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A Murine Recombinant Retrovirus Containing the *src* Oncogene Transforms Erythroid Precursor Cells In Vitro

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A murine retrovirus (MRSV) containing the *src* gene of Rous sarcoma virus has been shown to cause an erythroproliferative disease in mice (S. M. Anderson and E. M. Scolnick, J. Virol. 46:594–605, 1983). We now demonstrate that this same virus can transform erythroid progenitor cells in vitro. Infection of fetal liver cells or spleen and bone marrow cells from phenylhydrazine-treated adult mice gave rise to colonies of erythroid cells which grew in methylcellulose under conditions not favorable for the growth of normal erythroid cells. The presence of pp60^{src} in the transformed erythroid cells was demonstrated by an immune complex protein kinase assay. The time course of appearance and subsequent differentiation of erythroid colonies indicated that the target cell for MRSV was a 6- to 8-day burst-forming unit. Differentiation of the erythroid progenitors was not blocked by the presence of pp60^{src}, and the cells retained sensitivity to the hormone erythropoietin. In fact, the transformed cells exhibited increased hormone sensitivity since the number, the size, and the extent of hemoglobinization of the colonies were all increased by the addition of small amounts of erythropoietin. MRSV was not susceptible to restriction by the Fv-2 locus, as MRSV could transform hematopoietic cells from C57BL/6 mice. These results indicate that (i) the erythroid proliferation observed in vivo is caused by a direct effect of MRSV on erythroid progenitors and (ii) the transformed erythroid precursors acquire a growth advantage over uninfected cells without losing the ability to differentiate and respond to physiologic regulators.

The generation of a murine retrovirus (MRSV) (2) which contains the *src* gene of the avian retrovirus Rous sarcoma virus has allowed the investigation of the effect of *src* upon cell systems which were not previously amenable to research. The inability of most strains of RSV to infect mammalian cells (5, 30) or to replicate efficiently in mammalian cells (3, 6, 28, 29) has effectively limited the majority of research with RSV to avian cells. MRSV is a defective murine retrovirus lacking a functional *env* gene resulting from the fact that the *src* gene was inserted into the middle of the *env* gene by using recombinant DNA techniques (2). In the presence of an appropriate helper virus, MRSV can infect, transform, and replicate in cells from a variety of species (2). This has allowed recent investigations which document *src*-induced alterations in long-term bone marrow cultures (4) and transformation of murine lymphoid cells (25).

Intravenous administration of MRSV into adult mice is characterized by an erythroproliferative disease accompanied by splenomegaly and mild anemia (2). The spleens of these animals contained erythroid cells typical of all stages of the erythroid lineage from erythroblast to normoblast. Subsequent studies indicated that MRSV caused fibrosarcomas and splenomegaly in newborn mice (25). It was possible that the dramatic effect upon erythropoiesis might be secondary to the effects MRSV had upon other nonhematopoietic cells. We therefore investigated the effects of MRSV upon murine erythropoiesis in vitro to (i) test whether MRSV exerts a direct erythroproliferative action on hematopoietic cells, (ii) characterize the target cell, and (iii) elucidate the kinetics and temporal aspects of the erythroproliferative actions of MRSV.

The assay we have used detects the formation of large colonies of erythroid cells called bursts after infection of bone marrow cells or fetal liver cells with an oncogenic virus. This assay was originally developed to analyze the erythroproliferative effects of the Friend virus complex (10, 13, 16, 17, 21). With this assay, a number of transforming retroviruses have been shown to induce erythroid bursts with pleotropic developmental potentials characteristic for each virus (9, 11, 13).

In this report, we demonstrate that MRSV exerts a direct transforming effect upon hematopoietic targets to induce growth of erythroid colonies in vitro. Although MRSV-induced erythroid bursts have some important similarities to those induced by the Friend virus complex, striking differences were observed with respect to the time of colony appearance, morphology, and proliferative potential; another distinguishing characteristic of the MRSV-induced colonies was their sensitivity to erythropoietin.

MATERIALS AND METHODS

Animals. Ten-day pregnant NIH Swiss and C57BL/6 mice were obtained from the Small Animal Facility, National Institutes of Health. They were killed on day 12 of gestation; the fetuses were removed, and fetal livers were excised. Adult NIH Swiss mice (4 to 6 weeks old) were also obtained from the Small Animal Facility, and they were treated with phenylhydrazine to stimulate erythropoiesis as described previously (9, 10).

Virus. The origin of MRSV has been described previously (2). All MRSV stocks used herein were from nonproducer clone 2-1 superinfected with Moloney murine leukemia virus which serves as a helper virus for the replication-defective MRSV. The MRSV pseudotypes were collected as a fresh 24-h cell-free supernatant fluid before each experiment.

Erythroid transformation assay. Hematopoietic cells were infected and cultured in methylcellulose as previously described (10). Briefly, single-cell suspensions from fetal liver,

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bone marrow, or spleen were prepared in Dulbecco modified Eagle medium with 10% fetal calf serum. The cells were incubated with freshly harvested virus for 2 h at 4°C. Methylcellulose-containing growth medium was added to the virus-cell mixture to give final concentrations of 0.75% methylcellulose and 30% fetal calf serum. In some cases, erythropoietin was added at a final concentration of 0.1 U/ml. Plates were monitored in situ for the appearance of red (hemoglobinized) bursts or, alternatively, on glass slides after being clotted and stained with benzidine and hematoxylin (23). Nonerythroid colonies (lymphoid, myeloid, or fibroblast) were not quantitated. CFU and burst-forming units (BFU) have been defined previously (12, 13). Alternatively, MRSV-induced erythropoiesis was monitored by the incorporation of iron into heme as assessed by the uptake of $^{35}FeCl_3$ as described previously (9, 18). Erythropoietin (step 3 purification) was obtained from Connaught.

Presence of pp60^{src} in bursts. The presence of pp60^{src} in erythroid bursts was determined by the immune complex protein kinase assay. Erythroid bursts were picked from the methylcellulose, washed and lysed in RIPA buffer (150 mM NaCl, 50 mM Tris [pH 7.4], 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.5% sodium dodecyl sulfate) with 1% Kallikrein inactivator (Calbiochem-Behring) at a concentration of 10^6 cells per 1.0 ml. The lysates were disrupted with a Vortex mixer for 30 s and then spun at 40,000 rpm for 30 min in a Beckman Ti70.1 rotor. A 100-μl portion of the lysate, representing 10^6 cells, was mixed with 5 μl of normal rabbit serum or tumor-bearing rabbit serum and incubated on ice for 1 h. Fifteen microliters of a 50% suspension of protein A-Sepharose (Pharmacia Fine Chemicals) in RIPA buffer was then added, and these ingredients were mixed gently on a rocking platform for 1 h at 4°C. The immune complexes were washed five times with 1.0 ml of RIPA buffer and once with kinase buffer (60 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid; pH 7.0], 150 mM KCl, 5 mM dithiothreitol, 10 mM magnesium acetate). To the dry pellet was added 20 μl of kinase buffer and 1 μl of [γ^{32}P]ATP (3,000 Ci/mmol; Amersham Corp.). The reaction was for 10 min at 37°C and was terminated by adding an equal volume of 2× sodium dodecyl sulfate–gel sample buffer. Reaction products were displayed on an 8% sodium dodecyl sulfate–polyacrylamide gel (20).

RESULTS

Characterization of cells in the spleens of diseased animals. NIH Swiss mice (4 weeks old) were injected intravenously with 0.5 ml of MRSV. When the animals developed splenomegaly, the spleens were removed, single-cell syspensions were made, and the cells were plated in methylcellulose. Colonies developed, and cells from these bursts were isolated and stained with hematoxylin and benzidine. Analysis revealed these cells to be benzidine-positive erythroblasts. The numbers of CFU at 3-days and BFU at 6 to 8 days were determined in the methylcellulose colony assay (Table 1). Spleen cells from MRSV-infected mice had elevated numbers of both 3-day CFU and 6- to 8-day BFU colonies. CFU and BFU from infected mice appeared both in the presence and absence of erythropoietin. Spleen cells from normal mice formed 3-day CFU only in the presence of erythropoietin. No 6- to 8-day BFU were observed in the spleen cells of normal mice. These data indicate that the spleens of MRSV-infected mice contain elevated numbers of both CFU and BFU and that these cells are capable of growing in vitro in the absence of erythropoietin.

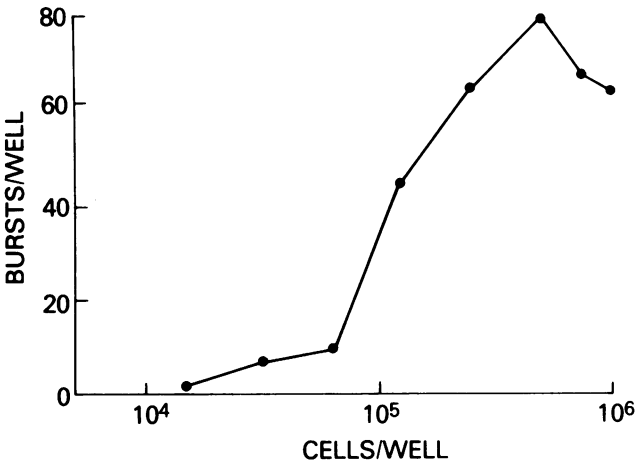

FIG. 1. Effect of cell number on burst production. Single-cell suspensions of 12-day-old fetal liver cells were prepared. MRSV (3×10^5 FFU) was incubated with 5×10^5 fetal liver cells, and 2 h later the cells were diluted and plated in methylcellulose at the indicated cell concentration with 0.1 U of erythropoietin per ml. The number of bursts formed was determined 1 week later.

In vitro erythroid transformation by MRSV. Fetal liver cells from 12-day old fetuses were exposed to MRSV in vitro and cultured in the erythroid burst assay. Very large irregularly-shaped colonies were observed after 5 days. Hemoglobinization of the colonies was enhanced by the presence of added erythropoietin (see below). The cells present in these bursts were identified as hemoglobin-containing erythroblasts by benzidine-hematoxylin staining.

To optimize burst formation by MRSV, the seeding density of fetal liver cells per well versus the number of bursts produced at constant virus input was studied (Fig. 1). A sigmoidal curve was produced with a linear region between 64,000 and 500,000 cells per well. A maximum of 70 to 80 bursts was observed with 500,000 fetal liver cells. No further increase in bursts was observed at cell concentrations higher than 500,000 cells per well. In these assays, an inoculum of 3×10^5 focus-forming units (FFU) of MRSV, as judged by titration on NIH 3T3 cells, was used; the multiplicity of infection was 0.6. The plateau in the number of bursts observed could be due to a change in the culture conditions caused by the high concentrations of cells, such that development of more bursts does not occur, or because it is impossible to distinguish individual bursts at a high cell-seeding density.

The number of bursts produced was linear with respect to virus input (Fig. 2). The single-hit kinetics indicate that infection with only one infectious virus is necessary to produce a burst. Since Moloney murine leukemia virus alone does not produce bursts, these results suggest that the helper virus plays no role in the transformation of these cells. The formation of erythroid colonies is dependent upon biologically active virus since heat treatment of the virus at 56°C for 30 min abolished the burst-forming activity of MRSV.

Effect of erythropoietin on bursts. Because erythroid cells from MRSV-infected mice appeared to retain their sensitivity to erythropoietin (Table 1), we sought to study the effect of erythropoietin upon cells infected in vitro with MRSV. The effect of erythropoietin upon MRSV-induced burst formation and hemoglobin synthesis was studied with vari-

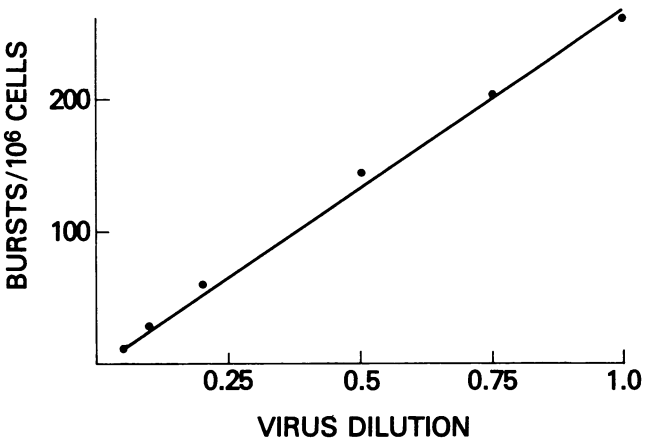

FIG. 2. Effect of virus dilution on burst formation. A 24-h harvest of MRSV was diluted with Dulbecco modified Eagle medium and used to infect fetal liver cells and plated at 125,000 cells per well with 0.1 U of erythropoietin per ml. The number of bursts was determined after 7 days.

ous concentrations of added erythropoietin. The concentration of erythropoietin had a noticeable effect upon the number of bursts (Fig. 3). Concentrations of erythropoietin above 0.05 U/ml increased the number of MRSV-induced bursts, and a plateau was observed at approximately 0.5 U/ml. In the absence of added virus, no bursts were observed.

The effect of erythropoietin upon hemoglobin synthesis was determined by the incorporation of $^{59}FeCl_3$ into heme (Fig. 4). The response to erythropoietin followed a sigmoidal curve with the maximum amount of ^{59}Fe incorporation occurring at 0.5 U of erythropoietin per ml. This concentration is approximately 10- to 20-fold higher than that needed to cause the maximum number of erythroid bursts (Fig. 3). The erythropoietin must be added on either day zero or day 1 postinfection to produce the maximum effect on the number of bursts or ^{59}Fe incorporation. In the absence of added erythropoietin, very little ^{59}Fe incorporation was observed. We believe that the difference in the amount of erythropoietin required for maximal burst formation as compared with maximal ^{59}Fe incorporation reflects the relative amount of erythropoietin necessary for maintaining cell viability or cell proliferation or both versus the amount required to cause hemaglobinization to occur.

To demonstrate a direct effect of erythropoietin on MRSV-infected fetal liver cells, MRSV-induced bursts, orginally formed in the presence of 0.1 U of erythropoietin per ml, were picked and replated in methylcellulose in the presence or absence of 0.1 U of erythropoietin per ml. The

TABLE 1. Numbers of CFU and BFU in spleen cells from MRSV-infected mice

Spleen cells	3-day CFU (per 10^6 cells)		6-to-8-day BFU (per 10^6 cells)	
	Without epo	With epo[a]	With epo	Without epo[b]
MRSV infected	120	1,180	16	48
Control	0	340	0	0

[a] 0.5 U of erythropoietin (epo) per ml.
[b] 0.1 U of epo per ml.

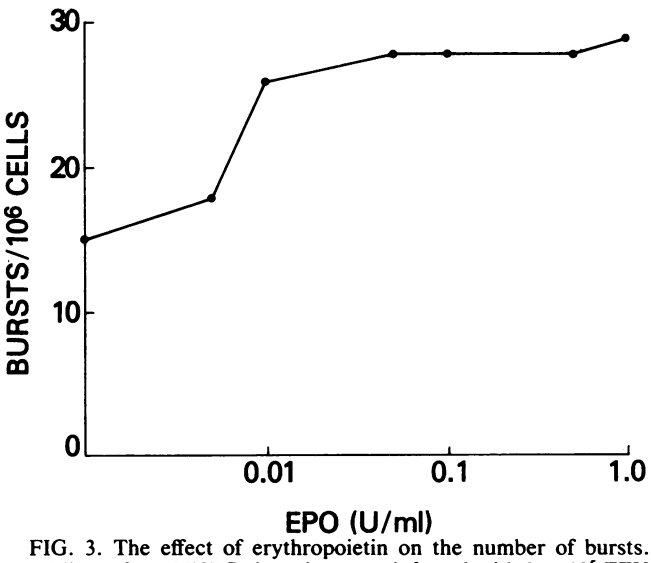

FIG. 3. The effect of erythropoietin on the number of bursts. Fetal livers from NIH Swiss mice were infected with 3×10^5 FFU of MRSV per 5×10^5 fetal liver cells for 2 h on ice and then plated at 125,000 cells per well. Various concentrations of erythropoietin were added, and the number of bursts produced was scored on day 7 postinfection.

number of secondary colonies per 10^6 cells was fourfold higher (4,100 colonies per 10^6 cells) in the presence of 0.1 U of erythropoietin per ml than in the absence of the hormone (900 colonies per 10^6 cells). Thus, erythropoietin increased the replating efficiency of these MRSV-transformed erythroid cells. In contrast, the addition of medium conditioned by WEHI cells (final concentration 5%), which contains burst-promoting activity for normal bone marrow cells (8, 26), did not increase the number of secondary colonies even when used in combination with erythropoietin. Our attempts to maintain the transformed erythroid cells in long-term cultures either in methylcellulose or in suspension cultures in Dulbecco modified Eagle medium with fetal calf serum were uniformly unsuccessful. Thus, no immortalized

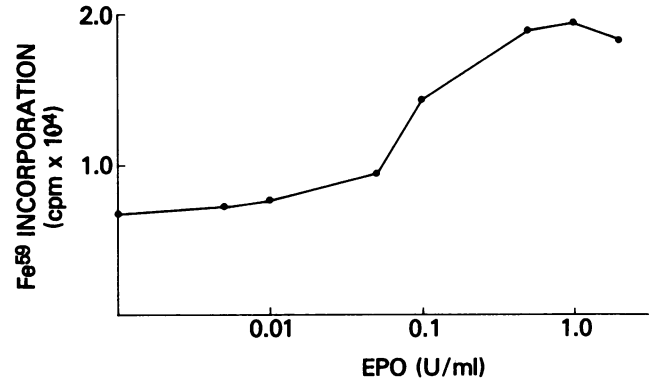

FIG. 4. The effect of erythropoietin upon the incorporation of $^{59}FeCl_3$ into heme. Bursts were produced by infection of 5×10^5 fetal liver cells with 3×10^5 FFU of MRSV for 2 h on ice. The cells were plated in methylcellulose at 125,000 cells per well, and the concentration of erythropoietin is indicated in the abscissa. On day 6, 0.5 μCi of ^{59}Fe was added, and the amount incorporated into heme was determined the following day (day 7 postinfection).

erythroid cells were isolated from the MRSV-transformed bursts.

Presence of pp60^{src} in erythroid bursts. The cells in erythroid bursts induced by MRSV contained elevated levels of pp60^{src} as determined by the immune complex protein kinase assay (Fig. 5). The antibody used in this assay was capable of detecting both the viral and cellular forms of pp60^{src}. Erythroid bursts induced by Harvey sarcoma virus did not contain elevated levels of pp60^{src} kinase activity (Fig. 5). This indicates that the increase in *src* kinase activity in MRSV-induced erythroid bursts is probably due to the presence of viral-encoded *src* and not due to the activation of the cellular *src* gene.

Time course of burst formation. The time course of burst formation after in vitro infection of fetal liver cells with MRSV was similar in the presence or absence of added erythropoietin (Fig. 6). In either case, MRSV-induced bursts were observed on day 4 postinfection, with the maximum being scored after 7 days. After day 8, the number of colonies gradually declined due to the lyses of terminally differentiated erythroid cells, and only rare bursts were obtained subsequent to day 14. The extent of hemoglobinization, maximal in the presence of erythropoietin, also reached a peak at days 7 through 9.

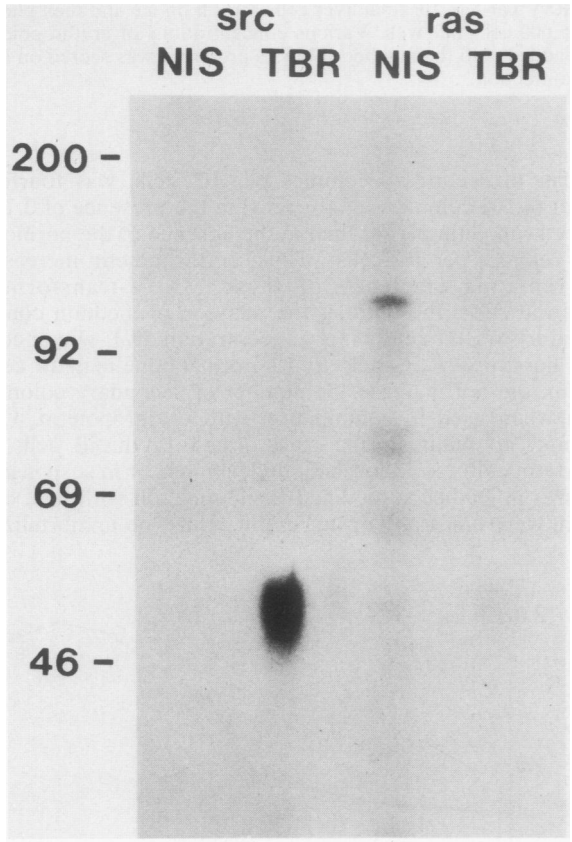

FIG. 5. Presence of pp60^{src} in burst cells. Colonies were picked from either cultures of MRSV-infected fetal liver cells or Harvey sarcoma virus-infected fetal liver cells. The cells were washed and lysed at 10^6 cells per ml in RIPA buffer. Immune complex protein kinase assay was as described in Materials and Methods. Immunoprecipitation was performed with either nonimmune rabbit serum (NIS) or tumor-bearing rabbit serum (TBR) which recognizes both pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$. Lysates of the MRSV-induced bursts are in the left two lanes, and those from Harvey sarcoma virus-induced bursts are in the right two lanes. The presence of pp60^{src} is indicated by the phosphorylation of the heavy chain of TBR immunoglobulin G (IgG) molecules. Molecular weights in thousands are indicated on the left of the figure.

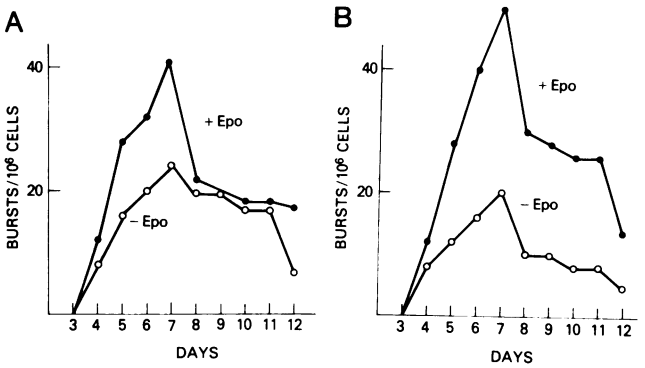

FIG. 6. Time course of erythroid burst formation in fetal liver cells. Single-cell suspensions of 12-day-old fetal liver cells from either NIH Swiss mice (A) or C57BL/6 mice (B) were infected with MRSV at 3×10^5 FFU of MRSV per 5×10^5 fetal liver cells for 2 h and plated at 125,000 cells per well in the presence (●) or absence (○) of 0.1 U of erythropoietin per ml. The numbers of bursts were determined each day by in situ inspection.

Similar experiments were performed with fetal liver cells from C57BL/6 mice (Fig. 6). C57BL/6 mice are homozygous Fv-2^{rr}. The Fv-2 locus is related to the ability of Friend spleen-focus-forming virus to induce its characteristic erythroid disease in mice (22). Infection of C57BL/6 fetal liver cells with MRSV gave rise to erythroid bursts with essentially the same time course as that observed with NIH Swiss fetal liver cells. The burst first appeared 4 days after infection in both the presence and absence of erythropoietin, and the maximum number of bursts was observed at 7 days postinfection. The sizes of the colonies and the extent of hemoglobinization also peaked at 7 days as previously seen with NIH Swiss cells. In vivo studies have shown that newborn C57BL/6 mice are susceptible to infection with MRSV and give rise to the same disease as described previously (S. Anderson, unpublished results).

Effect of MRSV on adult bone marrow and spleen cells. Spleen and bone marrow cells from phenylhydrazine-treated adult NIH Swiss mice were infected with MRSV, and the formation of erythroid bursts was monitored for 2 weeks (Fig. 7). Phenylhydrazine treatment induces anemia and elevates the number of erythroid precursors (14). Formation of erythroid bursts occurred with a time course similar to that observed with fetal liver cells, with the maximum number and maximum size of bursts occurring at 7 to 8 days postinfection. The effect of erythropoietin upon the number of bursts and the incorporation of $^{59}FeCl_3$ into heme was the same as that described for the MRSV-infected fetal liver cells in Fig. 3 and 4, respectively. The presence of erythropoietin had a much more dramatic effect upon the number of bursts formed by MRSV-infected bone marrow cells than upon that in MRSV-infected spleen cells. The number of colonies formed after infection of adult bone marrow and spleen cells was much lower on a per cell basis than that observed with fetal liver cells.

DISCUSSION

We have demonstrated that the transduced *src* gene present in MRSV can transform hematopoietic cells to give rise

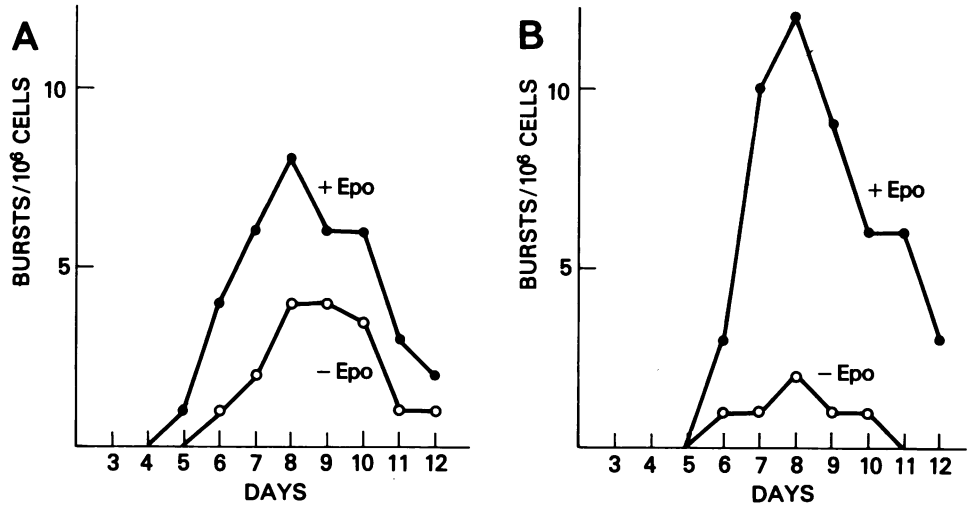

FIG. 7. Time course of burst formation after infection of adult mouse spleen (A) or bone marrow (B) cells. Four-week-old NIH Swiss mice were treated with phenylhydrazine and 48 h later used as a source of target cells. The cells were infected with MRSV at 3×10^5 FFU of MRSV per 5×10^5 spleen or bone marrow cells. The infections were for 2 h on ice, and the cells were then plated in methylcellulose at 125,000 cells per well in the presence (●) or absence (○) of 0.1 U of erythropoietin. The numbers of bursts were determined by in situ inspection at the indicated times.

to colonies of erythroid cells. Target cells are readily detected in fetal liver cells and in bone marrow and spleen cells from phenylhydrazine-treated adult mice. These colonies, called bursts, grow in the absence of added erythropoietin, but in the presence of 0.1 U of exogenously supplied erythropoietin per ml, the number, the size, and the extent of hemoglobinization of the colonies all increased. Uninfected hematopoietic cells from these tissues do not form erythroid colonies under these conditions. Thus, it would appear that *src* can stimulate the growth of these erythroid precursors and that these cells are still sensitive to erythropoietin and can fully differentiate. MRSV-transformed fetal liver cells, as do those transformed by Harvey and Kirsten sarcoma viruses (12), require less erythropoietin than do uninfected cells but more than do Friend virus- or Abelson leukemia virus-infected fetal liver cells (13, 17) to exhibit maximum hemoglobin synthesis.

The enhancement of MRSV-induced burst formation by erythropoietin may be because the hormone can increase the efficiency of transformation. For example, since retroviral integration requires a dividing target cell (27), stimulation of the target cell replication should enhance the infection process. Alternatively, erythropoietin may act as a viability factor, maintaining the cells and preventing their demise before or after infection. The elevation in ^{59}Fe incorporation could reflect the combination of the effect of the hormone on cell division and on hemoglobin synthesis per se.

From the time course of burst development in experiments with several hematopoietic tissues, we observe that a peak occurred at 6 to 8 days postinfection. It is interesting to note that all the oncogene-containing retroviruses we have examined to date seem to have a similar time course. The viruses studied include Harvey and Kristen sarcoma viruses (12), myeloproliferative sarcoma virus (11), and Abelson leukemia virus. In contrast, the non-oncogene-containing retroviruses Friend leukemia virus and Rauscher leukemia virus induce bursts in 4 to 5 days postinfection. Since MRSV-induced bursts appear later, are larger, and contain more cells than Friend virus-induced bursts, it is reasonable to speculate that these different viruses "see" different windows in erythroid differentiation.

Analysis of the erythroid precursors present in the spleens of animals infected with MRSV indicates the presence of elevated numbers of CFU and BFUe which grow in culture without added erythropoietin. The presence of these transformed cells in vivo indicates that the in vitro system accurately reflects at least some aspects of the in vivo disease process. In additon, diseased animals can actively respond to erythropoietin by incorporating increased amounts of [^{3}H]thymidine into their cellular DNA. This indicates that the in vivo-transformed erythroid cells, like their in vitro counterparts, can still respond to erythropoietin. Our inability to observe the 3-day CFU as target cells after in vitro infection suggest that the transformed 3-day CFU detected in vivo are probably derived from more primitive *src*-transformed cells (e.g., 6- to 8-day BFU) which are still able to differentiate into the more mature 3-day CFU. Collectively, our data suggest that the in vitro system accurately reflects the in vivo disease and that *src* has a direct effect upon an erythroid precursor, probably the 6- to 8-day BFU. The present data provide further evidence that *src* that does not block the ability of erythroid progenitor cells to continue to differentiate. Furthermore, our earlier in vivo data that the production of mature erythrocytes continues in MRSV-infected animals even 6 months postinfection (2) suggest that these transformed cells were not blocked in differentiation.

While this manuscript was in preparation, two other groups reported that the *src* gene present in Rous sarcoma virus could transform avian bone marrow cells and give rise to colonies of transformed erythroid cells (15, 24). In contrast to avian erythroblastosis virus-induced transformation, the differentiation of Rous sarcoma virus-transformed erythroid cells was not blocked. The growth of these colonies is dependent upon the presence of anemic chicken serum which presumably serves as a source of erythropoietin-like activity. We believe therefore that the studies on the transformation by *src* of avian and murine erythroid precursors are analogous. However, Kahn et. al. (15) suggest that, unlike the *src*- and *fps*-infected avian bone marrow cells, murine erythroid cells infected with *ras*- and *abl*-containing viruses are not transformed because the murine

cells do not have a lifespan as long as that of the avian cells. We suggest that such discrepancies may reflect differences in cell cycle or the hemopoietic factors used to support the growth of virus-injected cells. Whereas we use serum plus partially purified erythropoietin, Kahn et. al. (15) and Palmieri (24) used anemic chicken serum. The latter may contain many more growth factors that just an erythropoietin-like substance. However, all studies agree that *src* does not block the differentiation of these erythroid cells.

It is interesting to speculate on the mechanism by which *src* enhances erythroid growth. We believe that the growth advantage results from a direct effect of *src* upon the erythroid progenitors instead of on secondary cells, although our demonstration of high levels of $pp60^{src}$ kinase activity in the erythroid bursts admittedly does not absolutely prove this contention. With regard to this kinase activity, it should be noted that the receptors for both epidermal growth factor and platelet-derived growth factor possess a tyrosine-specific protein kinase activity. It is tempting to suggest that *src* may function by replacing the need for some growth factor by mimicking an activated receptor. Since the erythroid cells respond to very low levels of erythropoietin, *src* could be altering the requirement for this growth factor. Alternatively, *src* may replace the need for some other growth factor which at some point must operate through a tyrosine kinase in eliciting its cellular response. It would be interesting to search for these putative receptors by using antibodies directed against phosphotyrosine. For example, a comparison of phosphotyrosine-containing proteins in *src*-transformed and *ras*-transformed erythroid cells might yield interesting results. Similarly, we believe other oncogenic retroviruses may replace the need for or minimize the requirement for some growth factor(s) in erythroid progenitor cells.

There is still an apparent paradox which is unresolved which concerns the ability of *src* to block differentiation in some systems and not to do so in others. In several differentiating avian cell systems, including avian neuroretinal cells (7) and chicken chondroblasts (1), *src* blocks the differentiation of these cells. In this study and in the study on long-term bone marrow cultures (4), as well as those with RSV on avian bone marrow cells (15, 24), it has been shown that *src* can stimulate the proliferation of hematopoietic cells but does not arrest their differentiation. Whether this paradox is due to intrinsic differences in these cell systems remains to be determined.

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