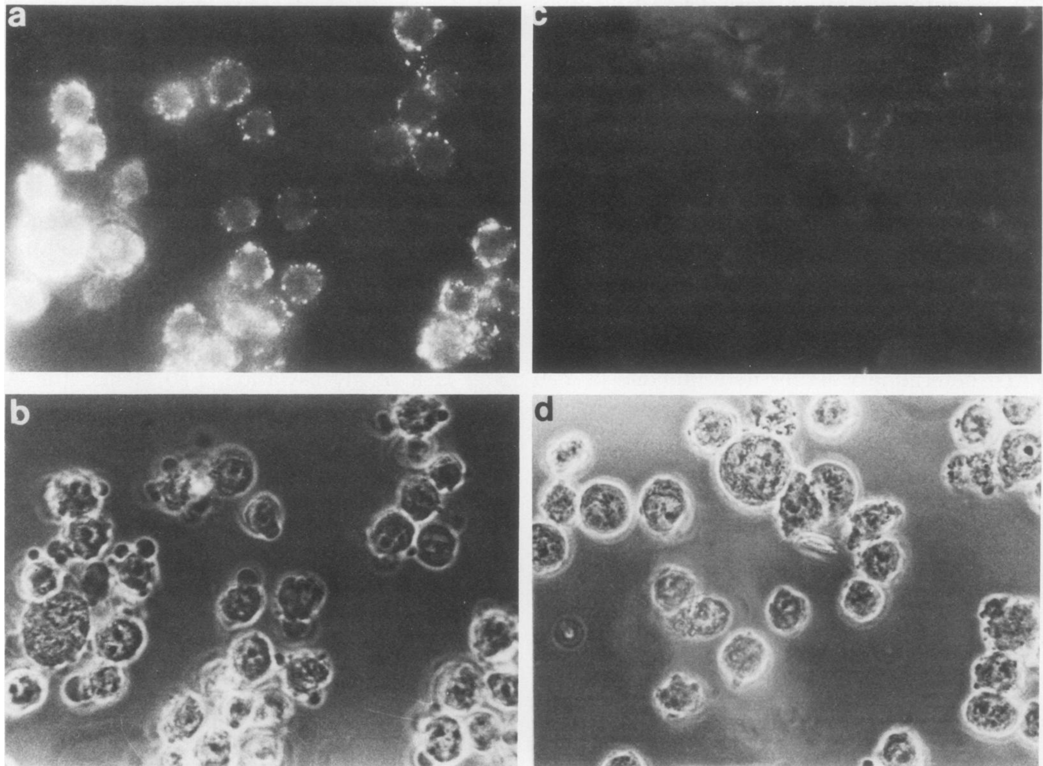

FIG. 6. Detection of P50 protein as an A-MuLV cell surface antigen by immunofluorescence. Intact, viable 2M3 cells were tested in a two-stage immunofluorescence assay, using normal mouse or TB serum at a final dilution of 1:20. After unbound antibody was washed away, fluoresceinated goat anti-mouse immunoglobulin G at a 1:20 final dilution was used and wet mounts were photographed. (a) Cells treated with TB serum, fluorescence; (b) cells treated with TB serum, phase contrast; (c) cells treated with normal serum, fluorescence; and (d) cells treated with normal serum, phase contrast.

same pattern (lane f). Thus, these sera precipitate indistinguishable proteins.

DISCUSSION

When A-MuLV-induced lymphoid tumor cells are growing in mice, the animals frequently form antibodies to one host-encoded protein, P50. The protein can be found in small amounts in normal lymphoid cells and non-A-MuLV-induced tumors, but is found at greatly accentuated levels in most A-MuLV-induced tumor cells (and certain other tumor cell lines; V. Rotter and D. Baltimore, unpublished data). Of all the A-MuLV-induced lymphoid cell lines examined, only the L1-2 tumor of C57L origin was lacking sufficient P50 to yield a dark band after [^{35}S]methionine labeling under a standard set of conditions. Even fibroblastic cells transformed by A-MuLV had a significant amount of P50. Accumulation of P50 in A-MuLV-transformed cells could be a result of either a stabilization of a labile P50 protein found in normal tissues or induction of increased production of this protein in the A-MuLV-transformed cells.

The recognition of P50 by both TB sera and the monoclonal antibody RA3-2C2 has been used to study this protein. The immunofluorescence of A-MuLV-induced tumor cells with both TB sera and RA3-2C2 suggests that some P50 molecules are at least partly exposed on the outer surface of cells. Also, the demonstration of the RA3-2C2 determinant on the surface of normal B-lymphocytes (Coffman and Weissman, in preparation) suggests that P50 is a normal cell membrane protein. It is curious but perhaps significant that RA3-2C2 does not label the surface of thymocytes (Coffman and Weissman, in preparation), but P50 is easily detected in biosynthetically labeled thymocytes by using RA3-2C2 antibody.

Among the more remarkable properties of P50 is its close similarity to the A-MuLV P120 protein. Although the two proteins share no known immunological determinants (certain C57L anti-P120 sera do not precipitate P50, and RA3-2C2

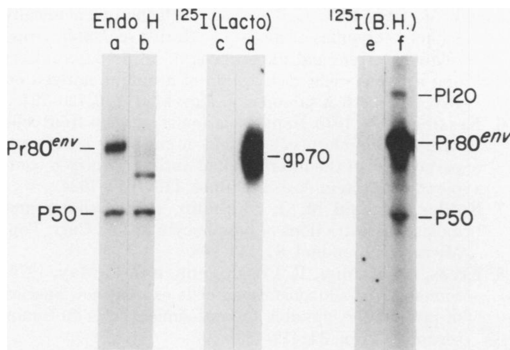

FIG. 7. *Iodination of P50 and resistance to endo-β-N-acetylglucosaminidase H (endo H) treatment. [^{35}S]methionine-labeled 2M3/M cells were immunoprecipitated with TB serum and (a) left without enzyme or (b) treated with endo-β-N-acetylglucosaminidase H. The digestion products were analyzed by electrophoresis through a polyacrylamide-sodium dodecyl sulfate gel. 2M3/M cells were labeled with Na^{125}I by the lactoperoxidase technique, and a cell lysate was immunoprecipitated with (c) normal serum or (d) TB serum. 2M3/M cells were labeled with the ^{125}I-labeled Bolton-Hunter reagent, and cell lysates were immunoprecipitated with (e) normal serum or (f) TB serum.*

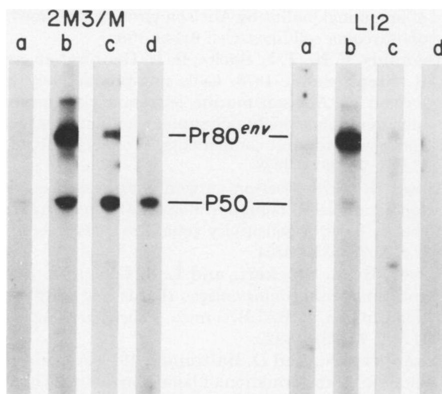

FIG. 8. *Immunoprecipitation of P50 protein with antibodies obtained from various sources. Lysates of [^{35}S]methionine-labeled 2M3 cells and L1-2 cells were immunoprecipitated with: (lane a) normal serum; (b) TB serum; (c) serum of a BALB/c mouse that rejected a 2M3/M tumor; and (d) RA3-2C2 hybridoma antibody.*

does not precipitate P120), they have similarities in that: (i) they are both partly exposed on the surface of cells; (ii) they are both phosphorylated in cells; (iii) they both lack detectable asparagine-linked carbohydrate of the type that is sensitive to endo-β-*N*-acetylglucosaminidase H; (iv) neither protein can be labeled by lactoperoxidase-catalyzed iodination of live cells; and (v)

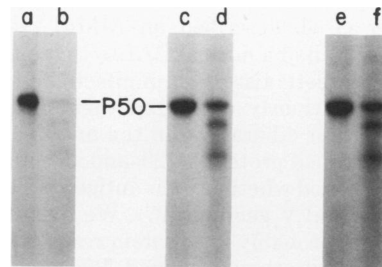

FIG. 9. *Comparison of partial digestion products by S. aureus V-8 of P50 protein immunoprecipitated by different antibodies. Extracts of [^{35}S]methionine-labeled 2M3 cells were immunoprecipitated with (a) RA3-2C2 hybridoma antibodies, (c) TB serum, or (e) serum from a BALB/c mouse that rejected a syngeneic 2M3/M tumor. Peptide digestion was obtained by treating the above proteins with 0.025 μg of S. aureus V-8 for 30 min at 37°C. Partial digestion products of P50 immunoprecipitated by (b) RA3-2C2 hybridoma antibodies, (d) TB serum, or (f) serum from a BALB/c mouse that rejected a syngeneic 2M3/M tumor were compared.*

P50 and the normal cell equivalent of P120, called NCP150, are both most readily demonstrable in normal thymocytes. P50, however, does not become phosphorylated by incubation with [γ-^{32}P]ATP in an immunoprecipitate (unpublished data), whereas under such conditions P120 is phosphorylated at a tyrosine residue (27).

A striking coincidence in our data is that the one A-MuLV-induced tumor that is rejected by syngeneic mice is L1-2 (28), the only A-MuLV-induced tumor that lacks a detectable band of biosynthetically labeled P50. L1-2 cells appear to have some P50 by immunofluorescence analysis, but the level must be quite low. Other A-MuLV-induced lymphoid cell lines from C57L mice have P50 and are lethal to syngeneic hosts (Rotter, Rosenberg, and Baltimore, unpublished data); thus, the uniqueness of L1-2 lies in the properties of the cell line and not in the genetics of C57L animals. How a low level of P50 might potentiate tumor rejection is not clear, and further investigation of this coincidence will be required before we can know if the low P50 content of L1-2 causes its rejection by C57L animals.

Whatever the role of P50 in tumor rejection, it is striking that mice often respond to this protein even in a syngeneic immunization. Presumably the concentration of the protein in the A-MuLV-induced cell lines is high enough to break tolerance. Were it not for the widespread occurrence of P50, it might be considered a "tumor antigen" because of the selective increase in antibody to the protein in tumor-bearing or rejecting animals.

Risser et al. described an A-MuLV-related antigen that had a normal BALB/c counterpart in hematopoietic tissues of uninfected mice (16, 17). We previously compared their data with data from our laboratory on the one known A-MuLV-encoded protein, P120, and its congeners and questioned whether their antigen is encoded by the A-MuLV genome (27). We now believe that P50 is probably the protein responsible for the antigenicity they described. P50 is found on the cell types they found to be positive and is, by cell surface analysis, a B-lymphocyte differentiation antigen readily apparent in BALB/c mice (Coffman and Weissman, in preparation).

Because of the approximate coincidence of size, we have investigated whether P50 might be the same antigen described by DeLeo et al. (4, 5) as a 53,000-molecular-weight protein elevated in chemically induced BALB/c tumor cells, or whether it might be related to the 54,000 to 56,000-molecular-weight protein that is elevated in simian virus 40-transformed cells and binds to the simian virus 40 T-antigen, or both (2, 8, 10-12, 25). Preliminary experiments have shown that the three proteins are closely related and probably identical (Rotter and Baltimore, unpublished data). Thus, P50 is elevated in a variety of tumor cells and may be involved in the malignant state of tumor cells induced by a variety of carcinogenic stimuli.

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