JOURNAL OF VIROLOGY, June 1983, p. 993–1002
0022-538X/83/060993-10$02.00/0

Vol. 46, No. 3

In Vitro Transformation of Murine Pre-B Lymphoid Cells by Snyder-Theilen Feline Sarcoma Virus

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Received 10 December 1982/Accepted 17 March 1983

Snyder-Theilen feline sarcoma virus (ST-FeSV) codes for a protein kinase with specificity for tyrosine residues (Barbacid et al., Proc. Natl. Acad. Sci. U.S.A. **77**:5158–5163, 1980), properties analogous to those of the transforming gene product of Abelson murine leukemia virus (Witte et al., Nature (London) **283**:826–831, 1980). In the present report, ST-FeSV was demonstrated to transform murine hematopoietic cells under in vitro assay conditions which detect lymphoid cell transformation by Abelson murine leukemia virus. Bone marrow colony formation was shown to require ST-FeSV, follow single-hit kinetics, and require the presence of mercaptoethanol in the agar medium. ST-FeSV-induced colonies could be established in culture as continuous cell lines that demonstrated unrestricted self-renewal capacity and leukemogenicity in vivo. The hematopoietic blast cells transformed by ST-FeSV in culture appeared to be at an early stage of B cell differentiation. They possessed Lyb 2 surface antigens, were dependent on mercaptoethanol for growth, and contained only low levels of terminal deoxynucleotidyl transferase. Moreover, a large fraction of the lines synthesized immunoglobulin μ chain in the absence of light chains. Thus, the phenotype of ST-FeSV hematopoietic transformants was indistinguishable from that of the pre-B lymphoblast transformants induced by Abelson murine leukemia virus. These findings indicate that the in vitro functional similarities in the *onc* gene products of ST-FeSV and Abelson murine leukemia virus may reflect a common pathway by which they exert their oncogenic potential.

The study of RNA tumor viruses, designated transforming retroviruses, has provided a potentially important approach for elucidating mechanisms involved in malignant transformation. Such viruses have arisen in nature by recombination of replication-competent type C retroviruses with evolutionarily well-conserved cellular genes. These latter sequences, termed *onc* genes, have been shown to be required for the induction and maintenance of the viral transformed state (for review, see references 7 and 8). Certain independent virus isolates of the same, and even different, species have transduced closely related *onc* genes (2, 4, 6, 13–15, 17, 18, 45, 48). These findings have suggested that vertebrates contain only a limited number of cellular genes that can acquire transforming properties when recombined with retroviral sequences.

There is emerging evidence for the existence of an even smaller number of *onc* gene families. The avian retroviruses Rous sarcoma virus, Fujinami, Y73, and PRC II, as well as the mammalian-derived Abelson murine leukemia virus (MuLV) and Snyder-Theilen feline sarcoma virus (ST-FeSV), code for phosphoproteins that have associated protein kinase activities with specificity for phosphorylation of tyrosine residues (3, 12, 16, 21, 26, 32, 34, 35, 50, 52). The results of molecular hybridization studies indicate that most of these *onc* genes have arisen from different cellular genes (7, 8, 44). Yet, nucleotide sequence analysis has revealed that the putative products of some of these genes possess amino acid sequence homology (20, 29, 44, 49; E. P. Reddy, personal communication).

In the present report, we sought to determine whether the in vitro functional similarities between the transforming proteins of two mammalian transforming retroviruses within this family, ST-FeSV and Abelson-MuLV, might reflect a common pathway by which they exert their oncogenic potential. As an approach to this question, we took advantage of the availability of hematopoietic cell culture systems for analysis of the array of cellular targets whose growth and differentiation could be altered by different retroviral *onc* genes.

MATERIALS AND METHODS

Cells and viruses. Cell lines included NIH/3T3 (25), a clonal ST-FeSV nonproducer transformant of mink

cells (24), and an Abelson-MuLV-transformed nonproducer clone of NIH/3T3 cells (ANN-1) (43). MuLV pseudotypes of ST-FeSV and Abelson-MuLV were obtained by superinfection of appropriate nonproducer cells with a clonal strain of the mouse type C helper virus, amphotropic (Amph)-MuLV (23, 38). The titers of defective transforming viruses, expressed as focus-forming units (FFU) per milliliter, were determined on NIH/3T3 cells as previously described (25).

Murine hematopoietic cell lines included a clonal Rauscher-MuLV-transformed NIH/Swiss pre-B cell line, 6ELB, that has been shown to synthesize μ chain (39), and a clonal Moloney-MuLV transformed NIH/Swiss T cell line, 19-1-2, that is Thy 1.2 antigen positive (39). Abelson-MuLV-transformed hematopoietic cell lines were isolated and propagated after Abelson-MuLV infection of NIH/Swiss (NFS/N) bone marrow cells according to the methods described by Rosenberg and Baltimore (40) and Rosenberg et al. (41).

Hematopoietic colony formation. Bone marrow suspensions were prepared from the femurs and tibias of 2- to 3-week-old NFS/N mice. Single-cell suspensions were washed and suspended at a density of 2×10^6 nucleated cells per ml in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 20% heat-inactivated fetal calf serum (Reheis Chemical Co., Phoenix, Ariz.), and 5×10^{-5} M mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.).

Single cell suspensions of NFS/N bone marrow were exposed to an equal volume of virus for 1 h at 37°C in the presence of 4 μg of Polybrene (Aldrich Chemical Co., Milwaukee, Wis.) per ml. After centrifugation, the cells were mixed with semisolid agarose medium containing RPMI 1640 medium supplemented with 20% fetal calf serum, 5×10^{-5} M mercaptoethanol, and 0.47% Sea Plaque agarose (FMC Corporation, Rockland, Maine) and plated at 10^6 cells per ml into 35-mm petri dishes in a volume of 2 ml. Plates were cooled at 4°C to allow solidification of the agarose, and the cultures were incubated at 37°C in a 5% CO_2-humidified incubator. Agarose medium (1 ml) was overlaid at 5-day intervals. Visible colonies were scored at 12 to 18 days after infection.

Establishment of ST-FeSV and Abelson-MuLV hematopoietic cell lines. Individual colonies were removed from the agarose plates and suspended in 1 ml of RPMI growth medium in 24-well microtiter plates (Costar, Cambridge, Mass.). When the cell number in an individual well reached around 10^6 cells, the cells were transferred at a 1:2 split ratio. After an initial adaptation period of 1 to 6 weeks, the suspension lines could be maintained by transfer at 2- to 3-day intervals (split ratio of 1:20 to 1:100).

In some cases, adherent bone marrow cell feeder layers were utilized during an initial adaptation period needed to establish hematopoietic cell lines. For preparation of feeder layers, NFS/N bone marrow cell suspensions (10^6 nucleated cells per ml) were plated in 60-mm tissue culture plates in RPMI 1640 medium supplemented with 20% horse serum (Flow Laboratories, Rockville, Md.) and 5×10^{-5} mercaptoethanol. Nonadherent cells and medium were removed after 5 days, and medium containing 15% fetal calf serum was used thereafter. Secondary adherent bone marrow cultures were generated from 15-day-old primary cultures. Cells were trypsinized and plated at a final concentration of 2×10^5 cells per ml in 60-mm tissue culture dishes. Tertiary adherent bone marrow cultures were derived from confluent secondary cultures in a similar manner. Any clones that were exposed to normal bone marrow feeder layers were recloned in soft agar before further characterization.

Soft agar cloning efficiency of ST-FeSV-transformed hematopoietic cell lines. Cells from established lines were suspended at various concentrations in 5 ml of soft agar medium containing 20% fetal calf serum and 5×10^{-5} M mercaptoethanol and plated in 60-mm petri dishes. Plates were incubated at 37°C in a 5% CO_2-humidified incubator. Visible colonies were scored at 12 days after plating.

Analysis of tumor induction. Clonal hematopoietic cell lines were washed twice in phosphate-buffered saline and suspended at a final concentration of 2×10^7 cells per ml. For tumorigenicity testing, NFS/N weanlings were inoculated subcutaneously with 5×10^6 cells in a volume of 0.25 ml. Virus alone was inoculated subcutaneously in the same volume.

Biosynthetic labeling and immunoprecipitation. The procedures for metabolic labeling with [^{35}S]methionine and immunoprecipitation were as previously described (4). Briefly, 10^7 transformed hematopoietic or NIH/3T3 cells were labeled with 200 μCi of [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) for a 3-h period. The cells were disrupted in 1 ml of Staph A lysing buffer and clarified, and 0.2-ml samples were incubated with 10 μl of rabbit anti-feline leukemia virus p15 serum (4) or normal rabbit serum overnight at 4°C. Antigen antibody complexes were precipitated with protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) by agitation for 15 min at 4°C, washed three times in Staph A buffer, suspended in sample buffer, and boiled for 2 min. Reduced samples were analyzed on sodium dodecyl sulfate-polyacrylamide gels. Gels were then fixed, dried, and exposed to Kodak X-Omat R film (Eastman Kodak Co., Rochester, N.Y.).

Radioimmunoassay for immunoglobulin μ and κ chains. The amount of mouse immunoglobulin μ or κ chain present in cells was determined by competition radioimmunoassay as previously described (39).

Immunofluorescence assays. Cells were washed twice in Hanks balanced salt solution containing 0.2% bovine serum albumin and 0.1% sodium azide and suspended at a concentration of 2×10^7 cells per ml. Around 10^6 cells were incubated with fluorescein-conjugated Thy 1.2 or unconjugated Lyb 2.1 monoclonal antibody (New England Nuclear Corp., Boston, Mass.) for 30 min at 4°C. In cases where unconjugated antibody was utilized, cells were washed twice and incubated with fluorescein-conjugated anti-mouse immunoglobulin G (IgG) (Cappel Laboratories, Cochranville, Pa.) for an additional 30 min at 4°C. Untreated cells were also incubated with the fluorescein-conjugated anti-mouse immunoglobulin as a control for nonspecific immunofluorescence. Cells were then washed twice, wet mounted on microscope slides, and examined for membrane-specific immunofluorescence. Surface Fc receptors were detected by immunofluorescence by incubating cells with heat-aggregated fluorescein-conjugated mouse IgG for 30 min at 4°C. Cells were then washed, wet mounted, and examined for membrane-specific immunofluorescence. Alternatively, the presence of Fc receptors was deter-

mined by rosette formation, essentially as described by Kerbel et al. (28).

Terminal deoxynucleotidyl transferase assay. The terminal deoxynucleotidyl transferase enzymatic assay was performed according to the method of Barton et al. (5) with minor modifications as previously described (36).

RESULTS

Induction of hematopoietic colonies in ST-FeSV-infected murine bone marrow cultures. To investigate the transforming potential of ST-FeSV for hematopoietic cells, bone marrow cell suspensions obtained from 2- to 3-week-old NFS/N mice were infected with ST-FeSV-(Amph-MuLV) and suspended in soft agar. By 8 to 12 days, growing colonies were observed in ST-FeSV(Amph-MuLV)-infected cultures but not in control cultures. Colony formation was found to be dependent on the presence of 50 μM mercaptoethanol in the agar medium, a phenomenon previously reported for in vitro transformation of lymphoid cells by Abelson-MuLV (40).

As shown in Table 1, three separate experiments revealed a linear relationship between the number of colonies induced and the amount of ST-FeSV(Amph-MuLV) inoculated. The one-hit titration pattern suggested that colony formation was dependent upon infection with a single virus particle. Amph-MuLV alone produced no detectable colonies, further implying that colony-forming activity was due to ST-FeSV. It has, however, been demonstrated that murine sarcoma virus-transformed nonproducer fibroblast cell lines can produce high levels of granulocyte-macrophage colony-stimulating factors (30). To examine whether hematopoietic colony formation observed in our assay system might be due to stimulatory factors present in our virus stocks, bone marrow cell suspensions were exposed to tissue culture fluids obtained from ST-FeSV-transformed mink fibroblast nonproducer cells and plated in soft agar medium. No hematopoietic colonies were detected in such cultures, further implying that colony formation was the result of ST-FeSV infection.

Induction of hematopoietic colonies by ST-FeSV was approximately 100-fold less efficient than by Abelson-MuLV based on the use of equivalent titers of fibroblast FFU. ST-FeSV-induced colonies were also slower to appear, first becoming visible 3 to 4 days later than Abelson-MuLV-transformed colonies under identical assay conditions. Colonies induced by ST-FeSV were compact in morphology and were round or oblong in shape (Fig. 1A). Such colonies were morphologically indistinguishable from those induced by Abelson-MuLV.

To characterize the ST-FeSV-induced colonies, cells from individual colonies were cytocentrifuged onto microscope slides and stained with Wright-Giemsa (Fig. 1B). The cells possessed characteristics of undifferentiated blast cells. The predominant cell type had large eccentric convoluted nuclei which comprised most of the cell volume. No granules were found in the scanty cytoplasm, although occasional vacuoles were seen. There were no apparent morphological differences between cells from several different colonies analyzed, although some variation in cell size was noted. Mitotic figures were frequently observed.

TABLE 1. In vitro hematopoietic colony formation induced by ST-FeSV

FFU added per plate	Colony formation (CFU per plate) induced by:			
	ST-FeSV(Amph-MuLV)			Abelson-MuLV-(Amph-MuLV)
	Expt 1	Expt 2	Expt 3	
2×10^5	10,15	10,8	7,12	>100
2×10^4	1,2	2,1	1,0	>100
2×10^3	0,0	0,0	0,0	18,12
2×10^2	0,0	0,0	0,0	2,1
FFU/CFU	1.3×10^4	1.3×10^4	4×10^4	1.3×10^2

[a] Bone marrow cell suspensions from NFS/N weanlings were infected with varying virus dilutions and plated at a concentration of 2×10^6 nucleated cells per plate into soft agar as described in the text. Hematopoietic colony formation was scored at day 18. Duplicate values are shown.

Establishment and characterization of transformed lines from ST-FeSV-induced hematopoietic colonies. The induction of hematopoietic colonies by retroviruses may result in continued cell proliferation or in short-term growth accompanied by terminal differentiation (22, 30, 36, 40, 51). To distinguish between these two alternatives, it is necessary to determine whether the cells from such colonies are capable of continued independent growth in culture. Moreover, if cell lines can be established, it is possible to characterize their hematopoietic cell phenotype and malignant potential. Thus, efforts were undertaken to develop clonal cell lines from colonies induced by ST-FeSV.

Individual colonies were transferred to liquid medium in microtiter wells and grown up to mass culture. The success rate for establishment of continuous cell lines from colonies was less than 5%, but could be significantly improved to greater than 75% with the initial use of secondary or tertiary adherent feeder layers. After an adaptation period of 1 to 6 weeks, the transformed lines could be propagated independently. Under the same experimental conditions, ST-FeSV infection of the feeder layer itself did not lead to detectable transformation. Two ST-FeSV-transformed hematopoietic cell lines analyzed were developed without the initial use of

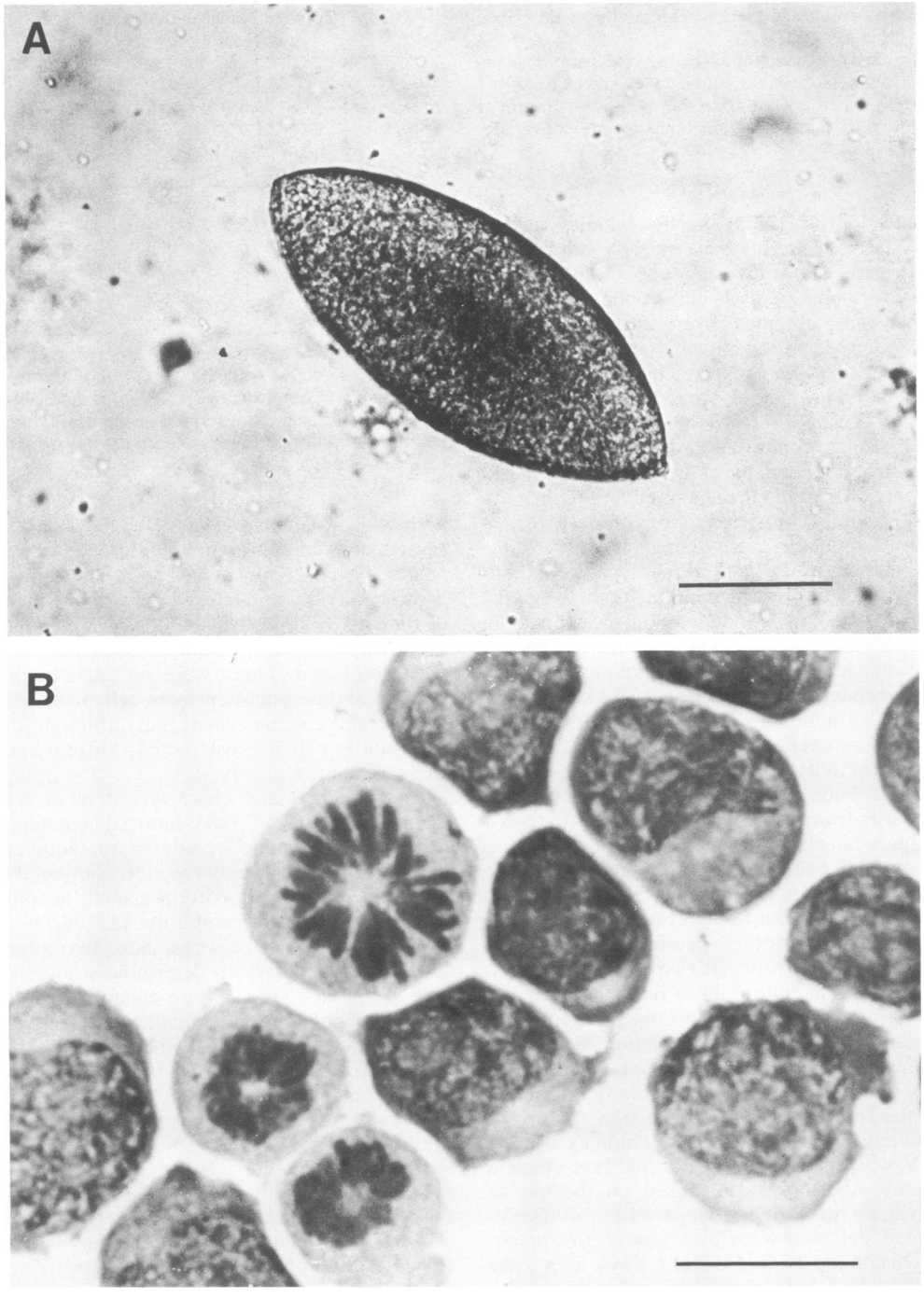

FIG. 1. (A) Morphology of ST-FeSV-induced hematopoietic colonies (bar equals 0.1 mm). (B) Wright–Giemsa-stained preparation of cells from ST-FeSV-induced colonies (bar equals 5 μm).

feeder layers, whereas the remaining lines were originally propagated in the presence of feeder layers with subsequent recloning before further characterization.

The ST-FeSV hematopoietic lines grew to saturation densities of around 2×10^6 cells per ml and had generation times of 10 to 12 h. Some clonal lines have been in continuous culture for

more than 6 months. Since colony formation in response to ST-FeSV occurred at a very low efficiency, it was unlikely that nonproductively transformed lines would be obtained. Moreover, it was likely that a large number of helper virus-infected cells would survive in agar cultures plated at high cell density, and it would be impossible to avoid such infected cells when picking colonies from these cultures. In fact, each cell line tested was found to release ST-FeSV as determined by the NIH/3T3 focus assay (data not shown). We are presently attempting to obtain non-virus-producing cell lines by performing assays under low-cell-density culture conditions.

To provide further evidence that ST-FeSV was responsible for cell transformation, the primary translational products of hematopoietic cell lines established from several ST-FeSV-induced colonies were analyzed. Cultures were labeled with [^{35}S]methionine, and virus-specific polypeptides were precipitated from cell extracts by using antiserum directed against feline leukemia virus p15. Antiserum directed against feline leukemia virus p15 precipitated a major polypeptide whose molecular weight corresponded with that of the putative ST-FeSV-transforming protein, p85 (37), from cell extracts of representative ST-FeSV-transformed hematopoietic cell lines (Fig. 2, lanes 4A and 5A). A similar pattern of immunoprecipitation was observed with ST-FeSV-transformed NIH/3T3 cells (Fig. 2, lane 3A). The additional polypeptides precipitated from the hematopoietic cell lines were shown to be Amph-MuLV related, as they were also precipitated from NIH/3T3 cells infected only with Amph-MuLV (Fig. 2, lane 2A).

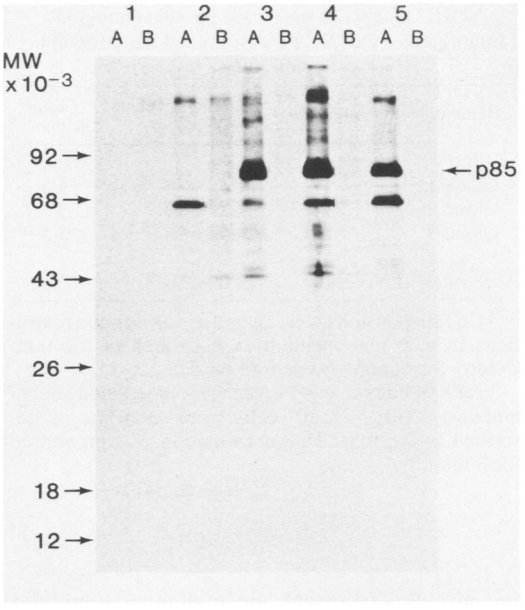

FIG. 2. Immunoprecipitation and electrophoretic analysis of translational products in ST-FeSV-transformed hematopoietic cell lines. Cell extracts from [^{35}S]methionine-labeled uninfected NIH/3T3 cells (lanes 1), Amph-MuLV-infected NIH/3T3 cells (lanes 2) ST-FeSV (Amph-MuLV) infected NIH/3T3 cells (lanes 3) and ST-FeSV-transformed hematopoietic cell lines (lanes 4 and 5) were treated with anti-FeLV p15 serum (A lanes) or normal rabbit serum (B lanes).

Oncogenicity of ST-FeSV-induced hematopoietic cell lines. The ability of cells to form colonies in soft agar is often an indication of their oncogenic potential. ST-FeSV-transformed hematopoietic cell lines were plated in soft agar medium at various concentrations, and their cloning efficiency was determined. As shown in Table 2, each ST-FeSV-transformed hematopoietic cell line tested formed colonies in soft agar at a very high efficiency, comparable to that of an Abelson-MuLV-transformed hematopoietic cell line.

To further analyze the malignant potential of ST-FeSV-induced hematopoietic cell lines, we inoculated NFS/N weanling mice subcutaneously with 5×10^6 cells from representative transformants. Each cell line tested was tumorigenic, forming very large tumors within 1 to 2 weeks. In contrast, subcutaneous inoculation of weanlings with 5×10^5 FFU of ST-FeSV(Amph-MuLV) yielded no detectable tumors. Histopathological analysis revealed the tumors to be comprised of undifferentiated hematopoietic blast cells. To confirm the donor origin of the tumors, cells from ST-FeSV-transformed hematopoietic clones derived from male mice were injected subcutaneously into 5-week-old female mice. The tumors that formed displayed a male karyotype (data not shown). These results established that the hematopoietic lines derived from ST-FeSV-induced colonies were tumorigenic.

Phenotype of ST-FeSV hematopoietic transformants. To investigate the phenotype of ST-FeSV-transformed hematopoietic cells, we analyzed clonal lines for markers associated with cells of various hematopoietic lineages. There was no evidence of hemoglobin synthesis, a characteristic of mature erythroid cells, nor could the cells be induced to synthesize hemoglobin after exposure to dimethyl sulfoxide or erythropoietin. The cells also lacked detectable nonspecific esterase and lysozyme and the ability to phagocytize latex beads, markers of cells within the myeloid series (data not shown).

The presence of mercaptoethanol in growth medium has been shown to be necessary for the maintenance of Abelson-MuLV-transformed lymphoblastic cell lines (41). This reducing agent has also been reported to enhance the survival

TABLE 2. Soft agar cloning efficiency and tumorigenicity of ST-FeSV-induced hematopoietic cell lines

Hematopoietic cell line	Colony-forming efficiency[a]	Tumor incidence[b]
ST-FeSV		
Clone 1	1.5×10^{-1}	5/5
Clone 2	7.5×10^{-2}	3/5
Clone 3	4.5×10^{-2}	3/5
Abelson-MuLV Clone 1	2.0×10^{-1}	5/5

[a] Cell suspensions were plated at various concentrations in soft agar medium as described in the text. Colony formation was scored on day 12.

[b] NFS/N mice (6 weeks old) were inoculated subcutaneously with 5×10^6 cells from each line as described in the text. Tumor formation was monitored for 3 months.

and growth of normal and malignant lymphoid cells in culture (10, 11, 33). As shown in Table 3, growth of the ST-FeSV lines required the addition of mercaptoethanol to the growth medium. None of the lines induced by ST-FeSV demonstrated detectable Thy 1.2 antigen as determined by direct immunofluorescence (Table 3). The presence of Fc receptors on the cell surface is a characteristic of many hematopoietic cell types, including B cells of lymphoid origin (27, 28). When assayed for Fc receptors by the rosette assay (28), a low percentage of rosette formation was observed in all of the lines analyzed. The presence of low levels of Fc receptors on these lines was confirmed by direct immunofluorescence (Table 3).

The alloantigen Lyb 2 (42) is expressed exclusively on B cells, a fraction of surface immunoglobulin-negative bone marrow cells, and on some pre-B cell tumors (47). A monoclonal antibody directed against Lyb 2.1 was utilized in an indirect immunofluorescence assay to determine whether this antigen was present on ST-FeSV-transformed hematopoietic cell lines. As shown in Table 3, all ST-FeSV-transformed lines tested were positive for Lyb 2.1 as determined by their membrane-specific immunofluorescence. The majority of Abelson-MuLV-transformed pre-B cell lines were also positive for Lyb 2.1, whereas a Moloney-MuLV-transformed T cell line was negative for membrane-specific immunofluorescence.

Pre-B lymphoid cells which synthesize immunoglobulin μ chain in the absence of detectable light chain appear to be the preferential target for Abelson-MuLV transformation (9, 46). When ST-FeSV transformants were analyzed for expression of mouse μ chain, 6 of 12 contained readily detectable levels as determined by competition radioimmunoassay (Fig. 3A). In comparison, 5 of 12 Abelson-MuLV hematopoietic lines transformed in vitro in our laboratory produced readily detectable amounts of μ chain. The levels of μ chain ranged from 10 to 220 ng/mg of protein in the ST-FeSV cell extracts. The amounts of μ chain present in Abelson-

TABLE 3. Characterization of ST-FeSV- and Abelson-MuLV-transformed hematopoietic cell lines

Lymphoid marker[a]	No. positive/no. tested for hematopoietic cell lines transformed by:	
	ST-FeSV	Abelson-MuLV
Mercaptoethanol dependence	6/6	6/6
Thy 1.2	0/6	0/6
Fc receptor	6/6	4/6
Lyb 2.1	6/6	4/6
Cytoplasmic μ	6/12	5/12
Cytoplasmic κ	0/12	0/12
High TdT	0/6	0/6
Low TdT	6/6	6/6

[a] The presence of Thy 1.2 antigen was determined by direct immunofluorescence as described in the text. A Moloney-MuLV T cell line exhibited >99% membrane-specific immunofluorescence, whereas a Rauscher-MuLV pre-B cell line demonstrated <1%. Exponentially growing clonal lines were transferred at a 1:20 split ratio into separate plates. Mercaptoethanol was omitted from the growth medium in one plate. Cultures were passaged every third day and maintained for several weeks. Viability was monitored every third day by trypan blue staining. Cultures were discarded when viability decreased to less than 5%. The presence of Fc receptors was determined by the rossette or immunofluorescence assay as described in the text. A cell line was considered positive if >5% rosette formation was reproducibly detected and membrane-specific immunofluorescence was observed. Neither sheep erythrocytes nor a Moloney-MuLV T cell line (19-1-2) yielded any rossettes, whereas a Rauscher-MuLV pre-B cell line (6ELB) demonstrated 65% rosette formation. The presence of Lyb 2.1 antigen was determined by indirect immunofluorescence as described in the text. Cells treated with fluorescein-conjugated rabbit anti-mouse IgG alone did not exhibit any membrane-specific immunofluorescence. The 19-1-2 cells exhibited no membrane-specific immunofluorescence, whereas >99% of 6ELB cells exhibited distinct membrane-specific immunofluorescence in the presence of anti-Lyb 2.1 serum. Cytoplasmic μ and κ chain synthesis was analyzed by radioimmunoassay as described (39). The sensitivity of detection was around 0.3 ng/mg of soluble cell protein. Cell lines were considered positive for low levels of TdT if they contained >0.01 U per 10^8 cells/h and <0.5 U per 10^8 cells/h, and positive for high levels of TdT if they possessed >0.5 U per 10^8 cells/h, as determined by enzymatic assay (5,36).

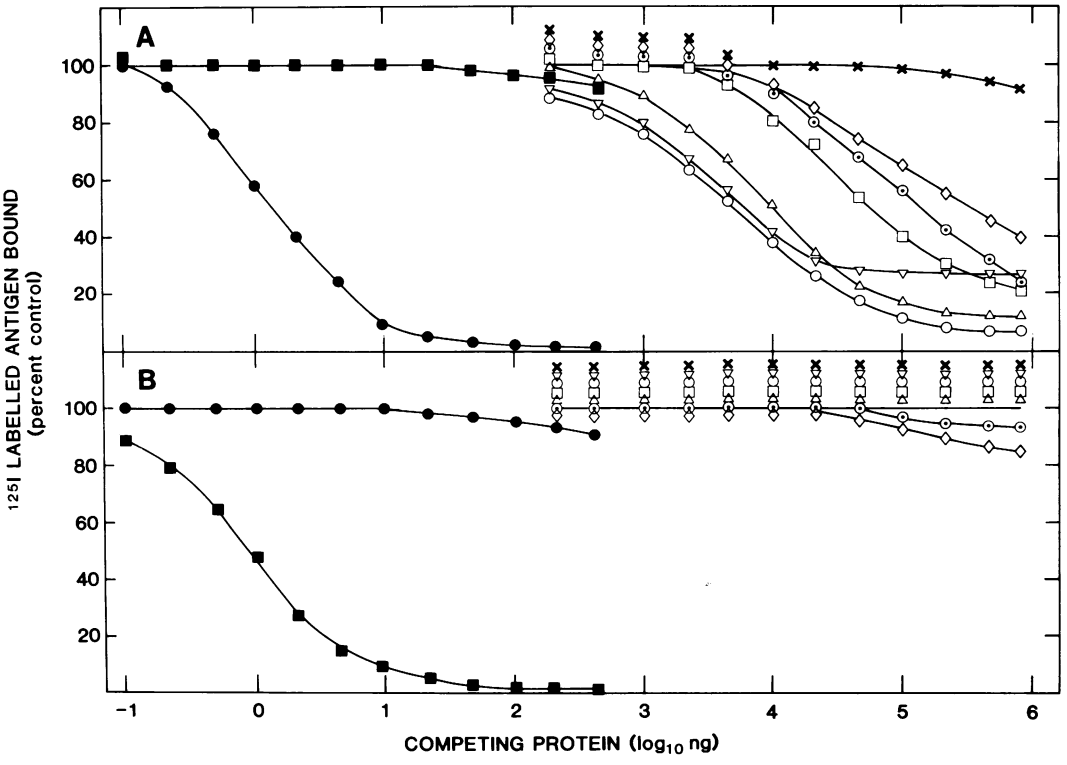

FIG. 3. Radioimmunological quantitation of immunoglobulin gene products in ST-FeSV-transformed hematopoietic cell lines. Cytoplasmic extracts were tested at serial twofold dilutions as competing antigens in radioimmunoassays utilizing limiting antibody to an immunoglobulin gene product to precipitate the homologous ^{125}I-labeled protein. Immunoassays were as follows: (A) μ chain (goat anti mouse μ:^{125}I-MOPC 104E) and (B) κ chain (goat anti mouse κ:^{125}I-MOPC 321). The filled symbols represent unlabeled immunoglobulins tested as sensitivity and specificity controls. These included purified myeloma proteins derived from tumor lines MOPC 104E (●) and MOPC 321 (■). Test samples included cytoplasmic extracts of ST-FeSV-transformed hematopoietic cell lines clone 1 (○), clone 2 (▽), clone 3 (△), clone 4 (□), clone 5 (⊙), clone 6 (◇), and a Moloney-MuLV-transformed T cell lymphoma, 19-1-2 (X).

MuLV-transformed hematopoietic cell lines were similar to those detected in ST-FeSV transformants (data not shown). In contrast, a Moloney-MuLV-induced T cell lymphoma, 19-1-2, contained no detectable μ chain. No κ light chain was detected in any ST-FeSV line analyzed (Fig. 3B).

Terminal deoxynucleotidyl transferase (TdT) is an enzyme that was first detected at high levels in immunoincompetent cortical thymocytes (31). More recently, it was demonstrated to be present at much lower levels in bone marrow-derived immature lymphocytes and Abelson-MuLV hematopoietic cell transformants (47). Analysis of six individual ST-FeSV transformants of both μ^+ and μ^- phenotypes for TdT activity by enzymatic assay revealed that they possessed low levels ranging from 0.02 to 0.15 U per 10^8 cells/h. The levels of TdT detected in most ST-FeSV-induced transformants was much lower than the amount found in normal thymus cells (2.35 U per 10^8 cells/h). Analysis of both μ^+ and μ^- Abelson-MuLV-transformed hematopoietic cell lines revealed levels of TdT ranging from 0.01 to 0.08 U per 10^8 cells/h, comparable to those detected in ST-FeSV clonal transformants. All of the above results argue that the ST-FeSV-transformed hematopoietic lines are at a stage of lymphoid cell differentiation similar to the pre-B lymphoid cell that is the preferential target for transformation by Abelson-MuLV (9, 46).

DISCUSSION

Investigation of the diversity of target cells susceptible to neoplastic transformation by a particular transforming retrovirus might be expected to provide insights into the relationship of the differentiated state of the cell to its

susceptibility to the action of a specific *onc* gene or family of *onc* genes. Abelson-MuLV is a virus known to induce nonthymic lymphomas and to transform pre-B lymphoid cells in tissue culture (1, 9, 46). ST-FeSV, whose *onc* gene arose from a cellular gene distinct from that of Abelson-MuLV, codes for a transforming protein with apparently similar in vitro functional properties (3, 52). In the present report, we investigated whether ST-FeSV induces transformation of hematopoietic cells phenotypically similar to those induced by Abelson-MuLV in culture.

We were able to demonstrate that ST-FeSV was capable of transforming murine hematopoietic cells under in vitro assay conditions. Evidence that transformation was a direct result of ST-FeSV infection was derived from the one-hit titration pattern for bone marrow colony formation, the absence of any detectable effect of helper virus alone, and the ability to demonstrate the presence of sarcoma virus in cell lines established from such colonies. ST-FeSV-induced colonies could be established in culture as continuous clonal lines with high proliferative capacity. Their ability to form rapidly growing tumors of donor origin and with the same hematopoietic blast cell morphology confirmed their malignant potential.

The hematopoietic blast cell transformants induced by ST-FeSV possessed none of the markers that identify mature cells within the known hematopoietic lineages. However, they possessed Lyb 2, a pre-B cell-specific antigen (42), were dependent on mercaptoethanol for growth, and synthesized only low or undetectable levels of TdT. Finally, a large number synthesized immunoglobulin μ chain in the absence of light chains. Thus, the hematopoietic cells transformed by ST-FeSV in culture appear to be at an early stage of B cell differentiation that is very similar to the pre-B cell phenotype of Abelson-MuLV hematopoietic cell transformants (9, 46).

It has recently been demonstrated that the growth-promoting action of Abelson-MuLV for hematopoietic cells in culture is not limited to its transforming effects on lymphoid cells. This virus also induces erythroid burst formation in early fetal liver embryo cultures (51). Whether ST-FeSV has similar effects on cells of the erythroid series remains to be resolved.

The similar phenotypes of the pre-B lymphoid cell transformants induced by ST-FeSV and Abelson-MuLV could be coincidental or might reflect similarities in the functional pathways by which their *onc* gene products exert their oncogenic potential. In this regard, several avian transforming retroviruses, whose *onc* gene products lack detectable tyrosine kinase-associated activity, preferentially transform cells at other stages of hematopoietic differentiation (19). Moreover, under conditions identical to those used in the present studies, BALB and Harvey murine sarcoma viruses, members of a different *onc* gene family (2, 15), specifically transform immature lymphoid cells that lack detectable immunoglobulin expression but demonstrate high levels of TdT activity (36). These transformants appear to be at an earlier stage of lymphoid cell differentiation than those of Abelson-MuLV or ST-FeSV transformants. Thus, we favor the possibility that the similar phenotype of ST-FeSV and Abelson-MuLV hematopoietic targets may reflect functional similarities in their *onc* gene products.

There is as yet no published evidence relating ST-FeSV and Abelson-MuLV transforming genes at a genetic level. Their *onc* genes are unrelated by molecular hybridization and anneal with different cellular genes (K. C. Robbins, personal communication). Moreover, attempts to detect immunological relatedness of their *onc* proteins have been unsuccessful (4). Recent results of nucleotide sequence analysis, however, have revealed high degrees of similarity at the amino acid sequence level in the predicted gene products of Rous sarcoma virus and ST-FeSV *onc* genes (20). Sequence analysis of the Abelson-MuLV transforming gene has also demonstrated a long stretch of homology in a region of the predicted *abl* product with those of both *src* and *fes* (E. P. Reddy, personal communication). These findings are consistent with the concept that the structural similarities detected between the putative products of these *onc* genes have a likely functional and evolutionary significance as well.

ACKNOWLEDGMENT

We thank Leslie Kalbfleisch for excellent technical assistance.

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