ML Antigen of DBA/2 Mouse Leukemias: Expression of an Endogenous Murine Mammary Tumor Virus

JANIS RACEVSKIS* AND NURUL H. SARKAR

Laboratory of Molecular Virology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 21 September 1981/Accepted 16 February 1982

Spontaneous, transplantable leukemias of DBA/2 mice express an antigen (ML) which cross-reacts with antigens of murine mammary tumor virus (MuMTV). The MuMTV cross-reactive antigen of the DBA/2 leukemias (ML cells) was found to be a glycoprotein of 78,000 molecular weight containing antigenic determinants of the major MuMTV glycoprotein gp52. No MuMTV particles were produced by the ML cells, although they did contain type A particles—the pronucleocapsids of MuMTV. The ML antigen appeared to be an aberrant form of the intracellular MuMTV env precursor molecule prgp70, which was not processed properly but instead acquired extra carbohydrate groups and was expressed in uncleaved form on the cell surface. Isolation of MuMTV core protein p28 from the leukemic cells and subsequent tryptic peptide mapping analysis showed that the p28 from leukemia cells differed from the p28 of MuMTV isolated from DBA/2 mouse milk. These observations indicate that the MuMTV expressed in DBA/2 leukemic spleen cells is of a different strain than the virus secreted in lactating mammary glands of DBA/2 mice and probably represents the expression of an endogenous DBA/2 provirus.

Murine mammary tumor virus (MuMTV) antigen expression on murine leukemia cells was first described during a study of cytotoxic antiserum prepared against spontaneous leukemias of DBA/2 mice (28). These antiseras were produced by immunizing C57Bl/6 mice with DBA/2 leukemias, followed by absorption in vivo in normal DBA/2 mice to remove alloantibodies. It was found that the antisera were cytotoxic for the DBA/2 leukemias, and that this activity could be absorbed by cells from mammary tumors and normal lactating mammary tissues of MuMTV-infected mice of various strains. These observations suggested that the antigen detected on the DBA/2 leukemias was derived from the MuMTV, and therefore it was designated ML for mammary leukemia antigen (28). More definitive proof that the ML antigen was induced by MuMTV was provided by the studies of Nowinski et al. (16).

Later studies of leukemic cells from another high mammary tumor incidence strain of mice, GR, also revealed the presence of MuMTV-related antigens on those malignant lymphoid cells (1, 8). The major component of the GR leukemic cells (GRSL cells) that could be isolated with anti-MuMTV serum was a molecule of about 73,000 molecular weight (31). More recently, this 73,000-dalton (73K) peptide has been shown to share antigenic determinants with MuMTV gp52 (19). Similarly, a study with the transplantable ascites L-1210 leukemia cells, a carcinogen-induced leukemia of DBA/2 mice has shown that these cells express 73K glycoproteins that react with anti-MuMTV serum (34).

Whether the 73K proteins found on GRSL cells (19, 31) and L-1210 cells (34) are also expressed on spontaneous leukemias of DBA/2 mice is not known, nor is it known with what molecules the ML typing serum (28) reacts. We therefore attempted to characterize the MuMTV antigens expressed on DBA/2 leukemias and to define the specificities of the ML typing serum. We found that DBA/2 leukemia cells (ML cells) expressed on their surface a glycoprotein of about 78,000 molecular weight which shared antigens with MuMTV gp52. Furthermore, the MuMTV proteins expressed in ML cells were found to differ from those of the MuMTV found in the milk of DBA/2 mice, and probably represented the expression of an endogenous virus.

MATERIALS AND METHODS

Cells and virus. Transplantable (ML antigen positive) leukemic cells of DBA/2 mice were obtained from E. A. Boyse of this institute. The ML-positive cells were passaged in 6- to 8-week-old DBA/2 male mice (Jackson Laboratories) every 12 to 14 days by intra-peritoneal injection of 2 × 10⁵ leukemic spleen cells. L-1210 cells were a gift from P. L. Chello of this institute and were propagated as ascites cells in DBA/2 mice. Tissue culture-adapted L-1210 cells, also obtained from P. L. Chello, were grown in suspension culture in RPMI 1640 medium supplemented with 10% fetal calf serum. Rauscher murine leukemia virus
(R-MuLV)-producing JLSV-9 cells were grown in minimal Eagle medium containing 10% fetal calf serum. Marker MuMTV was isolated from cell line Mm5mt/C1, which was obtained from J. Gruber through the Virus Cancer Program of the National Cancer Institute. Milk from DBA/2 mice was obtained from J. Holben of the Institute for Medical Research, Camden, N.J.

Antiserum. Anti-ML antiserum was prepared by immunizing C57Bl/6 mice with spontaneous leukemia cells of DBA/2 mice. The antiserum was then absorbed in vivo in normal DBA/2 mice to remove alloantibody as described (28). Cytotoxic tests with anti-ML serum were performed as described (28) on freshly prepared ML cells suspended in RPMI medium containing 1% fetal calf serum. Rabbit serum (a gift from U. Hammerling) was used as the source of complement, and cell viability was determined by trypan blue exclusion.

Anti-MuMTV antiserum was prepared by immunizing rabbits with detergent-solubilized RIII mouse milk virus, which was isolated and purified as previously described (23). MuMTV structural proteins gp52 and p28 were purified from solubilized RIII milk virus according to previously published protocols (12, 13) and were subsequently used to raise monospecific antisera in rabbits. Goat anti-R-MuLV gp70 and anti-R-MuLV p30 were obtained from Research Resources, Biological Carcinogenesis Branch of the National Cancer Institute.

Radiolabeling of cells and virus. Leukemic spleen cells (ML cells) were isolated by mincing leukemic DBA/2 mouse spleens in phosphate-buffered saline followed by passing through a nylon mesh screen. For metabolic labeling, the ML cells were washed three times with phosphate-buffered saline, suspended in methionine-deficient minimal essential medium, and preincubated in a shaking bath at 37°C. After a 30-min preincubation period, [35S]methionine was added (200 μCi/ml; New England Nuclear Corp.), and incubation was continued for 60 min. After the labeling period, cells were washed three times in cold (4°C) Earle balanced salt solution and then lysed in extraction buffer. Cell surface proteins were labeled with 125I by the noninvasive enzymatic lactoperoxidase iodination technique (32). The carbohydrate moieties of cell surface glycoproteins were labeled by tritiated borohydride reduction after oxidation with galactose oxidase (6). Extraction of isotopically labeled cells and immunoprecipitation were carried out according to the methods of Schultz et al. (25), using Sepharose-protein A (Pharmacia Fine Chemicals, Inc.) as the immunoadsorbent. Labeled leukemic spleen cell extracts were preadsorbed for 60 min with Sepharose-protein A before the addition of antiserum to remove labeled immunoglobulins. Immunoprecipitates were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and radioautography as previously described (22).

Radiolabeled MuMTV was obtained from dexa-methazone-treated cultures of Mm5mt/c cells after a 16-h exposure to a 14C-labeled amino acid mixture at a concentration of 12 μCi/ml in minimal Eagle medium containing 10% of the normal amino acid concentration (13). Labeled viruses were harvested and purified as previously described (13).

Two-dimensional electrophoresis. Two-dimensional electrophoresis was performed by using the combination of isoelectric focusing and SDS-gel electrophoresis as originally described by O’Farrell (20) with modifications according to Y. Bushkin of this institute. Isoelectric focusing was performed in 0.13-cm-thick slab gels, using pH 3.5 to 10 ampholytes (LKBI Instruments Inc.). Electrophoresis was done at 125 V for 15 min, followed by 160 V for 90 min and finally 200 V for 24 h. After electrofocusing, the appropriate gel lanes were cut, equilibrated for 1 h in equilibration buffer (20), and then placed on top of 0.15-cm-thick SDS-slab gels for analysis in the second dimension. The pH gradients formed in the isoelectric focusing gels were checked with a micro pH probe and were found to extend through a pH range of about 4 to 9. Routinely, pl calibration marker proteins (Pharmacia) were included with each run. Molecular weight marker proteins were also run on the edges of the second-dimension SDS-gels, alongside the isoelectric focusing strips.

Affinity chromatography. The immunoglobulin G fraction of the antiserum was purified by DEAE-cellulose (Whatman DE) chromatography (11) and bound to Sepharose 4B (Pharmacia) by the cyanogen bromide technique (11). Leukemic spleen cells were homogenized in a buffer containing 10 mM Tris-hydrochloride, pH 7.2, 0.5 M NaCl, 0.5% Sodium deoxycholate, and 0.5% Nonidet P-40, and the homogenates were cleared by centrifugation at 100,000 × g for 60 min. Supernatants were passed over the affinity column and eluted with 3 M KSCN. The eluate was dialyzed, lyophilized, and analyzed by SDS-gel electrophoresis. MuMTV proteins separated by SDS-gel electrophoresis were analyzed by tryptic peptide mapping according to the procedures of Elder et al. (5).

RESULTS

Virus-specific cell surface polypeptides of ML and L-1210 cells. Transplantable leukemic cells of DBA/2 mice (ML cells) were surface labeled with 125I by the lactoperoxidase method and then analyzed by immunoprecipitation and SDS-gel electrophoresis. When the iodinated ML cell extracts were immunoprecipitated with anti-MuMTV gp52 serum, the major component that was brought down was a peptide of about 78,000 molecular weight (gp78) which displayed a diffuse trailing edge on SDS-gels (Fig. 1, lane B), characteristic of a glycoprotein. A trace amount of MuMTV gp52 was also observed (Fig. 1, lane B), which was found to vary from experiment to experiment. The amount of gp52 present on ML cells was sometimes difficult to estimate because of a high background caused by the coprecipitation of labeled immunoglobulin heavy chains which had not been completely removed by the preadsorption procedure with Sepharose-protein A. No peptides from the surface-labeled ML cells were recognized by anti-MuMTV p28 serum (Fig. 1, lane C). Similarly, the ascitic transplantable L-1210 cells, which were originally shown to absorb out anti-ML activity from anti-ML serum (28), were also surface labeled with 125I and analyzed by immunoprecipitation. Anal-
(D) L-1210 cells, anti-R-MuLV extracts. (A) DBA/2 serum. gp52 serum 78,000 molecular cells, anti-gp52 of labeled L-1210 beled since these gp70 serum, not contain C viruses known that L-1210 wide proteins Anti-MuMTV also immunoprecipitated though also

The detection of gp52 on surface-labeled L-1210 cells was not hindered by a high background of labeled immunoglobulin G heavy chains, since these ascites cell preparations do not contain immunoglobulin-synthesizing cells. Anti-MuMTV p28 serum did not precipitate any MuMTV-specific proteins from the surface-labeled L-1210 cells (Fig. 1, lane F). Since it is known that L-1210 cells release low levels of type C viruses (10), the L-1210 cell extract was also immunoprecipitated with anti-R-MuLV gp70 serum, resulting in the precipitation of a wide diffuse band of MuLV gp70s (Fig. 1, lane D). As shown below (Fig. 2, lane C), ML cells were also found to express MuLV gp70, although at a lower level than L-1210 cells.

Specificities of the antisera. The MuLV gp70s expressed on ML cells appeared to be a less heterogeneous population than those found on L-1210 cells, and they migrated in a relatively lower-molecular-weight region of the gel (Fig. 2, lane C). Although the ML cell surface proteins precipitated with anti-MuMTV gp52 serum (Fig. 2, lane A) were clearly not the same as the proteins precipitated with anti-R-MuLV gp70 serum (Fig. 2, lane C), all of the antisera were tested against [35S]methionine-labeled R-MuLV-infected JLSV-9 cells (Fig. 2, lanes D through I) to rule out the possible presence of cross-reactive specificities in the antisera. The R-MuLV-specific proteins labeled during the 60-min pulse period of the JLSV-9 cells were the envelope protein precursor prp59 (Fig. 2, lane G) and the core protein precursor prp65 (Fig. 2, lane H). Neither of the anti-MuMTV antisera (anti-gp52 serum (Fig. 2, lane F) and anti-detergent-disrupted MuMTV serum [Fig. 2, lane E]), showed any activity against R-MuLV-specific proteins. Originally, the ML antigen was defined by the cytotoxic activity of anti-ML sera (28). Anti-ML serum was prepared by immunizing C57Bl mice (a strain that does not express MuMTV antigens) with leukemic cells of DBA/2 mice and then absorbing the antiserum of alloantibodies in normal DBA/2 mice. The antiserum prepared in this manner demonstrated a very strong cytotoxic activity against the ML cells, and this activity could be largely absorbed out with MuMTV-producing cells. When tested against the JLSV-9 cell extract (Fig. 2, lane D), the anti-ML serum precipitated a low-molecular-weight protein of about 12,000 molecular weight and none of the R-MuLV precursor polyproteins. The same 12K protein was also precipitated by anti-ML serum from labeled ML cells (Fig. 3, lane B). The 12K protein did not comigrate with R-MuLV p15E, and it was not a core protein since prp65 was not precipitated by the serum. When tested against purified 14C-amino acid-labeled MuMTV (Fig. 2, lane J), the precipitating activity of the anti-ML serum was shown to be directed solely against antigenic determinants of MuMTV gp52. The monospecific anti-MuMTV gp52 (Fig. 2, lane K) and anti-MuMTV p28 (Fig. 2, lane L) sera showed no cross-reactivity with each other, and the anti-MuMTV serum was directed primarily against gp36 and p28 (Fig. 2, lane N). Anti R-MuLV gp70 serum showed no activity against any MuMTV protein (Fig. 2, lane M).

Metabolic labeling of ML cells. Metabolic labeling of the carbohydrate moiety of gp78 of the ML cells with labeled sugar precursors proved to be difficult, because the ML cells were not viable in vitro long enough to permit adequate incorporation of label. The carbohydrate groups of the cell surface gp78 and gp52 could, howev-
er, be labeled by the nonmetabolic technique (6) of tritiated borohydride reduction after oxidation with galactose oxidase (Fig. 3, lane A). Metabolic labeling of DBA/2 leukemia cells with [35S]methionine and subsequent analysis by immunoprecipitation and gel electrophoresis revealed that the ML cells synthesized both MuMTV env prgp70 (Fig. 4, lanes B and C) and MuMTV gag prp75 (Fig. 4, lane E) precursor molecules. The env precursor molecules immunoprecipitated with anti-ML serum (Fig. 4, lane B) and anti-gp52 serum (Fig. 4, lane C) displayed a long diffuse trailing edge. As will be shown below, the diffuse material migrating with an apparent higher molecular weight than prgp70 (Fig. 4, lanes B and C) was the cell surface gp78 species. For an equivalent amount of serum, the anti-ML serum was not as potent a precipitating antiserum as was the anti-gp52 serum. In addition, anti-gp52 serum also precipitated a prominent band of 35,000 molecular weight (Fig. 4, lane C), which was a breakdown product of gp52 (26) and was not recognized by the ML serum (Fig. 4, lane B). Since anti-ML serum was prepared by immunization with whole cells, it is possible that the 35,000-molecular-weight segment was sequestered in the membrane or in the three-dimensional configuration of gp78 in such a way that it did not elicit an immune response. Anti-p28 serum precipitated the gag precursor prp75 (Fig. 4, lane E) as well as two intracellular processing intermediates of MuMTV core pro-
protein, which was the major component precipitated by the anti-MuMTV gp52 serum, displayed a very heterogeneous isoelectric pattern consisting of about eight or nine discrete bands, focusing in a pH range of about 6.5 to 8 (Fig. 4). The charge heterogeneity of the 78K protein was characteristic of glycoproteins and was probably due to heterogeneity in the carbohydrate moiety (15, 18). Relatively little MuMTV gp52 was found on the ML cells (Fig. 1B); the bands visible in the basic half of the pH gradient, migrating in a molecular weight range of 50,000 to 55,000, were labeled immunoglobulin heavy chains which were not completely predesorbed in this particular experiment. MuMTV gp52 migrates in a more acidic pH range (18) and can be detected after longer exposure times of the gel.

The MuMTV polypeptides immunoprecipitated with anti-gp52 serum from the [35S]methionine-labeled ML cells were also analyzed by two-dimensional gel electrophoresis (Fig. 5). The most heavily labeled proteins migrated with a molecular weight of about 70,000 and focused in three spots in a pH range of about 8 to 8.3. These proteins were the intracellular MuMTV env precursor molecules prgp70 which we observed to form a similar pattern, with the same isoelectric points, when isolated from MuMTV-producing cell lines (not shown). The characteristic feature of ML cells, not observed in MuMTV-producing epithelial cell lines, was the presence of protein bands (gp78) migrating with a higher apparent molecular weight and in a more acidic pH range than prgp70 (Fig. 5). When the autoradiographic film of Fig. 5 was superimposed on the film of Fig. 4 and aligned with markers, we observed that the bands of the [35S]methionine-labeled gp78 coincided directly with the 125I-labeled gp78 bands and concluded that both sets represented the same cell surface species. The 35,000-molecular-weight breakdown product of gp52 focused at the basic end of the pH gradient to a single spot (Fig. 5). Two-dimensional gel analysis of the immunoprecipitate obtained with anti-ML serum (not shown) displayed the same pattern for prgp70 and gp78 as seen in Fig. 5.

Closer inspection of the env precursor prgp70 and cell surface gp78 (Fig. 5) revealed the discrete nature of the multiple species making up these polypeptides. The transition from intracellular prgp70 to cell surface gp78 presumably resulted from the addition of extra carbohydrate residues and was seen to occur in discrete steps, both in change to higher apparent molecular weights and in change to more acidic isoelectric points (Fig. 5).

Tryptic peptide mapping of intracellular MuMTV protein. To determine the nature of the MuMTV antigens expressed in DBA/2 leukemia
cells, MuMTV-specific proteins were isolated from ML cells with an affinity column prepared from immunoglobulins derived from an anti-MuMTV serum. SDS-gel analysis of the isolated proteins showed the presence of proteins of 52,000, 35,000, and 28,000 molecular weight, variable amounts of smaller proteins, and relatively little of higher-molecular-weight species (not shown). By far the most abundant protein was the one comigrating with p28, the major MuMTV core protein. The relatively small amounts of large-molecular-weight MuMTV precursor proteins probably resulted from the action of proteases during the isolation procedure. The abundance of p28 is consistent with the observations that the core protein precursors are quite susceptible to proteolysis (27, 29), whereas p28 is resistant to further cleavage (4).

The p28 band was excised, iodinated with $^{125}$I, and analyzed by tryptic peptide fingerprint mapping. The tryptic peptide map of the band was characteristic of a MuMTV p28 core protein (Fig. 6A). However, comparison of this map with one derived from p28 of the virus isolated from the milk of DBA/2 mice (Fig. 6B) clearly showed that the two were not the same. Hence, the MuMTV proteins that were synthesized in the leukemic spleen cells of the DBA/2 mouse were not from the same strain of virus that was present in the milk of DBA/2 mice.

**DISCUSSION**

Our studies indicate that ML-positive, DBA/2 leukemic spleen cells express a cell surface MuMTV gp52 cross-reactive antigen, which we estimate to be about 78,000 molecular weight on the basis of migration in SDS-gels. This cell surface antigen migrates in SDS-gels with an apparent higher molecular weight than the intracellular MuMTV env precursor molecule, which we have estimated to be 70,000 molecular weight (22). The 70K env precursor protein is synthesized in ML cells as it is in MuMTV-producing epithelial cell lines. In MuMTV-producing cell lines, however, the env precursor molecule has not been observed to be expressed on the cell
surface (19, 24, 33). The major MuMTV cell surface component of MuMTV-producing cells is gp52 (19, 24, 33), which we found to be expressed only in trace amounts on ML cells. When isolated by electrophoresis on SDS-gels, the 78K cell surface protein migrates as a wide diffuse band, indicating that it is probably glycosylated. The glycoprotein nature of the 78K protein was confirmed after labeling the ML cell surface carbohydrate residues with tritiated borohydride and immunoprecipitation with anti-MuMTV gp52 serum. Analysis of the 78K protein by two-dimensional gel electrophoresis shows that it focuses into eight or nine discrete bands in a pH range of about 6.5 to 8. This charge heterogeneity is characteristic of glycoproteins and is due to variable carbohydrate content (15, 18).

In vitro metabolic labeling of the leukemic spleen cells with $^{35}$S-methionine followed by immunoprecipitation and SDS-gel analysis revealed that both MuMTV gag and env precursor molecules are synthesized in the cells. The ML cells were also found to contain breakdown products of gp52 and processing intermediates of MuMTV core proteins. All of these findings are consistent with the earlier observation (17) that these cells do not produce mature virus, but that they do contain numerous intracytoplasmic type A particles, the pronucleocapsids of mature virions.

Upon two-dimensional gel analysis of the metabolically labeled proteins precipitable with anti-gp52 serum, we observed that the intracellular env precursor prgp70 was composed of three charged species focusing in a pH range of 8 to 8.3. The cell surface gp78 bands are more numerous and migrate with an apparent higher molecular weight and in a more acidic pH range. The slower migration in SDS-gels and focusing in a more acidic pH range of gp78 probably reflect the acquisition of additional carbohydrate residues including the negatively charged sialic acid groups. Closer inspection of these polyproteins reveals that the transition from the intracellular env precursor molecules prgp70 to the cell surface species gp78 takes place in gradual discrete steps. Some additions increase the apparent molecular weight of the prgp70 molecules, whereas others confer a more acidic isoelectric point on the protein.

In an MuMTV-producing cell, the general

**FIG. 5.** Two-dimensional electrophoresis of proteins immunoprecipitated by anti-MuMTV gp52 serum from ML cells metabolically labeled with $^{35}$S-methionine for 90 min in vitro.
mature envelope glycoproteins gp52 and gp36, which embed in the cell membrane and form the budding sites for virus maturation (4, 14, 19, 22). In the case of the ML cells, however, because of some unknown defect or mutation, a portion of the envelope precursor molecules is degraded intracellularly, whereas other envelope precursors acquire additional carbohydrate groups, migrate to the membrane, and are expressed on the cell surface in an uncleaved form. These cell surface, extraglycosylated MuMTV envelope precursor molecules represent the ML antigen. This conclusion was verified by using an anti-ML serum that was prepared the same way as the original sera that were used to define the ML antigen (28). The anti-ML serum showed a very strong cytotoxic activity against the ML cells, and its precipitating activity was found to be directed against MuMTV gp52 of purified virus and against MuMTV prgp70 and cell surface gp78 of ML cells.

To date, the expression of MuMTV antigens by transformed lymphoid cells has been reported for three high mammary tumor incidence mouse strains: DBA/2 (16, 17, 28–30, 34), GR (1, 8, 19, 31), and ICRC (9). An important unanswered question relating to these observations is the relationship between the MuMTV expressed in the lymphoid cells and the milk-borne MuMTV that induces mammary tumors in the respective mouse strains. Since tryptic peptide maps of MuMTV core proteins can be used as characteristic strain-specific markers of the MuMTV strain (7), we isolated viral proteins from ML cells for peptide mapping analysis. Among the virus-specific proteins isolated, a protein comigrating with MuMTV p28 was the major component retrieved from leukemic spleen homogenates by affinity chromatography on a column prepared from immunoglobulins purified from an anti-MuMTV serum. The relative excess of MuMTV core proteins relative to envelope proteins isolated from the ML cells might have been due to the fact that ML cells accumulate A particles (17) which contain the core protein precursor (21, 27, 29), whereas envelope polyproteins are either degraded or transported to the outer cell membrane, from where they are probably shed (31). The large amount of p28 and relative absence of larger proteins probably resulted from proteolysis of the gag precursor molecules during the isolation procedure. A similar phenomenon of MuMTV precursor molecule proteolysis and accumulation of p28 has been observed in vitro with purified MuMTV A-particle preparations (27, 29).

The isolated ML cell p28 was analyzed by tryptic peptide mapping and compared with a similarly treated p28 of a MuMTV purified from

---

FIG. 6. Tryptic peptide maps of $^{125}$I-labeled MuMTV proteins. (A) Protein isolated from ML cells by affinity chromatography, comigrating with MuMTV p28 on SDS-polyacrylamide gel electrophoresis; (B) p28 of MuMTV isolated from the milk of DBA/2 mice. Corresponding peptide spots common to both maps A and B are numbered.

scheme for viral glycoprotein biosynthesis and maturation is as follows: envelope precursor molecules prgp70 are synthesized on membrane-bound polysomes and are subsequently cleaved and further glycosylated to give rise to the two
the milk of lactating DBA/2 mice. The ML cell p28 displayed a characteristic MuMTV p28 tryptic peptide map (7), which was clearly different from the peptide map of the DBA/2 milk MuMTV p28. This pronounced difference in peptide maps leads us to conclude that the MuMTV expressed in the leukemic spleen cells of the DBA/2 mouse is not of the same strain as the MuMTV found in the milk of DBA/2 mice. The MuMTV gene products that are expressed in the spleen cells are probably derived from an endogenous DBA/2 MuMTV provirus (2), whereas the virus secreted in the milk is the infectious exogenous MuMTV which causes mammary tumor formation in these mice. Whether the endogenous MuMTV that is expressed in the leukemic spleen cells of the DBA/2 mouse plays a role in the etiology of leukemia in these mice is an intriguing possibility that remains to be investigated.

ACKNOWLEDGMENTS

We thank E. A. Boyse and P. L. Chello of this institute for their gift of the cells used in this study. We are indebted to Y. Bushkin for advice on two-dimensional gel electrophoresis. Our thanks are also due to E. Stockert and E. A. Boyse for reviewing the manuscript.

This work was supported by Public Health Service grants CA-16599, CA-17129, and CA-08748 from the National Cancer Institute.

LITERATURE CITED


29. Tanaka, H. 1977. Precursor-product relationship between nonglycosylated polypeptides of A and B particles of...