Interleukin-3 (IL-3) Inhibits Erythropoietin-induced Differentiation in Ba/F3 Cells via the IL-3 Receptor α Subunit*

(Received for publication, February 14, 1996, and in revised form, July 10, 1996)

Jana Krosl‡, Jacqueline E. Damen‡, Gerald Krystal§§, and R. Keith Humphries¶¶**

From the ‡Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia V5Z 1L3, Canada and the Departments of §Pathology and ¶¶Medicine, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

Introduction of erythropoietin receptors (EpoRs) into the interleukin-3 (IL-3)-dependent murine hemopoietic cell line, Ba/F3, enables these cells to not only proliferate, after an initial lag in G1, but also to increase β-globin mRNA levels in response to erythropoietin (Epo). With IL-3 and Epo costimulation, IL-3-induced signaling appears to be dominant since no increase in β-globin mRNA occurs. Differentiation and proliferation signals may be uncoupled since EpoRs lacking all eight intracellular tyrosines were compromised in proliferative signaling but retained erythroid differentiation ability. Intriguingly, a chimeric receptor of the extracellular domain of the EpoR and the transmembrane and intracellular domains of IL-3Rβ3 chain (EpoR/IL-3Rβ3) was capable of Epo-induced proliferative and differentiating signaling, suggesting either the existence of a second EpoR subunit responsible for differentiation or that the α subunit of the IL-3 receptor (IL-3R) prevents it. Arguing against the former, a truncated EpoR lacking an intracellular domain was incapable of promoting proliferation or differentiation. An EpoR/IL-3Rα chimera, in contrast, was capable of transmitting a weak Epo-induced proliferative signal but failed to stimulate accumulation of β-globin mRNA. Most significantly, coexpression of the EpoR/IL-3Rα chimera with either EpoR/IL-3Rβ or wild-type EpoRs suppressed Epo-induced β-globin mRNA accumulation. Taken together, these results suggest an active role for the IL-3Rα subunit in inhibiting EpoR-specific differentiating signals.

Erythropoietin (Epo), the major in vivo stimulator of mammalian erythropoiesis (1), exerts its action by binding to the hemopoietin receptor superfamily and do not possess intrinsic tyrosine kinase activity (4). Nonetheless, within minutes of binding Epo, the EpoR and several intracellular proteins become tyrosine phosphorylated (5–7) through the action of an EpoR-associated tyrosine kinase, Jak2 (8). These Epo-induced tyrosine phosphorylations have been shown to correlate with both the expression of immediate-early response genes, such as c-jun and c-fos, and with mitogenesis (9). Moreover, tyrosine phosphorylation of the EpoR itself appears to be critical for activation of Stat5 and initiation of Epo-induced proliferation at physiological concentrations of Epo (10). In addition to its role in stimulating proliferation, Epo may have roles in preventing apoptosis (11, 12) and in stimulating erythroid differentiation (13–15).

Studies in both our laboratory (16) and others (17–20) have demonstrated that Ba/F3 cells engineered to express the EpoR rapidly accumulate β-globin mRNA upon exposure to Epo. Interestingly, the tyrosine kinase inhibitor genistein blocks both Epo-induced proliferation and β-globin mRNA accumulation in this model system. In contrast, inhibition of protein kinase C by Compound 3 suppresses only Epo-induced differentiation without affecting proliferation (16). These observations suggest that protein phosphorylation events play a critical role in Epo-induced differentiation and that the proliferative and differentiating functions of the EpoR can be uncoupled.

Interestingly, both the extra- and intracellular domains of the EpoR have been implicated in Epo-induced differentiation. Maruyama et al. (20), for example, showed that a chimeric receptor consisting of the extracellular and transmembrane domain of the epidermal growth factor receptor and the intracellular domain of the EpoR could induce hemoglobin synthesis in TSA-8 cells, consistent with a role for the cytoplasmic domain of the EpoR in differentiating signaling. In apparent contradiction to this, chimeric receptors consisting of the extracellular domain of the EpoR and the transmembrane and intracellular domain of the interleukin-3 receptor β subunit (EpoR/IL-3Rβ) were also found to be capable of increasing β-globin mRNA levels in Ba/F3 cells (18, 21). This latter result was particularly surprising in view of the observation that IL-3 inhibited the Epo-induced accumulation of β-globin mRNA in Ba/F3 cells expressing EpoRs.

To examine in more detail the regions within the EpoR that might be responsible for eliciting differentiation-specific signals, we have monitored the effects of various mutant and chimeric EpoRs on the induction of β-globin mRNA accumulation in Ba/F3 cells. Our results suggest that the cytoplasmic domains of the EpoR and the IL-3Rβ subunit are equivalent in mediating β-globin mRNA induction. Our results further suggest that the IL-3Rα subunit is responsible for the IL-3-induced suppression of β-globin mRNA accumulation and that, at least in this model system, late stage erythroid differentiation is contingent upon both Epo-mediated signaling and the absence of IL-3-mediated signaling.
IL-3 Receptor α Subunit Inhibits Erythroid Differentiation

MATERIALS AND METHODS

**Generation of EpoR Mutant and Chimeric cDNAs—**The mutant and chimeric EpoRs used in this study are depicted in Fig. 1. The null EpoR mutant in which all cytoplasmic tyrosines were replaced with phenylalanines was constructed using site-directed mutagenesis as described by Damen et al. (10). The EpoR/IL-3Rαβ, and EpoR/IL-3Rα chimeras and the C-terminal truncated EpoR (–230) contain the EpoR cDNA-derived extracellular domain encoded by a KpnI-Nhel fragment of pXM EpoR (provided by A. D’Andrea, Harvard Medical School, Boston, MA). The EpoR/IL-3Rαβ encompassing the extracellular domain of the EpoR and the transmembrane and cytoplasmic domains of the IL-3Rαβ, subunit was constructed by ligating a KpnI-Nhel fragment of the EpoR to a fragment spanning the transmembrane region of the IL-3Rαβ, subunit, generated by polymerase chain reaction amplification, and an Ndel-NorI fragment of pAIC2–26 (provided by A. Miyajima, DNAX Research Institute, Palo Alto, CA), encoding the intracellular domain of the IL-3Rαβ, subunit. To generate the hybrid EpoR/IL-3Rα gene, a 255-bp fragment encoding the transmembrane and intracellular domains of the IL-3Rα subunit was amplified by polymerase chain reaction using pSUT-1 (provided by A. Miyajima, DNAX Research Institute) as a template and ligated to the KpnI-Nhel fragment of the EpoR cDNA. The C-terminal truncated EpoR (–230) encompassing the extracellular and transmembrane domains of the EpoR was constructed using a cDNA-derived extracellular domain and a polymerase chain reaction-amplified transmembrane region.

**Generation of Retroviral Vectors Encoding Chimeric and Mutant EpoRs—**To generate the JZen EpoR/IL-3Rαβ, retroviral vector, a 2279-bp KpnI-NorI fragment encoding the coding region of the chimeric receptor was isolated from pBS-EpoR/IL-3Rαβ, and inserted by blunt end ligation into the XhoI site of JZen TK− upstream of the neo+ gene. Expression of the hybrid gene was thus under control of the myeloproliferative sarcoma virus long terminal repeat. To create MSCV-EpoR/IL-3Rα, a 1010-bp KpnI-NorI fragment encompassing the hybrid EpoR/IL-3Rα gene was isolated from the pBS-EpoR/IL-3Rα vector and inserted by blunt end ligation into the HpaI site of the MSCV PGKneo cassette. A MSCV-EpoR (–230) PGKneo retroviral vector was generated by subcloning a blunted 843-bp KpnI-BstBI fragment of pBS-EpoR (–230), encoding the truncated EpoR, into the HpaI site of the MSCV PGKneo vector upstream of the PGKneo cassette. The JZen null EpoR TK− vector was constructed as reported previously (10).

**Cell Lines—**The ectopic GP+ E-86 retrovirus packaging cell line (22) was obtained from Dr. A. Banks (Columbia University, New York) and was maintained in Dulbecco’s modified Eagle’s medium with 4500 mg/liter glucose and 10% heat-inactivated newborn calf serum, supplemented with 15 μg/ml hypoxanthine, 250 μg/ml xanthine, and 25 μg/ml mycophenolic acid (HXM selective medium). GP+ E-86 subclones transfected with recombinant retroviral vectors were selected and maintained in HXM medium containing 1 mg/ml G418 (Canadian Life Technologies, Burlington, Ontario, Canada) or 2 μg/ml puromycin (Sigma), as appropriate for selection of the virus-encoded selectable marker. The IL-3-dependent murine cell line, Ba/F3, was kindly provided by Dr. A. Miyajima (DNAX Research Institute) and was maintained in RPMI 1640 medium with 10% heat-inactivated fetal calf serum and 3 mmol/liter COS cell-derived murine IL-3. The retrovirally infected cells were maintained in the same medium, supplemented with 1.8 mg/ml G418 and/or 2 μg/ml puromycin. All media were obtained from StemCell Technologies Inc. (Vancouver, British Columbia, Canada).

**Viral Production and Infection of Ba/F3 Cells—**The generation of GP+ E-86 clones producing recombinant retrovirus and the infection of IL-3-dependent Ba/F3 cells were as described previously (10, 16). Clones expressing various chimeric and mutant EpoRs were selected by plating the infected cells in standard methylessue (StemCell Technologies Inc.) supplemented with IL-3 and 1.8 mg/ml G418 or 2 μg/ml puromycin. Selection of clones coexpressing WT EpoR or EpoR/IL-3Rαβ, with the EpoR/IL-3Rα chimaera was carried out in the presence of 1.8 mg/ml G418 and 2 μg/ml puromycin.

**Proliferation Assays—**Cells were grown for 2–4 days in the absence of G418, washed, and then deprived of IL-3 and fetal calf serum for 6 h in RPMI 1640 supplemented with 0.1% BSA. Cells were then washed, resuspended in RPMI 1640 containing 0.1% BSA, and aliquoted into 96-well U-shaped microtiter plates at 2.5 × 104 cells/well, and growth factors were added to a final volume of 0.1 ml/well. Following 22 h of incubation at 37 °C in a humidified atmosphere containing 5% CO2, cells were pulsed with 1 μCi of [3H]thymidine ([3H]-Tdr, 2 Ci/mmol, DuPont NEN) for 2 h. The cells were then harvested onto filter mats using an LKB 1295-001 Skatron cell harvester, and [3H]-Tdr incorporation was determined in an LKB 1205 Betaplate liquid scintillation counter (LKB Wallac, Turku, Finland).

**Northern Blot Analysis—**Ba/F3 cells expressing chimeric or mutant EpoRs were incubated for 4 h in RPMI 1640 supplemented with 0.1% BSA. Cells were then washed with RPMI 1640 and resuspended in RPMI 1640 containing 0.1% BSA, or in the same medium supplemented with Epo (0.05 or 0.5 unit/ml) or 3 nmol/liter COS cell-derived IL-3, or a combination of 0.5 unit/ml Epo and 3 nmol/liter IL-3. After 18–20 h of incubation at 37 °C in a humidified atmosphere with 5% CO2, cells were lysed in RNAzol (Canarian Life Technologies), and the total cellular RNA was isolated as recommended by the manufacturer. Northern blot analysis was performed as described previously (16). Probes used for hybridization were a 295-bp Sau3AI-AccI fragment encompassing the first exon and intron of the murine β-major globin gene (provided by Dr. P. Lebouche, MIT, Boston, MA) and either a 1.3-kilobase pair Ps1 fragment of rat glyceraldehyde-3-phosphate dehydrogenase cDNA (provided by P. Jeanterre, Centre Paul Lamarque, Montpellier, France) or a 1.6-kilobase pair Ps1 fragment of chicken β-actin cDNA used to test for loading equivalence.

**RESULTS**

To examine the regions within the EpoR that might be inducing β-globin mRNA, we engineered retroviral vectors carrying coding regions for various mutant and chimeric EpoRs that could be compared with the WT EpoR. The forms of the various receptors studied are illustrated in Fig. 1. These included a full-length EpoR in which all eight intracellular tyrosines were exchanged for phenylalanines (null EpoR) (10), two chimeric EpoRs containing the extracellular domain of the EpoR and the transmembrane and cytoplasmic domains of the IL-3Rβ or IL-3Rα subunit (termed EpoR/IL-3Rβ or EpoR/IL-3Rα, respectively), and a C-terminal truncated EpoR (EpoR (–230)) in which the entire cytoplasmic domain was replaced with two primer-derived arginine residues. Following retroviral infection, IL-3-responsive Ba/F3 cells expressing the various EpoR forms were then selected for assessment of Epo-
stimulated proliferation and differentiation.

EpoR Tyrosine Phosphorylation Is Not Required for EpoR-mediated β-Globin Gene Induction in Ba/F3 Cells—To determine the importance of EpoR tyrosine phosphorylation to Epo-induced β-globin gene expression, we tested the mutant EpoR in which all eight cytoplasmic tyrosines were substituted with phenylalanines (null EpoR). Several independent Ba/F3 clones were obtained following retroviral mediated gene transfer and assessed for expression of cell surface null EpoRs by both flow cytometry using biotin-labeled Epo and by Scatchard analysis using 125I-labeled Epo. Following selection of clones expressing similar numbers of cell surface WT or null EpoRs (approximately 3000/cell, as determined by Scatchard analysis), the Epo-induced proliferation and induction of β-globin message were compared. Null EpoR-expressing Ba/F3 cells proliferated in response to Epo as determined by 3H-Tdr incorporation assays but required approximately 5-fold higher concentrations of Epo to achieve levels of 3H-Tdr incorporation comparable with those obtained with WT EpoR-expressing cells (Fig. 2A). The null and WT EpoR-expressing cells accumulated comparable levels of β-globin mRNA upon stimulation with Epo, and for both, no induction of β-globin mRNA could be detected in response to IL-3 or to IL-3 plus Epo (Fig. 2B, representative Northern blot analysis). Interestingly, this Epo-induced differentiating response could be detected in both cell types at concentrations of Epo that stimulated proliferation of WT EpoR cells but were markedly less effective in promoting proliferation of null EpoR cells, consistent with recently published observations that Epo-induced differentiation of Ba/F3 cells can occur in the absence of proliferation (16, 19). Our results further suggest that tyrosine phosphorylation of the EpoR itself is not required for induction of β-globin mRNA.

The Intracellular Domain of the IL-3Rα Subunit Inhibits Erythroid Differentiation

IL-3 Receptor α Subunit Inhibits Erythroid Differentiation

FIG. 2. A, Epo-induced proliferative responses of WT and null EpoR-expressing Ba/F3 cells. Growth factor-deprived cells were resuspended in RPMI 1640 supplemented with 0.1% BSA and various concentrations of Epo. 3H-Tdr incorporation assays were performed as described under “Materials and Methods.” The graph shows results representative of five independent experiments. B, Northern blot analysis of β-globin mRNA levels in WT and null EpoR-expressing cells. Growth factor-deprived cells were incubated for 20 h in RPMI 1640 supplemented with 0.1% BSA in the presence of 3 nmol/liter IL-3 or IL-3 and 0.5 unit of Epo/ml or Epo alone (0.5 or 0.05 unit/ml). Lanes contained approximately 10 μg of total cellular RNA. Hybridization probes are listed on the right.

FIG. 3. A, Epo-induced proliferative responses of EpoR/IL-3Rβ1-3 subunit-expressing Ba/F3 cells. Levels of Epo-stimulated 3H-Tdr incorporation were determined as described for Fig. 2A. B, the Epo-induced accumulation of β-globin mRNA by cells of two representative Ba/F3 clones expressing EpoR/IL-3Rβ1-3 chimera. Growth factor-deprived cells were incubated for 20 h in RPMI 1640 supplemented with 0.1% BSA in the absence of growth factors (lane 0) or in the the presence of 3 nmol/liter of IL-3 or IL-3 and 0.5 unit of Epo/ml or Epo alone (0.5 or 0.05 unit/ml). Lanes contained approximately 10 μg of total cellular RNA. Hybridization probes are listed on the right. Clone 4 was subsequently used to assess the effect of coexpressing the EpoR/IL-3Rα (see Fig. 6). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
numbers of WT EpoRs (3000/cell as determined by Scatchard analysis) in response to Epo (Fig. 3A); moreover, the \( \beta \)-globin message was induced in these cells in response to Epo (Fig. 3B) but not in response to IL-3 or IL-3 plus Epo, consistent with results obtained by other groups (18, 21).

One possibility suggested by these observations is that a second as yet unidentified subunit associates with the extracellular domain of the EpoR and provides the differentiating signal. Another intriguing possibility is that the cytoplasmic domain of the IL-3 \( \beta \)-subunit on its own is permissive for differentiation, but signaling through the intact IL-3R suppresses differentiation, thus pointing to a specific inhibitory role for the \( \alpha \) subunit of the IL-3R. To discriminate between these two possibilities, we tested a C-terminal truncated EpoR (EpoR(−230)) possessing only 2 amino acids within the cytoplasmic domain (Fig. 1). The differentiating and proliferative capacity of this truncated EpoR was examined in several independent EpoR(−230)-transduced Ba/F3 clones, expressing between 8000 and 12,000 cell surface EpoRs (determined by flow cytometry and Scatchard analyses, data not shown). Viability of these cells decreased in Epo-supplemented medium within 24 h to approximately 25–30%, and no viable cells could be detected by 48 h (Fig. 4A). Moreover, no accumulation of \( \beta \)-globin mRNA by the EpoR(−230)-transduced cells could be detected in response to Epo or Epo plus IL-3 (Fig. 4B). This indicates that an EpoR lacking the intracellular domain is not capable of promoting the survival and differentiation of Ba/F3 cells in response to Epo and argues against differentiating signaling being activated through molecules associated with the extracellular domain of the EpoR, at least not in the absence of a functional cytoplasmic domain.

The \( \alpha \) Subunit of IL-3R Inhibits Epo-induced \( \beta \)-Globin Gene Expression—To test the hypothesis that the IL-3R \( \alpha \) subunit can suppress Epo-induced differentiation, we examined the differentiating and proliferative capacities of a chimeric receptor composed of the extracellular domain of the EpoR and the transmembrane and cytoplasmic domains of the IL-3R \( \alpha \) subunit (EpoR/IL-3R \( \alpha \)). Ba/F3 cells expressing this EpoR/IL-3R \( \alpha \) chimera proliferated in Epo-supplemented medium but required approximately 5-fold higher concentrations of Epo to achieve \(^{3}H\)-Tdr incorporation levels comparable with those obtained by WT EpoR-expressing cells (Fig. 5A). \( \beta \)-Globin mRNA levels were then examined in several independent EpoR/IL-3R \( \alpha \) clones, and no accumulation of \( \beta \)-globin mRNA could be detected in a variety of experimental conditions, including different concentrations of Epo (Fig. 5B) and different times of exposure to Epo (data not shown), suggesting that this EpoR/IL-3R \( \alpha \) chimeric receptor was not capable of promoting
expression of the EpoR/IL-3R chimera. Several clones expressing 2–4-fold higher numbers of cell surface EpoRs (than the parental WT EpoR cells) were identified by Scatchard analysis (i.e. 3300 WT EpoRs/cell plus 7000–12,000 EpoR/IL-3Rα/cell). These cells required 5–30-fold higher concentrations of Epo to achieve proliferation levels comparable with those obtained by the parental WT EpoR cells (Fig. 6B). More importantly, cells coexpressing WT EpoR and EpoR/IL-3Rα ceased to accumulate β-globin mRNA in response to Epo (Fig. 6C) suggesting that the IL-3Rα subunit inhibited the differentiating function of the EpoR.

**DISCUSSION**

In this study, we expressed various mutant and chimeric EpoRs in the IL-3-responsive cell line, Ba/F3, to examine the functional roles of the EpoR and IL-3R in regulating β-globin gene induction in Ba/F3 cells. Surprisingly, EpoRs totally lacking in potential tyrosine phosphorylation sites (null EpoR) were found to be capable of inducing β-globin mRNA as well as the normal EpoR. This finding is compatible with our previously published results showing that Epo-induced differentiation was blocked by genestein (16), since null EpoRs mediate tyrosine phosphorylation (and activation of Jak2) as well as WT EpoRs (10). Taken together, our data suggest that tyrosine phosphorylation of Jak2, but not the EpoR, is critical for Epo-induced differentiation. Null EpoRs, however, were severely compromised in their ability to promote proliferation of Ba/F3 cells, consistent with our previous results (10). It is conceivable that the reduced ability of the null EpoR to promote proliferation of Ba/F3 cells could create conditions permissive for differentiation. In this regard, Ba/F3 cells, although originally described as a pro-B cell line (23), express erythroid-specific transcription factors such as GATA-1 and NF-E2 (17) and very low levels of endogenous EpoRs (7) and may thus have evolved in culture toward an erythroid phenotype or have always been erythroid in nature. The Epo-induced delay in progression through G1 of the cell cycle, shown previously for the WT (16, 19) and currently for the null EpoR-expressing cells (data not shown), may simply be triggering a predetermined erythroid differentiating program, as seems to be the case for MEL cells engineered to express p53 (24). However, a simple delay in G1 of parental Ba/F3 cells does not induce β-globin mRNA (16), suggesting that induction of this gene depends on EpoR-mediated signaling.

The Epo-induced accumulation of β-globin mRNA in cells expressing the EpoR/IL-3Rα chimera is consistent with the previously published findings of Carroll et al. (18) and Chiba et al. (21). This observation pointed to the possibility that Epo-

**FIG. 6.** Epo-induced proliferative responses of Ba/F3 cells coexpressing EpoR/IL-3Rβ and EpoR/IL-3Rα chimeras (A) or WT EpoR and EpoR/IL-3Rα chimera (B). The β-globin and β-actin mRNA levels upon IL-3 or Epo stimulation of Ba/F3 cells expressing WT EpoR versus a representative clone coexpressing EpoR/IL-3Rα chimera, and Ba/F3 cells expressing the EpoR/IL-3Rβ alone (Fig. 3, clone 4) versus that clone coexpressing the EpoR/IL-3Rα chimera. Growth factor-deprived cells were stimulated with 3 nmol/liter of IL-3 or 0.5 unit of Epo/ml in RPMI 1640 containing 0.1% BSA for 1 day (WT EpoR and EpoR/RL-3Rβ) or for 1–3 days as indicated for cells coexpressing EpoR/IL-3Rα. Each lane contains approximately 10 μg of total cellular RNA. Hybridization probes are listed on the right.
specific signaling might depend on the interactions of the extracellular domain of the EpoR (25) with a second as yet unidentified subunit of the EpoR complex. However, cells expressing high levels of a truncated EpoR (−230) lacking the cytoplasmic domain neither survived nor accumulated β-globin mRNA upon Epo stimulation, suggesting that the cytoplasmic region of the EpoR is indispensable for EpoR function. The differentiating capacity of the EpoR/IL-3Rβ chimera, however, also suggested that the cytoplasmic domains of the EpoR and the βIL-3 subunit of the IL-3R were interchangeable in providing for the differentiating signal, which argues against the existence of a differentiation-specific domain within the cytoplasmic region of the EpoR and points to a permissive rather than an instructive role for the EpoR in Epo-induced differentiation.

Our finding that the EpoR/IL-3Rα chimera was capable of supporting proliferation of Ba/F3 cells was somewhat surprising since Kitamura and Miyajima (26) reported that the human IL-3Rα subunit alone was unable to support proliferation of IL-2-dependent CTLL-2 cells. These cells proliferated in response to human IL-3 only when engineered to coexpress human IL-3 or WT EpoRs. This mechanism seems unlikely since exposure to Epo of all EpoR/IL-3Rα subunits of receptors for IL-3, IL-5, and granulocyte macrophage colony-stimulating factor is essential for mitogenic signaling (27). It seems unlikely that a similar association occurs between the extracellular domains of IL-3Rα and EpoR. Our results are consistent with a steadily growing body of data suggesting that the membrane proximal region contained among α subunits of receptors for IL-3, IL-5, and granulocyte macrophage colony-stimulating factor is essential for mitogenic signaling (28–30) and Jak2 activation (31, 32). It is inhibitory effect of the EpoR/IL-3Rα subunits of the EpoR complex (28–30) and Jak2 activation (31, 32), and granulocyte macrophage colony-stimulating factor (33) receptors initiate distinct ligand-induced events.

The data presented in this study suggest both a permissive role for Epo in inducing β-globin mRNA in Ba/F3 cells expressing EpoRs and an active role for the IL-3Rα subunit in the IL-3-induced inhibition of Epo-induced differentiation. Based on these findings, ongoing studies are aimed at identifying effectors that are, through interaction with the IL-3Rα subunit, involved in suppression of β-globin gene expression in Ba/F3 cells.

Acknowledgment — We thank Patricia Rosten for helpful advice and technical assistance in generating chimeric EpoRs.

REFERENCES

1. Krantz, S. B. (1991) Blood 77, 419–434
2. Sawada, K., Krantz, S. B., Dai, C. H., Koury, S. T., Horn, S. T., Glick, A. D., and Dein, C. I. (1990) J. Cell. Physiol. 141, 219–230
3. Wognum, A. W., Landsorp, P. M., Humphries, R. K., and Krystal, G. (1990) Blood 76, 897–906
4. Bazan, J. F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6934–6938
5. Quele, F. W., and Wojchowski, D. M. (1991) J. Biol. Chem. 266, 609–614
6. Dauber, I., Teminou, N., Lacombe, C., Muller, O., Billat, C., Fischer, S., and Mayaux, P. (1992) J. Biol. Chem. 267, 10870–10875
7. Damen, J., Mui, A. L., Hughes, P., Humphries, K., and Krystal, G. (1992) Blood 80, 1923–1932
8. Wittthuhn, B. A., Quele, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ilhe, J. N. (1993) Cell 74, 227–236
9. Miura, O., Cleveland, J. L., and Ilhe, J. N. (1993) Mol. Cell. Biol. 13, 1788–1795
10. Damen, J., E. Wakah, H., Miyajima, A., Krosl, J., Humphries, R. K., Cutler, R. L., and Krystal, G. (1995) EMBO J. 14, 5557–5568
11. Koury, M. J., and Bondurant, M. C. (1990) Science 248, 378–381
12. Wickrema, A., Krantz, S. B., Winkelmann, J. C., and Bondurant, M. C. (1992) Blood 80, 1840–1849
13. Bondurant, M. C., Lind, R. N., Koury, M. J., and Ferguson, M. E. (1985) Mol. Cell. Biol. 5, 675–683
14. Koury, M. J., Bondurant, M. C., and Mueller, T. J. (1986) J. Cell. Physiol. 126, 259–265
15. Minegishi, N., Minegishi, M., Tsuchiya, S., Fujie, H., Nagai, T., Hayashi, N., Yamamoto, M., and Konno, T. (1994) J. Biol. Chem. 269, 27700–27704
16. Krosl, J., Damen, J. E., Krystal, G., and Humphries, R. K. (1995) Blood 85, 50–56
17. Liboi, E., Carroll, M., D’Andrea, A. D., and Mathey-Prevot, B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11351–11355
18. Carroll, M., Mathey-Prevot, B., and D’Andrea, A. (1994) Proc. Soc. Exp. Biol. Med. 206, 289–294
19. Carpen, M., Zhu, Y., and D’Andrea, A. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2869–2873
20. Maruyama, K., Miyata, K., and Yoshimura, A. (1994) J. Biol. Chem. 269, 5576–5580
21. Chiba, T., Nagata, Y., Kishi, A., Sacamakki, K., Miyajima, A., Yamamoto, M., Engel, J. D., and Todokoro, K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11593–11597
22. Markowitz, D., Goff, S., and Bank, A. (1988) J. Virol. 62, 1120–1124
23. Palacios, R., and Steinmetz, M. (1985) Cell 41, 727–734
24. Johnson, P., Chung, S., and Benchimol, S. (1993) Mol. Cell. Biol. 13, 4239–4243
25. D’Andrea, A. D., Lodish, H. F., and Weng, C. G. (1989) Cell 277, 257–275
26. Kitamura, T., and Miyajima, A. (1992) Blood 80, 84–90
27. Miyajima, A., Mui, A. L., Ogorechi, T., and Sacamakki, K. (1993) Blood 82, 1060–1074
28. Sacamakki, K., Miyajima, I., Kitamura, T., and Miyajima, A. (1992) EMBO J. 11, 3541–3549
29. Weiss, M., Yokoyama, C., Shikama, Y., Naugle, C., Druker, B., and Siff, C. A. (1993) Blood 82, 3289–3306
30. Polotskaya, A., Zhao, Y., Lilly, M. B., and Kraft, A. S. (1994) J. Biol. Chem. 269, 14677–14681
31. Correllis, S., Fache, F., Van der Heyden, J., Guisez, Y., Tavernier, J., Devos, R., Fiers, W., and Plaetinck, G. (1995) Eur. J. Immunol. 25, 1857–1864
32. Takagi, S., Kawanaka, H., Shiba, M., and Takata, K. (1996) Mol. Cell. Biol. 14, 7404–7413
33. Eder, M., Ernst, T. J., Ganser, A., Kubinsky, P. T., Inhorn, R., Hoelzer, D., and Griffin, J. D. (1994) J. Biol. Chem. 269, 30173–30180