JOURNAL OF VIROLOGY, Jan. 1980, p. 340-348
0022-538X/80/01-0340/09$02.00/0

Vol. 33, No. 1

Abelson Murine Leukemia Virus Mutants with Alterations in the Virus-Specific P120 Molecule

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Abelson murine leukemia virus (A-MuLV) is a replication-defective virus that transforms both fibroblasts and hematopoietic cells in vitro. The virus encodes a 120,000-molecular-weight protein (P120) that is composed of Moloney murine leukemia virus-derived *gag* gene sequences and A-MuLV-specific sequences. This protein is the only A-MuLV-encoded protein that has been detected, and thus P120 is a candidate for the transforming protein of A-MuLV. We now report isolation and characterization of three new A-MuLV isolates that do not synthesize P120 but do produce analogous proteins of larger (160,000 molecular weight) and smaller (100,000 and 90,000 molecular weight) size. All of these A-MuLV isolates transform fibroblasts and lymphoid cells in vitro. Because the different A-MuLV proteins vary in the A-MuLV-specific region of the molecule, these variants may set a maximum limit on the size of the A-MuLV transforming protein.

Abelson murine leukemia virus (A-MuLV) is a replication-defective, transforming retrovirus that arose in a steroid-treated BALB/c mouse injected with Moloney murine leukemia virus (M-MuLV) (1). The virus induces a fatal nonthymic lymphosarcoma 3 to 4 weeks after injection into neonatal mice and transforms both fibroblasts and hematopoietic cells in vitro (18, 21). A-MuLV stocks always contain a replication-competent virus (such as M-MuLV) that provides the defective A-MuLV with enzymatic functions and structural proteins (9, 21).

The defective nature of A-MuLV was demonstrated directly by Scher and Siegler (21) when they isolated an A-MuLV-transformed nonproducer cell line called ANN-1. A-MuLV rescued from ANN-1 cells with cloned helper virus induced typical Abelson lymphosarcomas, indistinguishable from the disease induced by the uncloned virus prepared from tumor extracts. ANN-1-derived virus thus became widely used and served as the prototype cloned A-MuLV strain.

Heteroduplex mapping and S1 nuclease mapping studies using ANN-1-derived A-MuLV show that the 5.6-kilobase A-MuLV RNA genome has two regions of precise homology with M-MuLV: a 730-base region at the 3′ end and a 1,320-base region at the 5′ end (D. Baltimore, A Shields, G. Otto, S. Goff, P. Besmer, O. Witte, and N. Rosenberg, Cold Spring Harbor Symp. Quant. Biol., in press; A. Shields, G. Otto, S. Goff, M. Paskind, and D. Baltimore, submitted for publication). The 3.5-kilobase region in the center of the A-MuLV genome is not homologous to M-MuLV, and hybridization studies show that this region is not present in other replication-defective murine retroviruses (8; Baltimore et al., in press). These A-MuLV sequences are present in normal mouse DNA and probably represent a portion of the genome of the BALB/c mouse from which A-MuLV was originally isolated. Because these sequences distinguish A-MuLV from its M-MuLV parent and other murine retroviruses, they may be responsible for the unique biological properties of A-MuLV.

Analysis of the proteins expressed in ANN-1 cells and A-MuLV-transformed cells derived using virus recovered directly or indirectly from ANN-1 cells has identified a single protein of 120,000 molecular weight (P120) that is encoded by the 5.6-kilobase genomic RNA of A-MuLV (18, 19, 26). This protein contains some of the determinants of the M-MuLV *gag* gene products (p15, p12, and a part of p30), and the remaining 90,000 molecular weight is made up of A-MuLV-specific sequences (25; Baltimore et al., in press). These A-MuLV-specific sequences share serological determinants with a normal mouse cellular protein of 150,000 molecular weight (NCP-150) that is present in spleen, thymus, and bone marrow (25a).

The P120 protein is the only A-MuLV-encoded protein that we have detected by using a variety of serological reagents including an anti-Abelson tumor (anti-AbT) serum. In addition, this molecule is expressed in all of the A-MuLV-

transformed cells we have examined (26; Rosenberg and Witte, unpublished data). Thus, P120 is a candidate for the transforming protein of A-MuLV. However, mutants expressing altered P120 are needed to directly assess the role of this molecule transformation.

We now report the isolation of three A-MuLV strains varying in the size of the A-MuLV-specific protein. Although all three of these variants transform fibroblasts and lymphoid cells in vitro, the variants set a maximum size for the A-MuLV-specific sequences that may be responsible for cellular transformation. One of the A-MuLV variants expresses a larger, 160,000-molecular-weight (P160) protein. This A-MuLV strain appears to be a naturally occurring variant that is present in a second non-ANN-1-related A-MuLV virus pool maintained at the National Institutes of Health. The P160 molecule has also been observed by R. Risser and co-workers using these virus stocks (D. Grunwald and R. Risser, personal communication). The other two A-MuLV variants produce smaller proteins, 100,000 and 90,000 molecular weight (P100, P90), and were isolated from Ab-NRK cells (8). Proteins made by these variants are nonglycosylated phosphoproteins and express the same *gag* gene determinants as P120. The new isolates are stable upon cloning and transform both fibroblast and lymphoid cells. The lymphoid transformants are similar morphologically and with respect to expression of the lymphoid-specific markers immunoglobulin and terminal deoxynucleotidyl transferase (TdT).

MATERIALS AND METHODS

Cells and viruses. Routine maintenance of cell cultures has been described elsewhere (16, 17). Ab-NRK cells were originally a gift of E. Scolnick, National Institutes of Health. ABPC-22 cells (9) were a gift of M. Potter, National Institutes of Health, and RAW309Cr cells (11) were a gift of P. Ralph, Memorial Sloan-Kettering Institute. Lymphoid cells transformed by the different A-MuLV isolates were derived from individual foci of infected bone marrow cells by using the agar transformation assay (16) and adapted to grow in liquid culture as previously described (17). Clones of A-MuLV-transformed fibroblasts were derived by plating the infected cells in microtiter wells after a 1.5-h adsorption period. The trypsinized cells were plated at a concentration of 0.3 to 0.4 cell per well.

All virus stocks were prepared from 24-h culture fluids and passed through 0.45-μm filters before use. The titers of focus-forming virus in A-MuLV stocks were determined using NIH/3T3 cells (21). Titers of helper virus were determined by assay on S+L- cells (2) or by the XC plaque assay (20).

A-MuLV(P120) (i.e., A-MuLV producing a 120,000-molecular-weight A-MuLV-specific protein) was prepared using ANN-1 cells superinfected with M-MuLV clone 1 (5). A-MuLV(P160) was prepared using RAW309Cr cells or NIH/3T3 cells transformed by filtered culture fluids from RAW309Cr. The RAW309Cr cells used in these experiments release A-MuLV in combination with an NB-tropic, XC plaque-positive helper virus (data not shown) that is probably related to the M-MuLV in the original A-MuLV tumor extracts. A-MuLV(P100) and A-MuLV(P90) were derived from clones of Ab-NRK cells that had been treated with 10 μg of fluorodeoxyuridine per ml for 4 days before cloning. The A-MuLV was rescued from these nonproducer clones by using the amphototropic virus isolate 292 (12) and passed onto NIH/3T3 cells. The resulting transformed cultures of NIH/3T3 cells were used for routine virus production.

Protein labeling studies. All radiochemicals were purchased from New England Nuclear Corp., Boston, Mass. Techniques for labeling, immunoprecipitation, and gel electrophoresis have been described in detail elsewhere (22, 24, 25). Briefly, rapidly growing cells were labeled with 50 μCi of [^{35}S]methionine per ml in serum-free medium lacking methionine. Cells were labeled in complete medium with [^{3}H]glucosamine at 100 μCi/ml. Endoglycosidase H reactions were as described (27). A cell extract was prepared from 10^7 cells by using lysis buffer (10 mM $NaPO_4$, pH 7.5, 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) at 0°C and clarified at 100,000 × *g* for 2.5 h. Antisera were added to the extract, and the immunoprecipitates were collected with *Staphylococcus aureus* (6) and analyzed on SDS-polyacrylamide gels (7) developed by fluorography (3).

Antisera used were rabbit anti-M-MuLV reverse transcriptase/p15, rabbit anti-M-MuLV gp70, goat anti-Rauscher MuLV p12, goat anti-Rauscher p10, goat anti-M-MuLV virion, mouse anti-AbT, rabbit anti-mouse immunoglobulin (a gift of V. Sato), and rabbit anti-calf TdT serum. The characterization of these reagents has been described elsewhere (22, 24-26; A. Silverstone, L. Sun, O. N. Witte, and D. Baltimore, submitted for publication). Virus proteins and TdT were examined by electrophoresis of immunoprecipitates through 10% polyacrylamide gels; immunoglobulin molecules were examined in the presence of 2-mercaptoethanol on 12.5% gels and in the absence of 2-mercaptoethanol on 5 to 20% gradient gels.

RESULTS

Detection of a strain of A-MuLV producing a larger form of P120. The A-MuLV-encoded P120 protein has been expressed in all of the more than 100 different lymphoid and fibroblast transformed cells we have examined (26; Rosenberg and Witte, unpublished data). These cell lines were all derived in vitro in a similar fashion, either by cloning infected cells at limiting dilution, in the case of the fibroblasts, or by using the agarose transformation assay (16) for lymphoid cells. In the case of lymphoid cells, all of the cell lines expressed a similar set

of differentiation markers and are probably analogous to primitive cells related to B lymphocytes (22, 23). The A-MuLV used in all of these experiments was derived either directly or indirectly from ANN-1 cells.

To investigate the universality of P120 expression, we examined two A-MuLV-transformed cell lines isolated from tumor tissue by two other laboratories. Two cell lines expressing different phenotypic markers were used: the ABPC-22 cell line, a typical immunoglobulin-secreting plasmacytoma (9), and RAW390Cr, a macrophage line (11). [^{35}S]methionine-labeled cell extracts from these cell lines were immunoprecipitated with antisera specific for M-MuLV and A-MuLV proteins and analyzed by electrophoresis (Fig. 1). The previously described lymphoblastoid 2M3 cell line (17, 26) produced P120 that reacted with anti-p15, anti-p30, and anti-AbT sera (Fig. 1C). However, the ABPC-22 myeloma cell line (Fig. 1D) and the macrophage cell line RAW309Cr (Fig. 1A) both did not produce P120, but did produce a 160,000-molecular-weight protein (P160) that was precipitated with anti-p15, anti-p30, and anti-AbT sera. The weak precipitation of P160 with anti-p30 serum was not observed consistently. A typical lymphoblastoid cell line isolated by infecting spleen cells with virus from RAW309Cr, the R5 cell line (Fig. 1B), also produced P160. Thus, P160 expression was not related to the highly differentiated phenotypes expressed by RAW309Cr and ABPC-22 cells.

A more complete survey of the serological determinants present on P160 is shown in Fig. 2. Immunoprecipitation of P160 from the R5 spleen cell line showed that the protein contained determinants of p15, p12, and p30, but not p10. Anti-AbT serum (lane 7) but not normal mouse serum (lane 6) also precipitated P160. In

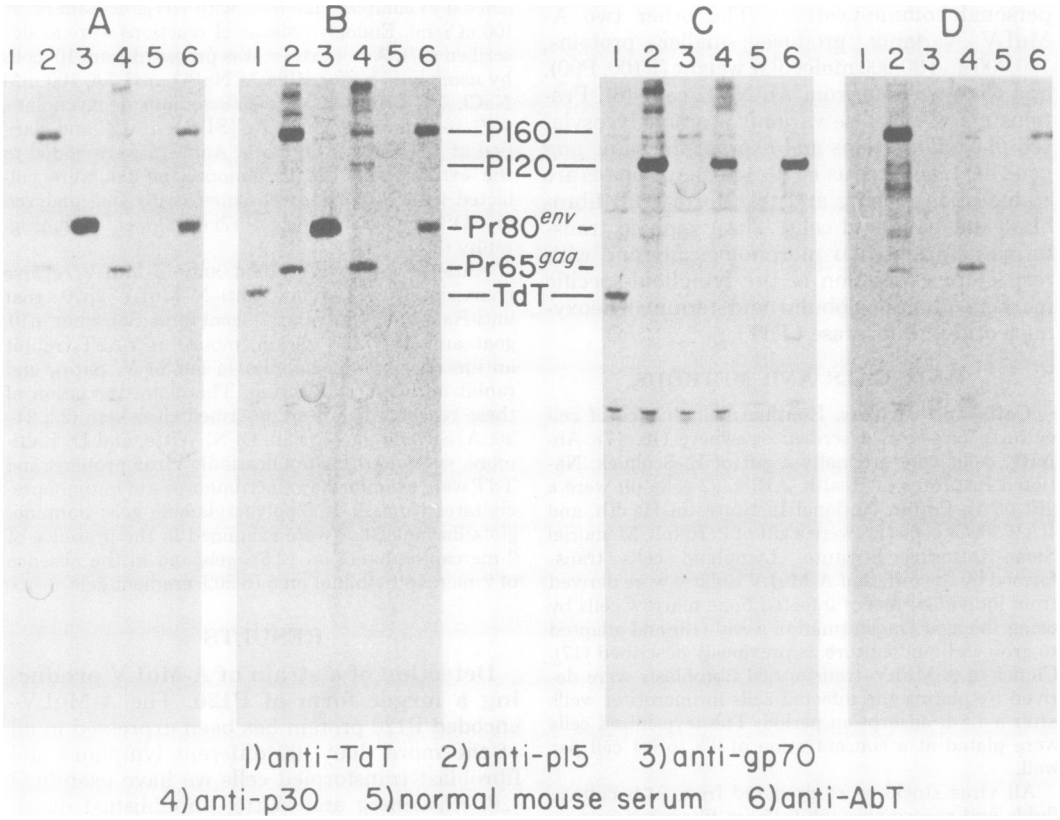

FIG. 1. *Analysis of virus-specific proteins in A-MuLV-transformed cell lines. Cell lines RAW309 (A), R5 (B), 2M3 (C), and ABPC22 (D) were labeled with [^{35}S]methionine (100 μCi/10^7 cells, 1 h, 37°C) and extracted as previously described (24). A volume of 10^6 cells was immunoprecipitated with 5 μl of anti-TdT (lane 1), anti-M-MuLV p15 reverse transcriptase (lane 2), anti-M-MuLV gp70 (lane 3), anti-M-MuLV p 30 (lane 4), normal mouse serum (lane 5), and mouse anti-Abelson MuLV tumor regressor serum (AbT) (lane 6). Precipitates were collected with S. aureas, denatured, and analyzed on a 10% SDS-polyacrylamide gel developed by fluorography (3, 6, 7).*

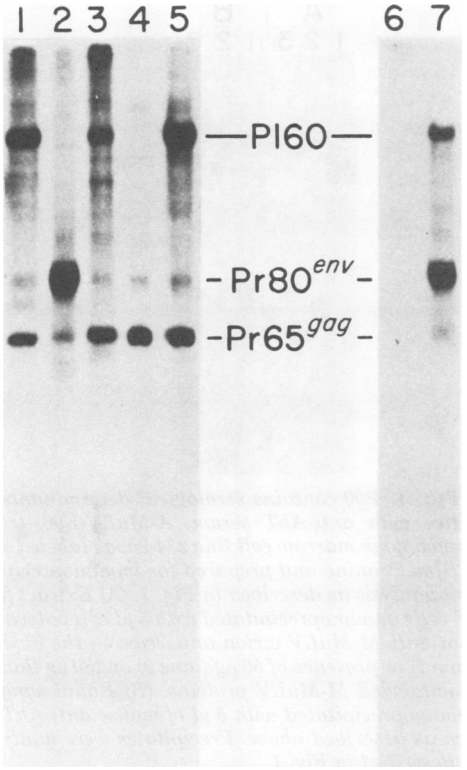

FIG. 2. *Further serological analysis of P160. The R5 cell line was labeled, extracted, immunoprecipitated, and analyzed as described in Fig. 1. Lane 1, Anti-M-MuLV p15/reverse transcriptase; lane 2, anti-M-MuLV gp70; lane 3, anti-M-MuLV p30; lane 4, anti-Rauscher MuLV p10; lane 5, anti-Rauscher MuLV p 12; lane 6, normal mouse serum; lane 7, anti-AbT.*

addition to the *gag* gene-related determinants, the P160 molecule also contained A-MuLV-specific determinants, because precipitation of P160 by anti-AbT was not blocked by excess M-MuLV virion protein (data not shown). No reactivity with anti-gp70 (Fig. 1 and 2) or with reverse transcriptase determinants was detected (data not shown). In addition, P160 was efficiently labeled with inorganic phosphate but not with [^{3}H]glucosamine, and the size of the molecule was not altered by treatment with endoglycosidase H (data not shown).

A-MuLV strains synthesizing smaller forms of P120. In another series of experiments, we attempted to isolate P120 mutants by selecting for flat variants of the Ab-NRK cells (a P120 producer) (8, 26). Ab-NRK cells were treated with 10 μg of fluorodeoxyuridine per ml for 4 days and cloned at limiting dilution. After 3 weeks, two clones were chosen for study. One of the clones, AbNRK-1, was morphologically distinct, being composed of elongated, refractile cells that formed an incomplete cell layer, one cell in thickness. The second clone, Ab-NRK-3, was similar in morphology to the parent AbNRK cell line, being composed of cells with a more regular epithelioid morphology that formed densely packed layers.

To determine whether the morphology of the two clones was controlled by the A-MuLV genome, the virus was rescued from them with the amphotropic wild mouse virus isolate 292 (12) and passed to NIH/3T3 cells. A-MuLV that transformed NIH/3T3 cells efficiently was recovered from both clones. The titers of focus-forming virus, measured in the NIH/3T3 cell transformation assay (21), were about the same: 10^5 focus-forming units for virus recovered from Ab-NRK-3 and 2×10^5 focus-forming units for virus recovered from Ab-NRK-1. In addition, the morphology of the NIH/3T3 cells transformed by both virus stocks was indistinguishable and typical of A-MuLV-transformed fibroblasts.

Focus isolates of cells transformed with virus derived from the two Ab-NRK cell clones were examined for expression of the A-MuLV-specific protein. All of the clones derived from virus rescued from AbNRK-1 synthesized, instead of P120, a smaller, 100,000-molecular-weight (P100) protein. The clones derived from virus rescued from AbNRK-3 fell into two groups, one synthesizing P120 and another synthesizing a 90,000-molecular (P90) protein. Serological analysis of the P100 and P90 molecules is shown in Fig. 3. Extracts from a nonproducer cell line synthesizing P90 were precipitated with the M-MuLV *gag* gene reagents anti-p15, anti-p12, and anti-p30 sera (Fig. 3A, lanes 2, 3, and 4), but not by anti-p10 serum (Fig. 3A, lane 5). P90 was also precipitated by anti-AbT serum (Fig. 3A, lane 6). A similar pattern of precipitation was obtained when extracts from a producer cell line synthesizing P100 were examined (Fig. 3B). This molecule reacted with anti-p15, anti-p30, and anti-p12 sera (Fig. 3B, lanes 2, 3, and 4) and anti-AbT (Fig. 3B, lane 6), but not with anti-p10 serum (Fig. 3B, lane 5). Both proteins reacted with anti-M-MuLV virion serum (lane 1), and neither reacted with anti-gp70 serum (lane 7).

The presence of A-MuLV-specific determinants in P90 was demonstrated in a blocking experiment (Fig. 4) which showed that P90 was precipitated with anti-AbT (Fig. 4B, lane 1) alone or with anti-AbT in the presence of 60 or 180 μg of M-MuLV virion proteins (lanes 2 and 3). P90 precipitation via *gag* gene determinants with anti-M-MuLV serum (Fig. 4A, lane 1) was blocked by the presence of 60 and 180 μg of M-

MuLV virion protein (Fig. 4A, lanes 2 and 3). P100 molecules also had A-MuLV-specific determinants that could be demonstrated in blocking experiments with anti-AbT serum (data not shown).

Retrospective examination of AbNRK-1 cells and the culture of NIH/3T3 cells transformed by mass infection with AbNRK-1-derived A-MuLV showed that all of these cells synthesized P100. The NIH/3T3 culture transformed by mass infection with Ab-NRK-3-derived A-MuLV synthesized both P120 and P90, but only P120 could be detected in Ab-NRK-3 cells. Thus, although a stable change in the A-MuLV genome in the fluorodeoxyuridine-treated cells does not appear related to the altered morphology of some of these cells, this screening procedure did result in the recovery of altered P120-like proteins.

Stability of the A-MuLV variants. Virus stocks from A-MuLV-transformed cells synthesizing the various sizes of proteins were used to transform a series of new clones to determine whether the size of the A-MuLV-specific protein was a stable, heritable trait. Clones of both fibroblasts and bone marrow cells derived using A-MuLV(P160) and A-MuLV(P100) synthesized the appropriate proteins (Table 1). In addition, a new series of ANN-1-derived A-MuLV clones were also examined, and all of these clones synthesized P120 (Table 1). Most of the clones derived using the mixed P90 and P120 A-MuLV stock expressed only P90. The high proportion of the P90 producers may reflect the ratio of A-MuLV(P90) and A-MuLV(P120) in the uncloned, transformed NIH/3T3 cells used to prepare the virus stock. The amounts of P90 and P120 in this cell line detected by metabolic labeling indicate that P90 is the predominant species (data not shown).

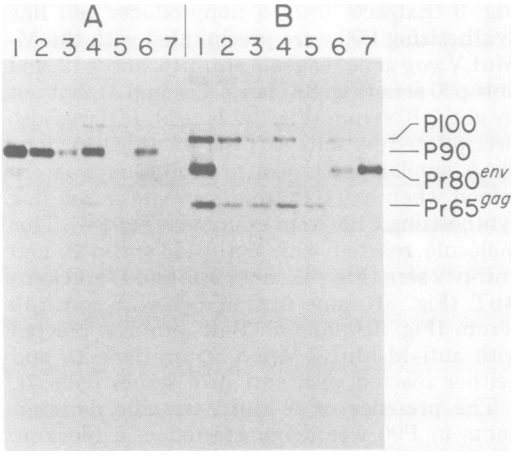

FIG. 3. *Analysis of A-MuLV P90 and P100 proteins. Bone marrow cells transformed with A-MuLV(P90) (A) and A-MuLV(P100) (B) were prepared and analyzed as described in Fig. 1. Lane 1, Anti-M-MuLV virions; lane 2, anti-p15/reverse transcriptase; lane 3, anti-p12; lane 4, anti-p30; lane 5, anti-p10; lane 6, anti-Abt; lane 7, anti-gp70.*

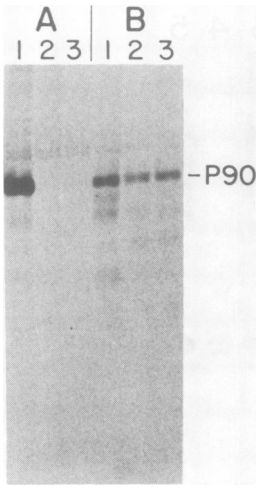

FIG. 4. *P90 contains serological determinants reactive with anti-AbT serum. A-MuLV(P90)-transformed bone marrow cell line 234-9 was labeled with [^{35}S]methionine and prepared for immunoprecipitation analysis as described in Fig. 1. (A) Extract from 10^6 cells immunoprecipitated with 5 μl of a polyvalent goat anti-M-MuLV virion antiserum in the absence (lane 1) or presence of 50 μg (lane 2) or 180 μg (lane 3) of unlabeled M-MuLV proteins. (B) Equal samples immunoprecipitated with 5 μl of mouse anti-AbT serum as described above. Precipitates were analyzed as described in Fig. 1.*

The P160 molecule is the largest A-MuLV-specific protein we have found; this A-MuLV strain may be the parental A-MuLV isolate. To determine whether P120 was a chance isolate in the ANN-1 nonproducer or represented a second major variant of A-MuLV, we analyzed the size of the A-MuLV-specific protein in a series of clones derived from a tumor extract similar to those used in the original Scher and Siegler (21) experiments that led to the isolation of ANN-1. The A-MuLV in this tumor extract (a gift of R. Siegler) descended from original material given to Dr. Siegler by H. T. Abelson and L. S. Rabstein and was maintained by animal passage in Boston for several years prior to the in vitro studies. Seven clones of A-MuLV-transformed fibroblasts and lymphoid cells were isolated using this tumor stock, and all seven clones synthesized P120 (Table 1). Therefore, the P120 strain of A-MuLV was the major component of the Boston lineage of A-MuLV, at least at the

TABLE 1. *Stability of the A-MuLV-specific protein upon cloning*[a]

Source of A-MuLV	Protein expressed by parent cell line	Protein expressed in clonally derived transformants			
		P90	P100	P120	P160
ANN-1	P120	0/50	0/50	50/50	0/50
RAW309Cr	P160	0/21	0/21	0/21	21/21
Ab-NRK-1	P100	0/12	12/12	0/12	0/12
Ab-NRK-3	P90, P120	12/16	0/16	4/16	0/16
Tumor extract		0/7	0/7	7/7	0/7

[a] Filtered A-MuLV stocks were used to infect both bone marrow cells and NIH/3T3 fibroblasts. After adsorption, the infected cells were cloned by using limiting dilution for NIH/3T3 cells and the agarose transformation assay (16) for bone marrow cells. Transformed clones were isolated, and the size of the A-MuLV-specific protein was determined by SDS-gel electrophoresis of [^{35}S]methionine-labeled immunoprecipitates.

time in passage at which this extract was prepared.

Lymphoid-specific markers expressed by A-MuLV-transformed clones. Previous work has established the presence of lymphoid-specific gene markers in A-MuLV-transformed lymphoid cells (4, 9, 11, 22, 23). Direct biochemical and immunological assays have demonstrated the presence of the markers TdT and intracellular immunoglobulin in many cell lines (22, 23). The frequency of expression of immunoglobulin and TdT was monitored in A-MuLV-transformed bone marrow clones to determine whether the transformed cells derived using the different A-MuLV strains were similar. Clones derived using A-MuLV(P90) and A-MuLV(P160) were analyzed along with a new series of A-MuLV(P120) clones. Bone marrow cells from C57BL/6J, C57L/J, and BALB/cAn mice were used for the transformation studies. The presence of immunoglobulin and TdT was determined by SDS-polyacrylamide gel analysis of [^{35}S]methionine-labeled immunoprecipitates (Fig. 5). Consistent with observations described elsewhere, the anti-calf TdT serum used here precipitated a 60,000-molecular-weight protein from murine cell lines synthesizing TdT (Silverstone et al., submitted for publication). The anti-mouse immunoglobulin serum used here precipitated immunoglobulin M (IgM) and IgG from cell lines synthesizing these proteins (22).

Screening of a large number of lymphoid clones showed that most of them synthesized TdT (Table 2). About 60% of the clones synthesized immunoglobulin in the form of μ heavy chain (Table 1). No 7 to 8S IgM molecules or free light-chain molecules were detected in any of the clones analyzed here. The frequency of immunoglobulin-positive clones and the presence of μ heavy chain in the absence of light chain is consistent with earlier observations (22). As shown in Table 2, no strain-specific differences were seen when the frequency of immunoglobulin and TdT expression was tabulated according to the A-MuLV isolate used to transform the clones. In addition, when these data were reanalyzed with respect to the mouse strain

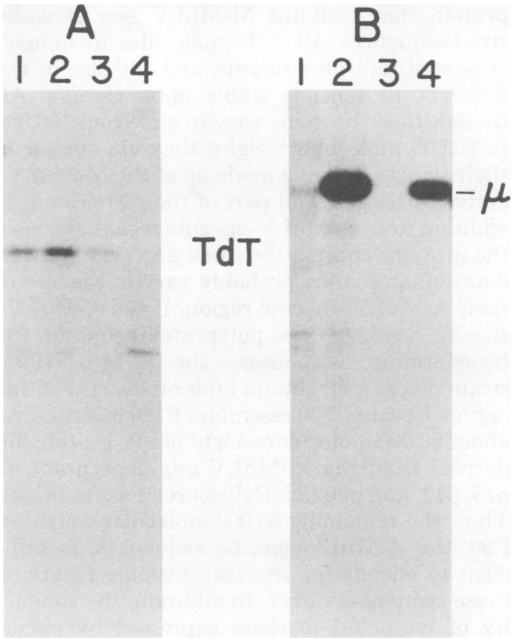

FIG. 5. *Immunoprecipitation analysis for intracellular immunoglobulin and TdT in clonal A-MuLV-transformed lymphoid cell lines. Individual clones of A-MuLV-transformed cells (lanes 1 to 4) were labeled and prepared as described in Fig. 1, then immunoprecipitated with 5 μl of rabbit anti-TdT (A) and a polyvalent rabbit anti-mouse immunoglobulin (B). Samples were processed as described in Fig. 1. TdT expression was analyzed on a 10% SDS-polyacrylamide gel, and immunoglobulin expression was analyzed on a 12.5% SDS-polyacrylamide gel.*

TABLE 2. *Frequency of A-MuLV-transformed bone marrow clones expressing lymphoid markers*[a]

Marker	Frequency in A-MuLV strain:		
	P160	P120	P90
μ	15/22	6/11	2/3
TdT	20/22	9/11	2/3

[a] Single-focus isolates of A-MuLV bone marrow cells were adapted to grow in liquid medium and examined for expression of immunoglobulin and TdT by SDS-gel electrophoretic analysis of [^{35}S]methionine-labeled immunoprecipitates (22, 23). The size of the A-MuLV-specific protein was confirmed in each of the clones by electrophoretic analysis of labeled immunoprecipitates.

used as a source of bone marrow cells, no mouse strain-specific differences in the frequency of marker expression were evident (data not shown).

DISCUSSION

All of the A-MuLV-transformed cell lines we have examined synthesize an A-MuLV-specific protein that contains M-MuLV *gag* gene-derived sequences. All of the molecules are nonglycosylated phosphoproteins, and the size of the different proteins is stable upon cloning. Although these proteins vary in size from 160,000 to 90,000 molecular weight, they are similar in their structure, being made up of the M-MuLV-derived p15, p12, and part of the p30 protein in addition to an A-MuLV-specific region. Because the proteins contain the same *gag* gene-derived determinants, they probably vary in the size of their A-MuLV-specific region. If the A-MuLV-specific region of these polyproteins contains the transforming sequences, the A-MuLV(P90) strain places a maximum limit on the size of this region. Because P90 resembles P120 in structure, about 30,000 molecular weight of P90 is probably derived from the M-MuLV *gag* gene products p15, p12, and p30 (26; Baltimore et al., in press). Thus, the remaining 60,000 molecular weight of P90, the A-MuLV-specific sequences, is sufficient to encode for any transforming functions these sequences direct. In addition, the similarity of lymphoid markers expressed by clones transformed with all of these isolates shows that the addition of sequences in A-MuLV(P160) and the loss of sequences in A-MuLV(P90) does not affect target cell selection.

The P160 strain of A-MuLV appears to be a naturally occurring isolate that probably is the predominant component of A-MuLV stocks maintained at the National Institutes of Health by passage of tumor extracts. R. Risser and D. Grunwald, using A-MuLV derived from the National Institutes of Health pool of virus, have identified a 160,000-molecular-weight protein that expresses p12 determinants in some of their A-MuLV-induced tumors (R. Risser, personal communication). This protein is probably the same as the P160 described here.

The P160 protein probably contains about 130,000 molecular weight of A-MuLV-specific sequences; based on the ability of A-MuLV(P90) to transform cells, over half of these sequences must not be required to transform cells in vitro. Whether these sequences are beneficial to the virus growth and oncogenicity in vivo is unclear. However, the predominance of A-MuLV(P120) in another tumor extract virus pool indicates that A-MuLV(P160) is not always selected by in vivo passage. Whether differences in the passage history of the Boston and National Institutes of Health pools of A-MuLV are important in the derivation of two A-MuLV strains cannot be ascertained at this time.

The genomes of the new A-MuLV strains have not been fully characterized. However, the 3.6-kilobase central region of the A-MuLV(P120) genome is large enough to encode 130,000 molecular weight of protein. Thus, if the A-MuLV(P160) genome is the same size as the A-MuLV(P120) genome, P160 would account for the entire coding capacity of the genome.

The origin of the shorter A-MuLV proteins is unclear. The possible role of fluorodeoxyuridine treatment in their origin must be considered. although neither P100 or P90 appears to be directly related to the morphological variations observed in the fluorodeoxyuridine-treated cells, 20 random Ab-NRK clones did not synthesize smaller forms of P120 (O. Witte, unpublished data). The Ab-NRK cells were originally isolated using virus from ANN-1 cells. We have shown that this strain of A-MuLV is stable through repeated cloning in mouse cells (26) (see above).

The lymphoid marker studies indicate that all the strains of A-MuLV transform similar types of cells. In the case of A-MuLV(P160), which was originally detected in cell lines of peculiar differentiation type for A-MuLV, it is clear that the P160 protein is not responsible for these altered phenotypes. Our data are consistent with those of Raschke et al. (11), who showed that A-MuLV produced by RAW309Cr, a macrophage cell line, produced typical Abelson lymphomas.

The percentage of A-MuLV clones expressing μ chain is similar to the frequency reported by Siden et al. (22) using the same method to isolate the transformed clones. The μ-only phenotype is the most common immunoglobulin phenotype expressed by A-MuLV-transformed lymphoid cells isolated in vitro using focal transformation assays. The relationships, if any, of these μ-only cells to normal lymphocytes is unclear. No normal μ-only cell has been detected, although suggestive evidence of μ-only cells exists (10; E. Siden and D. Baltimore, personal communication). Such a cell might be the precursor of the cytoplasmic IgM-positive pre-B cells. The ability to stimulate complete IgM synthesis in some μ-only A-MuLV-transformed lymphoid cell lines by using the B lymphocyte mitogen lipopolysaccharide (19) suggests that such a pathway may exist.

TdT expression by A-MuLV-transformed cells has been previously reported (4, 23), but no prospective surveys have been conducted. This

study shows that almost all A-MuLV-transformed cells express TdT. Many of the TdT-positive clones also synthesized μ chain. These results support the hypothesis suggested by Silvertone et al. (23), that TdT expression is not limited to committed pre-T-lymphocytes, but may characterize all types of immature lymphoid cells.

The lymphoid marker survey also indicates that the cells susceptible to A-MuLV in the bone marrow of BALB/cAn, C57BL/6J, and C57L/J mice are probably similar. This result is significant because these three strains differ in their susceptibility to A-MuLV-induced tumors, BALB/c being susceptible and C57BL/6J and C57L being resistant (15). Whereas previous results have shown that bone marrow cells from these three strains are susceptible to A-MuLV in vitro (16), the results described here demonstrate that similar A-MuLV-sensitive target cells are present in all three strains. Thus, the resistance of C57BL/6J and C57L adult mice to A-MuLV is probably not mediated by alterations in target cell population.

ACKNOWLEDGMENTS

We are grateful to Dan Clark for excellent technical assistance and to David Baltimore for his support and helpful discussions.

This work was supported by Public Health Service CA-24220 and Program Project grant CA-24530 from the National Cancer Institute (N.R.), by grant VC-4J from the American Cancer Society (to David Baltimore), and by Public Health Service grant CA-14051 from the National Cancer Institute (core grant to S. E. Luria). N.R. is a recipient of an American Cancer Society Research Scholar Award (Massachusetts Division). O.N.W. is a Helen Hay Whitney Postdoctoral Fellow.

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