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Induction of Erythropoietin Responsiveness in Murine Hematopoietic Cells by the *gag-myb-ets*-Containing ME26 Virus

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ME26 virus, which was generated by inserting the coding region of the acute avian leukemia-inducing virus E26 into a murine retrovirus vector, encodes a 135-kDa *gag-myb-ets* fusion protein. Amphotropic murine leukemia virus pseudotypes of ME26 virus induce a high incidence of erythroleukemia 2 to 4 months after injection into newborn NFS/N mice. Spleen cells from the majority of these mice proliferate to high levels in the presence of the erythroid hormone erythropoietin (Epo) and can easily be established as permanent Epo-dependent cell lines. The cell lines contain multiple copies of ME26 viral DNA and express viral message and protein. An Epo receptor mRNA of normal size can be detected in these cells, and binding studies reveal a single class of lower-affinity Epo receptor with an affinity for Epo that is in the range of that previously reported for erythroid cells. The ME26 virus-induced Epo-dependent cell lines, however, appear more immature than previously described erythroid cell lines and more closely resemble early hematopoietic precursor cells, suggesting that the virus may be activating the Epo receptor in hematopoietic cells that do not normally express it. Consistent with this idea, we are able to infect an interleukin-3-dependent myeloid cell line, FDC-P2, with ME26 virus and convert it to Epo dependence. The ME26 virus-infected FDC-P2 cells, even before growth on Epo, showed a large increase in the amount of Epo receptor mRNA. However, no ME26 viral integrations can be detected adjacent to the Epo receptor gene, indicating that the virus is not activating the Epo receptor gene by promoter/enhancer insertion. Our results are more consistent with the hypothesis that the *gag-myb-ets*-encoded viral fusion protein, which is known to bind DNA, is directly or indirectly activating the expression of the Epo receptor gene in these cells.

E26 virus is a defective avian retrovirus which predominantly induces erythroblastosis in infected chickens (16). In vitro, the virus can transform avian cells of both erythroid and myeloid lineages, suggesting that it may transform uncommitted erythroid/myeloid precursor cells (17, 19). Molecular analysis of the E26 virus has shown that it has transduced two proto-oncogenes, *myb* and *ets* (11, 18), which are expressed as a 135-kDa Gag-Myb-Ets fusion protein (2). Since *myb* sequences have previously been associated with myeloid leukemia, it is thought that the v-*ets* sequences are responsible for the erythroid-transforming potential of the virus in conjunction with the v-*myb* sequences.

To study the effects of the fused *myb* and *ets* sequences on mouse cells, a murine equivalent of the avian E26 virus, designated ME26 virus, was constructed by inserting the *gag-myb-ets* coding region of the E26 virus into a murine retrovirus vector derived from Abelson murine leukemia virus (MuLV) (30). Like E26 virus, ME26 virus encodes a 135 kDa Gag-Myb-Ets fusion protein. ME26 virus alters the growth of murine fibroblasts in tissue culture, and an amphotropic MuLV pseudotype of the virus (AMT/ME26), when injected into newborn NFS mice, induces a high incidence of leukemia within 100 days. While the majority of the mice are diagnosed as having erythroleukemia, myeloid and lymphoid leukemias can also develop. In contrast to mice injected with AMT/ME26 virus, mice injected with amphotropic MuLV alone develop a low incidence of lymphoid leukemia after 200 days. The ME26 virus, therefore, provides us with a new and convenient system for studying the oncogenic potential of the *myb* and *ets* genes in various hematopoietic cells of the mouse.

In this study, we examined the effects of ME26 virus on erythropoiesis. Other erythroleukemia-inducing viruses, such as Friend MuLV (Fr-MuLV) and Friend spleen focus-forming virus (Fr-SFFV), have been shown to transform erythropoietin (Epo)-dependent erythroid cells (for a review, see reference 20). Our results with the ME26 virus, however, suggest that this virus is not transforming Epo-dependent erythroid cells but is inducing Epo responsiveness in early hematopoietic cells that do not normally express the Epo receptor.

MATERIALS AND METHODS

Cells and viruses. The interleukin-3 (IL-3)-dependent myeloid cell line FDC-P2 (6) was grown in RPMI 1640 medium supplemented with 15% fetal calf serum and 5% WEHI-3 conditioned medium as a source of IL-3. HCD-57 cells, an Epo-dependent cell line established from a Fr-MuLV-induced erythroleukemia, were grown in Epo-containing medium as previously described (23).

Infectious ME26 virus preparations (designated AMT/ME26 virus or Fr/ME26 virus) were obtained from NIH 3T3 cells that had been stably transfected with ME26 DNA and then superinfected with amphotropic MuLV 4070 (AMT-MuLV) or Fr-MuLV, respectively. Virus preparations of the

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anemia-inducing strain of the SFFV (designated AMT/$SFFV_A$) were obtained from $SFFV_A$-L-transfected NIH 3T3 cells that had been superinfected with AMT-MuLV (21).

Infection of mice. NFS/N or NIH Swiss mice were inoculated intraperitoneally within 48 h of birth with AMT/ME26 virus (0.1 ml of culture supernatant), and mice with palpable spleens and low hematocrits (less than 35%) were sacrificed for further study. Other mice injected with AMT-MuLV alone were used as controls. In some experiments, adult mice (6 to 8 weeks of age) were injected intraveneously with virus after treatment 1 and 2 days previously with phenylhydrazine hydrochloride (60 mg/kg of body weight) or 4 days previously with 5-fluorouracil (150 mg/kg).

For comparative purposes, NIH Swiss mice were injected with the erythroleukemia-inducing AMT/$SFFV_A$ as previously described (21).

Establishment of Epo-dependent cell lines and infection of FDC-P2 cells. Spleen cell suspensions from virus-infected mice were seeded at 1×10^6 to 2×10^6/ml in Iscove's Dulbecco modified Eagle medium supplemented with 30% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, and 0.3 U of recombinant human Epo per ml. Rapidly proliferating cultures were established within 1 week.

FDC-P2 cells were washed and suspended at a concentration of 4×10^5/ml in virus or medium plus Polybrene (4 μg/ml). After 1 h at 37°C, the cells were pelleted and suspended in IL-3-containing medium. The cells were maintained in this medium for 5 days before being transferred to medium containing 0.3 to 1 U of Epo per ml in place of IL-3.

Agar cultures. Cells were plated at 10^3 cells per dish in 60-mm tissue culture dishes containing RPMI 1640 medium with final concentrations of 15% fetal calf serum and 0.35% SeaPlaque agarose. Cells were cultured in the presence of 5% WEHI conditioned medium or 1 U of Epo per ml as well as in medium with no added growth factors. Colony formation was measured after 14 days of incubation at 37°C.

Cell proliferation assays. Cells (10^5/ml) were incubated for 48 h in the presence or absence of growth factors and then pulsed overnight with 1 μCi of [^{3}H]thymidine per well as previously described (22). Incorporation was measured by harvesting cells onto glass fiber filters with a microharvester and determining counts on the filters.

Erythroid colony assays. Spleen cells taken from infected animals were cultured at 2.5×10^5 cells per ml in Iscove's Dulbecco modified Eagle medium containing 30% fetal calf serum, 1% deionized bovine serum albumin, 0.1 mM 2-mercaptoethanol, and 0.5 U of Epo per ml (25). Two days later, individual clots were harvested and stained with benzidine-hematoxylin. Benzidine-positive late erythroid CFU (CFU-E)-derived colonies were counted under the microscope.

Epo binding assays. Epo was iodinated, and ^{125}I-Epo binding assays were carried out as previously described (24). Cells were washed and cultured in the absence of Epo overnight to increase the receptor number and reduce the occupancy of receptors. Cells (10^7) were then incubated for 20 h at 0°C in concentrations of ^{125}I-Epo ranging from 0.1 to 10 U/ml (26 to 2,600 pM). Bound Epo was measured by separation of the cells from the binding medium by centrifugation through dibutyl phthalate oil. Nonspecific binding was measured in the presence of 100 U of unlabeled Epo per ml and was subtracted from specific binding. The data were plotted by the Scatchard method to determine the binding affinity.

Northern (RNA) and Southern (DNA) blot hybridization analysis. Total RNA was purified by using RNAzol (4). RNAs were denatured in 50% formamide–2.2 M formaldehyde, separated electrophoretically on 1.2% agarose gels containing 2.2 M formaldehyde, and transferred to nitrocellulose filters. RNA containing Epo receptor sequences was identified by hybridization with a random-primed probe prepared from a 1.9-kb fragment of the Epo receptor cDNA from plasmid pXM(ER) (5) (kindly provided by A. D'Andrea, Dana Farber Cancer Institute, Boston, Mass.). Hybridizations were also carried out by using a v-*ets* probe (E1.28) (27) to detect expression of the ME26 virus. Hybridizations were performed at 42°C for 24 h in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and were followed by two washes at room temperature with 2× SSC–0.1% sodium dodecyl sulfate (SDS) and two washes at 42°C with 0.2× SSC–0.1% SDS.

DNA was prepared as previously described (3). High-molecular-weight DNA was digested with restriction enzymes, electrophoresed in 1% agarose gels, and transferred to nitrocellulose filters. Filters were hybridized to either a ^{32}P-labeled probe to v-*ets* or to a probe prepared to a *Hin*dIII-*Xba*I fragment derived from the promoter region of the Epo receptor gene (p0.45GH) (29) (kindly provided by H. Youssoufian, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Hybridizations were performed for 24 h in 50% formamide–5× SSC and were followed by washing as described above for analysis of RNA.

RESULTS

Effects of ME26 virus on mouse erythroid cells. In an effort to better understand the effects of the ME26 virus on the Epo pathway, we analyzed spleen cells from leukemic NFS/N mice infected 2 to 3 months previously with AMT/ME26. Only mice that were anemic (hematocrit below 35%) and showed no lymph node involvement were chosen for this study. Since normal erythroid cells are dependent on the erythroid hormone Epo for proliferation and differentiation, we measured the effect of this hormone on the growth and differentiation of spleen cells from ME26 virus-infected mice. As shown in Fig. 1, spleen cells from mice infected with AMT/ME26, like those of erythroleukemic mice infected with AMT/$SFFV_A$, proliferated to high levels only in the presence of the erythroid hormone Epo. In contrast, spleens from mice infected with amphotropic helper virus alone (AMT) contain only a low number of Epo-responsive cells, like spleens from uninfected mice.

Spleen cells from virus-infected mice were also analyzed in semisolid medium for their ability to form CFU-E in the presence or absence of Epo. As shown in Table 1, spleens from mice infected with AMT/ME26 virus showed no increase in Epo-dependent CFU-E over that seen with spleens from mice infected with AMT-MuLV alone, indicating that the Epo-responsive cells proliferating to high levels in the AMT/ME26 virus-infected mice are blocked from differentiating into mature erythrocytes. This contrasts with spleens from mice infected with AMT/$SFFV_A$, which show a large increase in the number of Epo-dependent CFU-E.

Development and characterization of Epo-dependent cell lines from ME26 virus-infected mice. When spleen cells from ME26 virus-infected mice were put into culture in medium with or without recombinant human Epo, Epo-dependent cell lines could easily and reproducibly be obtained from these mice. Of 64 spleens cultured, 45 (70%) were successfully established as permanent cell lines. The lines were established within a few days of culturing and appeared to

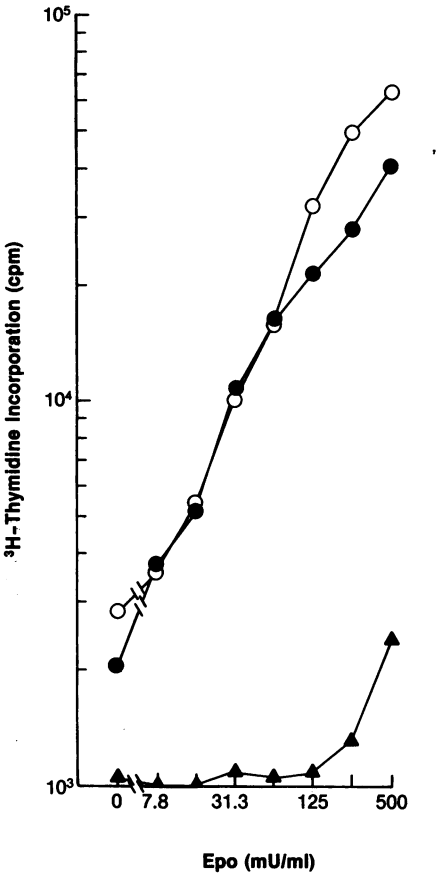

FIG. 1. Proliferation of spleen cells from ME26 virus-infected mice. Spleen cells from mice infected with AMT/ME26 virus (●), AMT-MuLV (▲), or AMT/SFFV$_A$ (○) were cultured for 48 h with medium lacking Epo or containing the indicated concentration of Epo. Cells were pulsed overnight with [^{3}H]thymidine and assayed for counts incorporated.

represent the major population of cells growing in the spleens of the infected mice rather than a rare cell selected out of the spleen cell population. In contrast, no lines could be established from the spleens of normal mice or mice infected with amphotropic helper virus alone. All but two of the lines required the continued presence of Epo for proliferation and each line failed to proliferate in response to any other growth factor tested (IL-1 through IL-7, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and colony-stimulating factor 1). All but

TABLE 1. CFU-E analysis of spleens of virus-infected mice[a]

Spleen cells from mice infected with:	CFU-E/10^5 spleen cells	
	−Epo	+Epo
Fr-MuLV/SFFV$_A$	20 ± 2	2,003 ± 76
AMT/ME26	0	16 ± 3
AMT	0	20 ± 2

[a] Spleen cells in plasma clots were cultured for 2 days in the presence or absence of Epo. Clots were harvested and stained, and benzidine-positive CFU-E-derived colonies were enumerated under the microscope. Spleen cells from two animals for each group were analyzed, and the mean number of colonies ± standard deviation was determined.

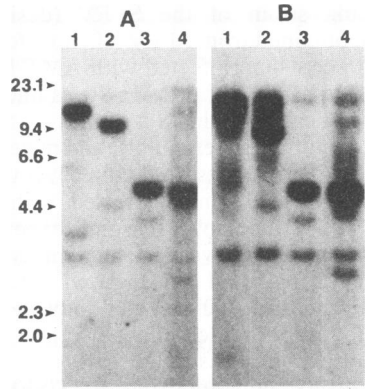

FIG. 2. Analysis of Epo-dependent cells derived from ME26 virus-infected leukemic mice for integrated viral DNA. DNAs from four independently derived Epo-dependent cell lines (A) and the spleens from which they were derived (B) were digested with *Hin*dIII and hybridized with an *ets*-specific probe. Positions of size markers are shown in kilobases on the left.

two of the lines have retained their Epo dependence for 6 to 18 months.

DNAs from the ME26 virus-induced Epo-dependent cell lines were analyzed for the presence of virus after digestion with *Hin*dIII, which cleaves once within the v-*ets* sequence of the virus, and were found to contain at least one copy of the ME26 viral genome (Fig. 2). Southern analysis using a v-*ets* probe showed similar hybridization patterns in DNA from the established cell lines and in the spleen cells from which they were derived, consistent with the idea that the cell lines are representative of the majority of the cells proliferating in the diseased spleens of the infected mice. The cell lines also express viral RNA and the 135-kDa protein product of the ME26 virus, and they produce virus that was active on fibroblasts in vitro (30) and was able to induce leukemia in mice (data not shown).

The Epo receptors on the ME26 virus-induced Epo-dependent cell lines were also analyzed and compared with the receptors on HCD-57 cells, an Epo-dependent erythroleukemia cell line induced by Fr-MuLV. All of the ME26 virus-induced cell lines examined express an Epo receptor mRNA (Fig. 3, lanes 1 to 5) of a size (2 kb) and level comparable to that expressed in HCD-57 cells (lane 6) and other erythroid cells, as well as a 66-kDa protein that reacts with an Epo receptor-specific antiserum (data not shown). When Epo binding studies were carried out on ME26 virus-induced cell lines (Table 2), it was found that these cells, like HCD-57 cells, expressed a single class of lower-affinity Epo receptors (K_d, 212 to 289 pM). The number of Epo receptors expressed by the ME26 virus-induced cell lines (610 to 990 per cell) was similar to that found on many erythroid cell lines, although the number was considerably lower than that detected on HCD-57 cells.

The ME26 virus-induced Epo-dependent cells lines that we have established are morphologically indistinguishable from one another but distinct from erythroleukemia cell lines derived from mice infected with other viruses such as Fr-MuLV or Fr-SFFV. As shown in Fig. 4, the cells have a large, round nucleus and a small granule-free cytoplasm and exhibit numerous mitotic figures, indicating their high proliferative rate. Unlike other erythroleukemia cell lines, they cannot be induced to differentiate into mature erythrocytes with dimethyl sulfoxide, hexamethylenebisacetamide, or

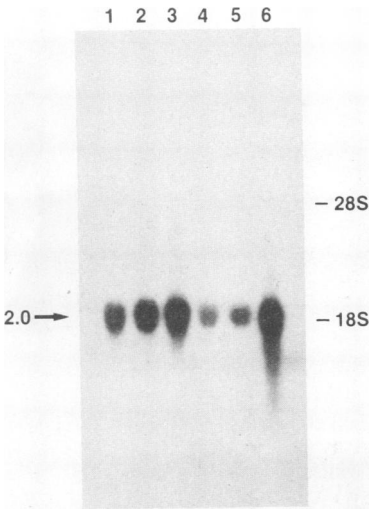

FIG. 3. Analysis of Epo-dependent cell lines derived from ME26 virus-infected mice for expression of the Epo receptor. Northern analysis using a probe specific for the Epo receptor was carried out on RNA isolated from independently derived cell lines (lanes 1 to 5) from ME26 virus-infected mice. RNA isolated from the mouse erythroleukemia cell line HCD-57 (lane 6) is shown for comparison. All lanes represent total RNA (10 μg per lane).

hemin (data not shown). They fail to express any cell surface markers specific for myeloid or lymphoid lineages or markers often, but not always, detected on hematopoietic stem cells (i.e., Thy-1 and Ly-6) (15a).

5-Fluorouracil treatment increases the target cells for ME26 virus. Unlike newborn mice, adult mice are not susceptible to ME26 virus-induced leukemia (30), suggesting that there may be fewer target cells for the virus in the adult mouse. In an effort to increase the target cells for ME26 virus, adult NFS/N mice were treated prior to virus infection with either phenylhydrazine, which leads to a large increase in late erythroid precursor cells (10), or 5-fluorouracil, which greatly increases the number of uncommitted hematopoietic progenitor cells (26). Sixty percent (9 of 15) of adult mice pretreated with 5-fluorouracil before being given ME26 virus developed leukemia within 150 days postinfection. Untreated adult mice or mice pretreated with phenylhydrazine failed to develop disease following infection with ME26 virus. The disease induced by ME26 virus in the 5-fluorouracil-treated mice was indistinguishable from that induced in newborns by the virus, and the cells from these diseased mice also grew as permanent Epo-dependent cell lines. This result, combined with the morphological characteristics of the cells transformed by the virus, suggest that ME26 virus may not be transforming a committed erythroid precursor cell but may be inducing Epo responsiveness in a less mature hematopoietic cell that does not normally express the Epo receptor.

TABLE 2. Epo receptors on ME26 virus-induced cell lines[a]

Cell line	No. of receptors/cell	K_d (pM)
ME26		
4	990 ± 413	254 ± 46
5	834 ± 81	274 ± 25
9	858 ± 274	289 ± 53
15	610 ± 15	212 ± 34
HCD-57	4,087 ± 652	542 ± 40

[a] Calculations are based on Scatchard analysis of Epo-binding data and represent the mean ± standard deviation of at least two separate experiments.

Effects of ME26 virus on an IL-3-dependent myeloid cell line. To test the possibility that ME26 virus was inducing Epo responsiveness in an hematopoietic cell that did not normally express the Epo receptor, an IL-3-dependent myeloid cell line, FDC-P2, was infected with ME26 virus. As shown in Table 3, FDC-P2 cells infected with AMT/ME26 or Fr/ME26 were able to grow in medium containing only Epo. Uninfected cells or cells infected with AMT-MuLV or Fr-MuLV helper virus alone grew only in the presence of IL-3, indicating that ME26 virus was responsible for inducing Epo responsiveness. ME26 virus-infected FDC-P2 cells failed to grow in the absence of either IL-3 or Epo, indicating that the virus had not rendered them factor independent. Furthermore, growth factors other than IL-3 or Epo were not able to sustain the growth of these cells, indicating that the virus was specifically inducing Epo responsiveness.

ME26 virus-infected FDC-P2 cells plated in soft agar in the presence of either Epo or IL-3 formed compact colonies (Table 3). In contrast, uninfected FDC-P2 cells or cells infected with helper virus alone formed diffuse colonies in the presence of IL-3 and no colonies in the presence of Epo. The cloning efficiency of ME26 virus-infected cells, when grown with either IL-3 or Epo, was significantly higher than that of the uninfected or helper virus-infected cells, suggesting that ME26 virus infection confers a growth advantage on these cells.

The ME26 virus-infected FDC-P2 cells express large amounts of ME26 viral mRNA (Fig. 5) as well as the ME26 virus-encoded protein (data not shown). Furthermore, the cells show a greatly enhanced level of Epo receptor mRNA compared with their uninfected counterparts (Fig. 5), indicating that ME26 virus is activating expression of the Epo receptor in these cells. ME26 virus infection appears to induce Epo responsiveness in FDC-P2 cells rather than select for a rare Epo-responsive cell in the population, since high levels of Epo receptor mRNA could be detected in the ME26 virus-infected cells even before the cells were grown on Epo.

Analysis of the Epo receptor gene in ME26 virus-infected, Epo-responsive hematopoietic cell lines. To determine whether ME26 virus was inducing the expression of the Epo receptor gene by promoter/enhancer insertion, a probe specific for the promoter region of the Epo receptor gene was used to analyze DNA from ME26 virus-induced Epo-dependent cell lines and ME26 virus-infected FDC-P2 cells for any rearrangements compared with the Epo receptor gene in normal NFS/N mouse tissues. DNAs were cut with *Ava*I, which cleaves at the 5′ end of the first exon within the Epo receptor gene, and Southern analysis was performed using a probe specific for the Epo receptor promoter region. As shown in Fig. 6, no rearrangement of the Epo receptor gene is apparent in any of the Epo-responsive, ME26 virus-infected cells tested, suggesting that the virus is not inducing Epo responsiveness in hematopoietic cells by integrating near the Epo receptor gene and activating its expression by promoter/enhancer insertion.

DISCUSSION

Our studies indicate that the *gag-myb-ets*-containing ME26 virus can induce Epo responsiveness in hematopoietic cells. This was first demonstrated by our observation that

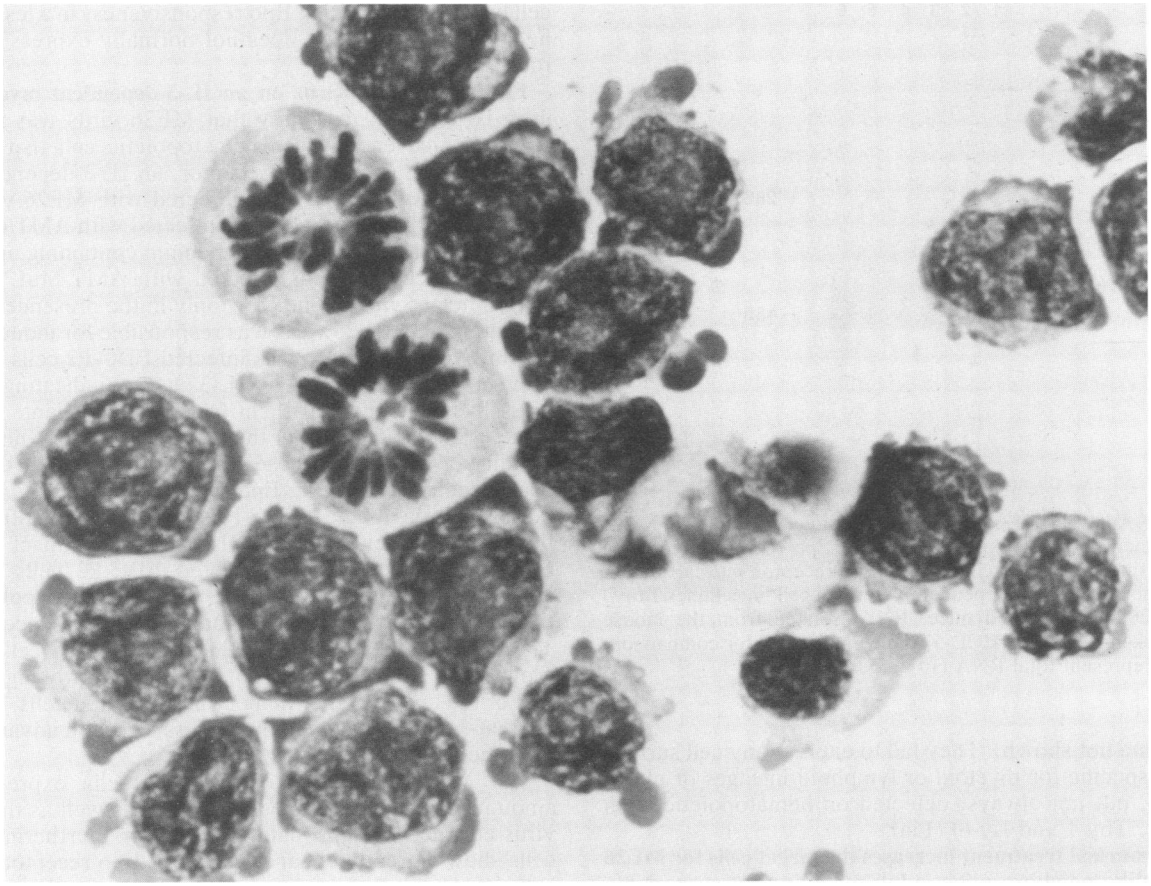

FIG. 4. Photomicrograph of cells from an Epo-dependent cell line derived from an ME26 virus-infected leukemic mouse. Cytocentrifuge preparations of proliferating cells were prepared and then fixed and stained with Dif-Quik (Fisher).

the disease induced in mice by ME26 virus was a hyperplasia of Epo-dependent cells which could easily be grown as Epo-dependent cell lines in culture. Furthermore, an IL-3-dependent myeloid cell line acquired the capacity to grow on Epo following infection with ME26 virus. Thus, unlike other erythroleukemia-inducing retroviruses, ME26 virus may be activating the expression of the Epo receptor in hematopoietic precursor cells that do not normally express it rather than transforming an Epo-dependent erythroid cell. This would represent a novel mechanism for retroviral pathogenesis.

TABLE 3. Induction of Epo responsiveness in FDC-P2 cells

Virus	Growth in liquid culture[a]			Agar colonies/10^3 cells[b]		
	No factor	IL-3	Epo	No factor	IL-3	Epo
None	−	+	−	0	21	0
AMT/ME26	−	+	+	0	86	84
AMT-MuLV	−	+	−	0	25	0
Fr/ME26	−	+	+		NT	
Fr-MuLV	−	+	−		NT	

[a] FDC-P2 cells grown in IL-3 were infected with virus. After growing in IL-3 for 5 days, the cells were transferred to medium containing either IL-3 or Epo as the sole growth factor. Experiments infecting cells with either AMT/ME26 or AMT-MuLV were carried out on four separate occasions with identical results, whereas experiments infecting cells with Fr/ME26 and Fr-MuLV were carried out only once.

[b] Colony assays were carried out on ME26 virus-infected cells that had been established in medium containing Epo. Data represent the averages of two separate experiments. NT, not tested.

The mechanism by which ME26 virus is inducing Epo responsiveness is not known. The virus does not appear to be activating the Epo receptor gene by promoter/enhancer insertion, because no rearrangement of the Epo receptor gene could be detected in various Epo-dependent cells transformed by ME26 virus. Since the ME26 viral gene product is localized to the nucleus (30) and contains two DNA-binding oncogenes, *myb* and *ets*, it is possible that the viral gene product, p135, is either directly transactivating the Epo receptor gene by binding to its promoter or indirectly activating it by transactivating another gene involved in Epo receptor gene regulation. Preliminary results (1) suggest that ME26 virus alone does not significantly activate the Epo receptor promoter after cotransfection into fibroblasts but may be transactivating the *GATA-1* gene, which encodes an erythroid-specific transcription factor which can transactivate a number of genes (7, 8, 12, 13, 28), including the Epo receptor gene (31).

The role of particular ME26 viral sequences in the induction of Epo responsiveness is under investigation. Recent studies with derivatives of the avian E26 virus have indicated that only those viruses expressing *myb* and *ets* sequences on a single fusion protein are leukemogenic (14). Studies in progress in our laboratory using mutants of ME26 virus that contain changes in either the *myb* or *ets* domain suggest that the intact virus may be needed for the induction

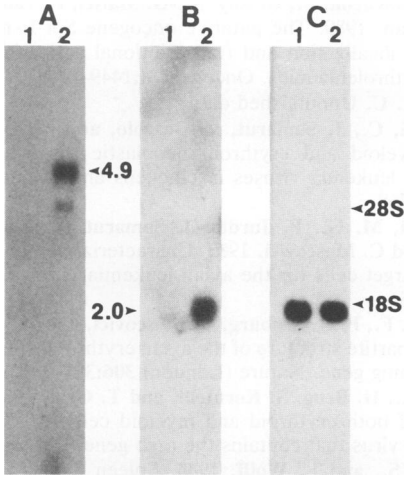

FIG. 5. Analysis of uninfected and virus-infected FDC-P2 cells for expression of the Epo receptor. Northern analysis was carried out on RNA isolated from uninfected FDC-P2 cells (lanes 1) or FDC-P2 cells infected with AMT/ME26 virus (lanes 2). Filters were hybridized to a probe specific for either v-*ets* (A), the Epo receptor (B), or actin (C).

of Epo responsiveness. The fusion protein of the virus may recognize unique DNA sequences that are not recognized by either *myb* or *ets* alone, and mutations throughout the virus could affect the conformation of the protein product in such a way that it can no longer interact with the appropriate sequences.

Although induction of Epo responsiveness by the ME26 virus is an important step in pathogenesis by the virus and results in the outgrowth of a unique population of early hematopoietic cells in infected mice, it is likely that this is not the only step involved in the generation of an immortal population of Epo-dependent cells. The cell lines described in this study were generated from the spleens of mice sacrificed 60 to 90 days after virus infection. Attempts to develop Epo-dependent cell lines from mice sacrificed between 30 and 60 days, even though their spleens contained a large population of Epo-responsive cells, were less successful. This finding suggests that additional changes are required to generate Epo-dependent cells capable of continuous growth in culture. As has been observed in other virus-induced hematopoietic neoplasms, it is possible that promoter/enhancer insertion, involving either the defective ME26 virus or its amphotropic MuLV helper, is responsible for their immortalization. These changes may also involve a differentiation block, because the immortal cell lines are unable to differentiate, while cells replicating in the animal early in the course of disease were able to differentiate into mature erythrocytes. Since the production of Epo is regulated by the level of mature erythrocytes being produced, the growth of the Epo-dependent, ME26 virus-infected cells would be favored by a block in differentiation, leading to high levels of Epo in response to the depletion of mature erythroid cells. Studies are currently under way to determine the role of the virus in immortalizing the Epo-dependent cells.

It is interesting that of the three viruses known to cause erythroleukemia in mice, two of them, Fr-SFFV and Fr-MuLV, are associated with the activation of *ets*-related genes *Spi-1* (9, 15) and *Fli-1* (1a), respectively, and the third, ME26 virus, expresses an *ets*-related protein. This suggests that abnormal expression of *ets*-related genes in erythroid precursor cells, either by insertional activation or by retroviral transduction of the gene, may lead to a malignant state. Further studies with the ME26 virus should provide insights into the role of one member of the *ets* gene family in the regulation of hematopoietic cell growth.

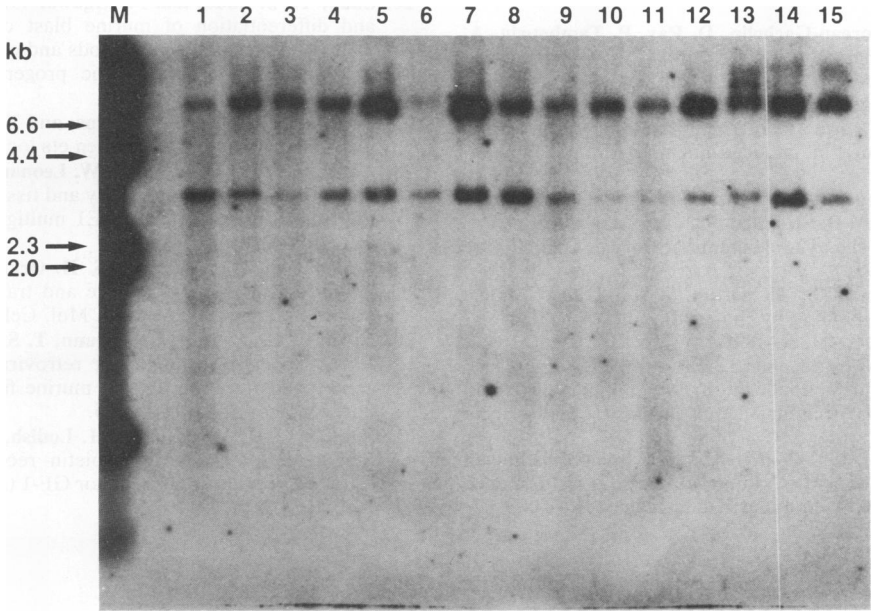

FIG. 6. Analysis of the Epo receptor gene in Epo-responsive, ME26-virus-infected cells. DNA was isolated from Epo-dependent cell lines derived from ME26 virus-infected mice (lanes 1 to 12), the liver of uninfected NFS/N mice (lane 13), uninfected FDC-P2 cells (lane 14), and Epo-responsive, ME26 virus-infected FDC-P2 cells (lane 15). After digestion with *Ava*I, Southern analysis was performed with a probe specific for the promoter region of the Epo receptor gene.

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