Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2374–2378, April 1984
Cell Biology

Hematopoietic cell transformation by a murine recombinant retrovirus containing the *src* gene of Rous sarcoma virus

(murine lymphoid progenitor cells/leukemogenesis/avian oncogene/terminal deoxynucleotidyl transferase/tumorigenesis)

JACALYN H. PIERCE*, STUART A. AARONSON*, AND STEVEN M. ANDERSON†

*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20205; and †Virus and Cell Biology, Merck, Sharp & Dohme Research Laboratories, West Point, PA 19486

Communicated by Raymond L. Erikson, December 16, 1983

ABSTRACT **A recombinant murine retrovirus (MRSV) containing the *src* gene of avian Rous sarcoma virus (RSV) was shown to induce hematopoietic colonies in infected mouse bone marrow. MRSV-induced colony formation followed single-hit kinetics and required mercaptoethanol in the agar medium. Cells from the colonies induced by MRSV could be established as continuous cell lines that demonstrated unrestricted self-renewal *in vitro* and tumorigenicity *in vivo*. The transformants, all of which expressed high levels of the Rous sarcoma virus transforming protein, pp60^{src}, appeared to be at an early stage in lymphoid cell differentiation. They lacked Fc receptors and detectable immunoglobulin μ heavy chain synthesis, markers normally associated with committed B cells. The majority of the MRSV-transformed cell lines contained high levels of terminal deoxynucleotidyl transferase, an enzyme present in lymphoid progenitor cells committed to the T-cell lineage. One cell line expressed Thy-1 antigen, but none expressed Lyt-1 and Lyt-2, markers of more differentiated T cells. These findings demonstrate that the *src* gene is capable of transforming cells of hematopoietic origin.**

Certain retroviral *onc* genes have been shown to induce a wide spectrum of neoplasia, including solid and hematopoietic tumors, while others appear to possess a more restricted transforming potential (1). The prototype avian sarcoma virus, Rous sarcoma virus (RSV), is known to induce solid tumors in avian species (2, 3) and to transform avian cells of connective tissue and epithelial origin *in vitro* (4–8). Efforts to demonstrate its capacity to transform avian hematopoietic cells have not been successful. For example, although RSV replicates efficiently in avian macrophages, it is unable to transform them in culture (9).

The ability to study the range of mammalian target cells susceptible to RSV transformation has been impaired by the fact that it does not productively replicate in mammalian cells (10, 11). With the advent of recombinant DNA technology, it has become possible to construct retroviruses containing a variety of foreign genes. By such an approach, the RSV *onc* gene, *src*, has been introduced into an amphotropic murine helper virus, yielding a recombinant virus designated MRSV (12). Although MRSV is replication defective, it can be efficiently rescued by mouse type C helper viruses and, thus, is capable of productively infecting mammalian cells. The generation of this virus has permitted a more extensive investigation of the types of mammalian cells that may be susceptible to the transforming effects of the *src* gene product. Because it has been reported that MRSV induces splenomegaly in adult mice (12), we examined the ability of this virus to alter the growth properties of mouse hematopoietic cells and characterized a novel lymphoid target for malignant transformation by MRSV *in vitro* and *in vivo*.

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MATERIALS AND METHODS

Cells and Viruses. Cell lines included NIH/3T3 cells (13); clonal NIH/3T3 nonproducer transformants of MRSV, clone 2-1 (12); and Abelson murine leukemia virus (A-MuLV), Ann-1 (14). Murine hematopoietic cell lines included Harvey murine sarcoma virus (MSV) and A-MuLV NFS/N lymphoid transformants (15), NIH/Swiss Rauscher MuLV pre-B-cell line, and Moloney-MuLV (M-MuLV) T-cell line (16). Mouse type C helper leukemia viruses included clonal strains of M-MuLV (16) and amphotropic MuLV (Amph-MuLV). Amph-MuLV 292 was obtained from N. Rosenburg (Tufts University, Boston, MA).

Antisera. Antisera included tumor-bearing rabbit serum from H. Opperman (University of California, San Francisco, CA); anti-pp60^{src} antiserum from T. Parsons (University of Virginia, Charlottesville, VA); monoclonal antibody to the murine Fc receptor from J. Unkeless (Rockefeller University, New York, NY); Fluorescein-conjugated monoclonal antibodies to Thy-1, Lyt-1, and Lyt-2 from Becton-Dickinson; and fluorescein-conjugated mouse IgG from Cappel Laboratories.

Hematopoietic Colony Formation. The colony-forming assay was performed as described (15).

Metabolic Labeling and Radioimmunoprecipitation. The procedure for biosynthetic labeling with [^{35}S]methionine and immunoprecipitation were as described (15).

Immunofluorescence Assays. Cell surface immunofluorescence was performed as described (15). Briefly, 10^6 cells were incubated with fluorescein-conjugated Thy-1, Lyt-1, or Lyt-2 monoclonal antibody for 30 min at 4°C. Cells were then washed, mounted wet, and examined for membrane-specific immunofluorescence. Surface Fc receptors were detected by direct immunofluorescence by incubating cells with heat-aggregated fluorescein-conjugated mouse IgG for 30 min at 4°C. Alternatively, the presence of Fc receptors was determined by indirect immunofluorescence utilizing mouse monoclonal anti-Fc receptor serum as described (17).

Terminal Deoxynucleotidyl transferase Assay. The terminal deoxynucleotidyl transferase enzymatic assay was performed by the method of Barton *et al.* (18) with minor modifications as described (15).

RIA for Immunoglobulin μ Chains. The amount of mouse immunoglobulin μ chain present in cells was determined by competition RIA as described (16, 19).

RESULTS

***src*-Containing Murine Recombinant Virus Induces the Growth of Murine Hematopoietic Colonies.** To investigate the hematopoietic-transforming potential of MRSV, we used a

Abbreviations: RSV, Rous sarcoma virus; MSV, murine sarcoma virus; ST-FeSV, Synder–Theilen feline sarcoma virus; MRSV, murine Rous sarcoma virus; MuLV, murine leukemia virus; Amph-MuLV, amphotroph-MuLV; M-MuLV, Moloney-MuLV; A-MuLV, Abelson-MuLV; ffu, focus-forming units.

colony-forming assay that detects hematopoietic cell transformation by a number of mammalian transforming retroviruses (15, 17, 20). Bone marrow cell suspensions obtained from 2- to 3-wk-old NFS/N mice were infected with either Amph-MuLV or M-MuLV pseudotypes of MRSV and suspended in soft agar medium. By 8–12 days, growing colonies were observed in virus-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. Infection of bone marrow cells with either Amph-MuLV or M-MuLV alone produced no detectable colonies, indicating that colony-forming activity was due to MRSV.

It has been reported that some murine sarcoma virus-transformed nonproducer fibroblast cells can release high levels of granulocyte/macrophage colony-stimulating factors into culture fluids (21). To determine whether the hematopoietic colony-forming ability of MRSV preparations was due to the presence of such factors, tissue culture fluids obtained from the MRSV-transformed nonproducer NIH/3T3 cell line, 2-1, were tested for the induction of bone marrow colonies. No hematopoietic colonies were detected in such cultures, suggesting that colony formation was the direct result of MRSV infection.

Induction of hematopoietic colonies by MRSV was approximately 1/10th as efficient as induction by A-MuLV, based upon the use of equivalent titers of fibroblast focus-forming units (ffu) (Table 1). Moreover, MRSV-induced colonies first became visible 2–4 days later than A-MuLV-transformed colonies under identical assay conditions. Colonies induced by MRSV were compact in shape and were indistinguishable from those induced by A-MuLV, except that they generally did not become as large. We also investigated whether such colonies could be detected upon infection of mouse fetal liver cells. Colony formation was observed in infected fetal liver cultures, and the efficiency of colony formation was comparable to that observed with mouse bone marrow (Table 1). Cells from individual colonies induced by MRSV were cytocentrifuged on microscope slides and stained with Wright–Giemsa. These cells demonstrated morphological characteristics of undifferentiated blast cells (data not shown).

MRSV Induces Hematopoietic Colony-Forming Cells in Infected Mice. Previous studies have indicated that MRSV induces an erythroproliferative disease characterized by splenic foci and splenomegaly in adult mice (12). When we inoculated newborn NFS/N mice intraperitoneally with MRSV (Amph-MuLV), they developed grossly enlarged spleens containing many pale, poorly defined foci within 3–5 wk. Histopathological analysis revealed that the majority of the cells within the spleen were immature cells within the erythroid series (data not shown). Extracts derived from enlarged spleens were shown to contain detectable levels of MRSV as determined by the NIH/3T3 focus-forming assay (data not shown). We also observed the appearance of solid tumors occurring at multiple sites in 8 of 10 infected animals. Histopathological analysis revealed that they were either fibrosarcomas or rhabdosarcomas. The presence of MRSV also was detected in extracts of these tumors (data not shown). There was no detectable pathology in animals infected with the helper virus alone at comparable times after infection or in uninfected controls.

To determine whether hematopoietic colony-forming cells, like those detected after *in vitro* infection with MRSV, were present in the inoculated animals, bone marrow cells from MRSV-infected animals were plated in soft agar. We observed the formation of compact colonies from only 1 of 10 animals tested. The frequency of colony formation from the bone marrow of this animal was 13 colonies per 10^6 nucleated cells plated.

Malignant Phenotype of MRSV-Transformed Hematopoietic Cell Lines. The induction of hematopoietic colonies by retroviruses has been reported to result either in continued cell proliferation or in short-term growth promotion accompanied by terminal differentiation (15, 17, 20–23). In order to distinguish between these two effects, it is necessary to determine whether such colonies are capable of continued, independent growth. Moreover, if cell lines could be established, it would be possible to characterize their phenotype with respect to tumorigenicity and stage of differentiation.

Individual MRSV-induced colonies were transferred to liquid medium in microtiter wells. Nonadherent blast cells could be propagated for several passages but would not continue to proliferate independently without the initial use of normal adherent mouse bone marrow feeder layers (24, 25). After passage in the presence of feeder layers for 1–3 months, cells could be adapted to propagate independently. Cell lines established in the presence of feeder layers were recloned in soft agar before further characterization. Infection of adherent bone marrow feeder layers with MRSV alone did not lead to detectable cell transformation.

Cells from the established lines demonstrated morphological characteristics indistinguishable from cells in the original colonies. Electron microscopy revealed typical lymphoblastoid morphology, and budding virus particles were frequently seen (data not shown). No difference in cellular morphology was observed among the seven clones examined. The cell lines grew to saturation densities of around 2×10^6 cells per ml and possessed generation times of 10–12 hr. Each of the

Table 1. *In vitro* hematopoietic colony formation induced by MRSV

	Induced colony formation†, cfu per plate					
	MRSV (Amph-MuLV)		MRSV (M-MuLV)		A-MuLV (Amph-MuLV)	
ffu*	Bone marrow	Fetal liver	Bone marrow	Fetal liver	Bone marrow	Fetal liver
ffu added per plate						
10^5	25, 19	20, 15	39, 22	NT	>100	>100
10^4	3, 0	2, 0	2, 2	NT	9, 18	16, 8
10^3	0, 0	0, 0	0, 0	NT	1, 2	2, 0
10^2	0, 0	0, 0	0, 0	NT	0, 0	0, 0
ffu/cfu ratio	6700	10,000	5000	—	740	830

NT, not tested; cfu, colony-forming units.
*ffu as determined on NIH/3T3 cells.
†Bone marrow cell suspensions from NFS/N weanlings were infected with various 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. Fetal liver suspensions from NFS/N 18-day-old embryos were infected in the same manner.

established MRSV-transformed cell lines demonstrated high cloning efficiency in soft agar (Table 2).

When MRSV-transformed cell lines were inoculated into syngeneic adult mice, the cells formed large tumors at the site of inoculation within 1–2 wk (Table 2). Histopathologic analysis revealed the tumors to be comprised of hematopoietic blast cells indistinguishable from the original MRSV transformants (data not shown). In contrast, subcutaneous inoculation of 10^5 ffu of MRSV yielded no detectable tumors in NFS/N adult mice. All of these results established that hematopoietic cell lines derived from MRSV-induced bone marrow colonies were capable of unrestricted self-renewal *in vitro* and were tumorigenic *in vivo*.

Presence of pp60^{src} in MRSV-Induced Hematopoietic Cell Lines. To substantiate that MRSV was responsible for hematopoietic cell transformation, we sought to detect the *src* gene primary translational product in each of several cell lines established from MRSV-induced colonies. Cell lines were tested for the presence of pp60^{src} by radioimmunoprecipitation (Fig. 1). MRSV-transformed hematopoietic clones 1, 2, 4-1, 5, and 37-5-3 all contained elevated levels of pp60^{src} (Fig. 1, lanes 4a–8a). Similar data were obtained for MRSV-transformed clones 4-2 and 7 (data not shown). The levels of pp60^{src} in the MRSV-transformed hematopoietic cell lines were comparable to those detected in the MRSV-transformed NIH/3T3 nonproducer cell line, 2-1 (Fig. 1, lane 2a). In contrast, pp60^{src} was not detected in control NIH/3T3 cells or in a Harvey MSV-transformed murine lymphoblastoid cell line, HS1C5 (Fig. 1, lanes 1a and 3a). The *src*-encoded transforming protein has been demonstrated to possess kinase activity capable of phosphorylating the heavy chain of IgG and other cellular substrates (26, 27). All pp60^{src} proteins present in the MRSV-transformed cell lines were active in the immune complex protein kinase assay (data not shown).

MRSV Hematopoietic Cell Transformants Are Lymphoid Progenitor Cells. To investigate the phenotype of MRSV transformants, we analyzed clonal lines for markers associated with cells of various hematopoietic lineages. Characteristics associated with erythroid cells, including spectrin and hemoglobin synthesis, were not detectable. Markers of cells within the myeloid lineage, including specific and nonspecific esterase, lysozyme, and myeloperoxidase were also negative (data not shown).

Mercaptoethanol has been shown to enhance normal lymphoid cell survival and growth in tissue culture (28, 29) as well as the growth capacity of certain lymphoblastic and myeloblastic tumor cell lines (30). Mercaptoethanol also has been shown to be critical for lymphoid cell colony formation by A-MuLV (20, 31), Synder–Theilen feline sarcoma virus (St-FeSV) (17) and Harvey and BALB MSVs (15). The growth of MRSV transformants was found to be strictly dependent on the presence of mercaptoethanol (Table 3).

Table 2. Soft agar cloning efficiency and tumorigenicity of MRSV-induced hematopoietic cell lines

Hematopoietic cell line	Colony-forming efficiency × 10^{-1}*	Tumor incidence†
MRSV (M-MuLV)		
Clone 1	1.2	5/5
Clone 5	0.25	5/5
MRSV (Amph-MuLV)		
Clone 2	0.36	3/5
Clone 4-1	1.1	5/5
Clone 4-2	1.5	5/5
Clone 37-5-3	2.1	5/5
Clone 7	1.8	5/5
MuLV (Amph-MuLV)		
Clone 1	2.3	5/5

*Cell suspensions were plated at 1:10 dilutions in semisolid agar medium. Colony formation was scored on day 12.

†NFS/N mice (16-wk-old) were inoculated subcutaneously with 5×10^6 cells from each line. Virus alone was inoculated at 10^5 ffu. Tumor formation was monitored for 3 months.

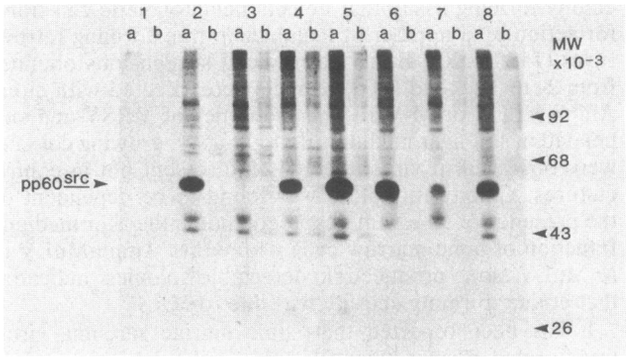

FIG. 1. Immunoprecipitation and electrophoretic analysis of translational products in MRSV-transformed hematopoietic cell lines. [^{35}S]Methionine-labeled cell extracts were treated with rabbit anti-pp60^{src} antiserum (lanes a) or normal rabbit serum (lanes b). Antigen–antibody complexes were precipitated with protein A-Sepharose CL-4B. Washed samples were suspended in sample buffer, boiled, and subjected to electrophoretic analysis on a 10% NaDodSO$_4$/polyacrylamide gel. Cell extracts were from uninfected NIH/3T3 cells (lanes 1); MRSV-transformed NIH/3T3 cells, 2-1 (lanes 2); Harvey MSV-transformed lymphoblastoid cell line HS1C5 (lanes 3); and MRSV-transformed hematopoietic clonal cell lines 1, 2, 4-1, 5, and 37-3-5 (lanes 4–8, respectively).

Lymphoid pre-B-cells, which synthesize μ heavy chain in the absence of detectable light chains (32), are the preferential target for transformation by both A-MuLV (33) and ST-FeSV (17). When MRSV transformants were analyzed for expression of mouse chain, none contained detectable levels as determined by competition RIA (Table 3). In contrast, more than half of the A-MuLV- and ST-FeSV-transformed hematopoietic cell lines established *in vitro* in our laboratory produced readily detectable amounts of μ chain (ref. 17; data not shown).

The presence of Fc receptors on the cell surface is a characteristic of many hematopoietic cell types, including B cells of lymphoid origin (34). The MRSV transformants demonstrated no detectable Fc receptors under conditions in which Rauscher MuLV-transformed μ^+ pre-B-cells expressed high levels of Fc receptors, and M-MuLV-transformed T cells were negative (Table 3). The absence of μ chain synthesis and surface Fc receptors indicated that the MRSV transformants were probably not immature or mature cells within the lymphoid B-cell series.

Terminal deoxynucleotidyl transferase is an enzyme present at high levels in murine immunoincompetent cortical thymocytes (18) as well as in lymphoid progenitor cells and immature T-cell lymphomas of both murine and human origin (35, 36). Although pre-B-cells have been shown to contain terminal deoxynucleotidyl transferase, the levels of the enzyme present in these cells are greatly reduced in comparison to cells within the T-cell lineage (15, 17). Most MRSV-induced hematopoietic cell transformants demonstrated terminal deoxynucleotidyl transferase levels that were as high or higher than those detected in normal thymus cells from 2 to 3-wk-old NFS/N mice or in a M-MuLV T-cell lymphoma (Table 4). By contrast, A-MuLV transformants invariably contained only very low or undetectable levels of this enzyme.

Lymphoid progenitor cells committed to the T-cell pathway are known to possess elevated levels of terminal deoxynucleotidyl transferase (18). Thus, the MRSV transformants were analyzed for the presence of T-cell surface markers

Table 3. Phenotypic characterization of MRSV-transformed hematopoietic cell lines

Lymphoid marker*	Number positive/ number tested
Mercaptoethanol dependence	7/7
Cytoplasmic μ chain	0/7
Fc receptor	0/7
TdT	7/7
Thy-1 antigen	1/7
Lyt-1 antigen	0/7
Lyt-2 antigen	0/7

*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 3 days and monitored by trypan blue staining. Cultures were discarded when viability decreased to <5%. Cytoplasmic μ-chain synthesis was analyzed by RIA as described (16). The sensitivity of μ-chain detection was 0.3 ng/mg of soluble cell protein. The presence of Fc receptors was determined by both direct immunofluorescence with fluorescein-conjugated mouse IgG and indirect immunofluorescence utilizing an anti-Fc receptor monoclonal antibody. Cell lines were considered positive for terminal deoxynucleotidyl transferase (TdT) if they contained enzyme levels greater than 0.05 units per 10^8 cells per hr. The presence of Thy-1 antigen was determined by complement-mediated lysis and direct immunofluorescence. The presence of Lyt-1 and Lyt-2 antigens was determined by direct immunofluorescence.

Table 4. Terminal deoxynucleotidyl transferase (TdT) activity in MRSV-transformed hematopoietic cell lines

Cell	TdT activity,* units/10^8 cells/hr
Normal thymus†	2.25
MRSV (Amph-MuLV)	
Clone 2	2.76
Clone 4-1	0.67
Clone 4-2	0.11
Clone 7	2.85
Clone 37-5-3	3.64
MRSV (M-MuLV)	
Clone 1	0.07
Clone 5	0.69
A-MuLV (Amph-MuLV)	
Clone 1	0.04
Clone 2	0.02
M-MuLV	
Clone 1	2.35

*TdT values represent the average of duplicate samples. One unit is equivalent to the incorporation of one nmol of dGTP into acid-insoluble material in 1 hr at 37°C.

†Normal thymus cells were obtained from 2- to 3-wk-old NFS/N weanling mice.

Thy-1 and Lyt. One of the seven lines tested, clone 4-1, possessed surface Thy-1 antigen as determined by complement-mediated lysis and direct immunofluorescence (Table 3). These cells demonstrated 70% complement-mediated lysis. Under the same conditions, three M-MuLV T-cell lymphomas showed >95% lysis, and a Rauscher MuLV pre-B-cell lymphoma showed <1% lysis. Immunofluorescence analysis revealed that 100% of the cells from clone 4-1 were positive for Thy-1 antigen, but the intensity of cell-surface fluorescence was not as strong as that demonstrated on three M-MuLV lymphomas. None of the MRSV-transformed hematopoietic cell lines, including the Thy-1-positive clone, 4-1, expressed Lyt-1 or Lyt-2 antigens (Table 3). These T-cell markers are associated with more functionally mature Thy-1-positive cells (37). Two of the three M-MuLV lymphomas were positive for Lyt-1 or Lyt-2, whereas the Rauscher MuLV pre-B-cell lymphoma was negative. All of these findings help to establish the identity of our MRSV transformants as immature cells within the lymphoid differentiation pathway, possibly progenitor cells of the T-cell lineage.

DISCUSSION

The present study demonstrates that a murine recombinant retrovirus (MRSV) containing the *src* gene of RSV is capable of transforming immature murine lymphoid cells *in vitro* and *in vivo*. Evidence that growth alterations of hematopoietic cells were a direct result of infection by MRSV was indicated by the single-hit kinetics of colony formation and by the inability of helper virus alone or factors released from nonproducer cells to cause colony formation. Moreover, all cell lines established from the MRSV-induced colonies were shown to contain the *src*-transforming gene product, $pp60^{src}$. Our findings that MRSV-induced colonies could be established as cell lines that possessed unrestricted self-renewal capacity *in vitro* and tumorigenicity *in vivo* demonstrate that the *src* gene of RSV is capable of inducing malignant transformation of hematopoietic cells.

The MRSV-induced hematopoietic transformants were shown to be at an early stage within the lymphoid differentiation pathway by analysis of appropriate markers. The presence of terminal deoxynucleotidyl transferase in many clones and Thy-1 on one clone indicates that these transformants may be progenitor cells committed to the T-cell lineage (18). It is not known whether this phenotype reflects that of the actual target cell infected. For example, evidence exists that avian erythroblastosis virus-induced hematopoietic transformants may continue to differentiate after infection (38). The fact that not all of the MRSV-transformed cell lines isolated possessed identical markers suggests the possibility that further differentiation may have occurred after infection with MRSV. However, there appears to be a stable block in the ability of these transformants to mature beyond a relatively primitive stage in lymphoid development. Construction of murine recombinant viruses containing temperature-sensitive *src* genes (39) could be useful in determining whether the apparent block in differentiation is reversible in hematopoietic cells transformed by *src*.

Other studies have demonstrated that RSV or MRSV can induce growth of erythroid colonies after infection of avian bone marrow (P. Kahn and T. Graf, personal communication) or murine fetal liver cells, respectively (W. D. Hankins, personal communication). In the murine system, erythroid bursts induced by MRSV grow in the absence of erythroprotein. However, addition of erythroprotein increases the number of these colonies as well as the extent of hemoglobinization of the cells in the bursts. Thus, MSRV induces growth promotion of erythroid cells without blocking terminal differentiation. We have not observed erythroid bursts in our assay system, and we presume that this is due to the different culture conditions required for these separate assay systems.

Recent studies have indicated that the retroviral long terminal repeat may influence the expression of the viral genome in different tissues and may play a role in the disease spectrum observed (40, 41). Thus, the structure of the MRSV long terminal repeat may be an important element in the ability of MRSV to transform murine lymphoid cells. It is also possible that the hematopoietic cell transformed by *src* in the mouse is not present in avian species or is not susceptible to RSV infection. Finally, other host factors or assay conditions critical for the growth promotion of transformed hematopoietic cells may differ in avian and murine systems.

It recently has been reported that cells from many A-MuLV-induced hematopoietic colonies are initially poorly oncogenic and that they become progressively tumorigenic and growth independent only after propagation on normal

adherent bone marrow feeder layers (24, 25). In tissue culture, the establishment of permanent cell lines from MRSV-induced colonies also required the initial use of feeder layers. These findings suggest that MRSV may, by itself, be sufficient to initiate transformation of hematopoietic cells but may require additional events, such as the activation of cellular *onc* genes, for expression of the fully transformed state. Evidence that MRSV-transformed lymphoid cells were detectable in only a minority of infected animals, despite the fact that all animals demonstrated splenomegaly, indicates that lymphoid cell transformation occurs relatively infrequently in the course of the MRSV-induced disease. This also would be consistent with the concept that a multistage process is required for development of the malignant phenotype of MRSV-altered lymphoid cells.

Transforming proteins encoded by *abl*, *src*, and *fes* are all tyrosine-specific protein kinases that share extensive homology in their predicted amino acid sequences (42, 43). Retroviruses containing these *onc* genes are capable of transforming murine lymphoid cells in culture but do not always arrest lymphoid cell differentiation at the same stage. Whereas *abl* and *fes* preferentially transform lymphoid pre-B-cells (17, 33), the targets of *src* appear to be progenitors of the T-cell lineage. Comparison of the predicted amino acid sequences has not revealed any closer homology of *abl* and *fes* gene products to each other than to $pp60^{src}$ (43). However, the transforming proteins encoded by *fes* and *abl* have been shown to be gag-onc fusion proteins, whereas the *src* gene product is not. It is possible that these structural differences may play a role in determining the hematopoietic cell specificity for transformation by these, otherwise, closely related *onc* gene products.

We thank Leslie Kalbfleisch and Charles Knicley for excellent technical assistance.

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