Effect of Activin A on Globin Gene Expression in Purified Human Erythroid Progenitors

By Li-en Shao, Normand L. Frigon, Jr, Arlene L. Young, Alice L. Yu, Lawrence S. Mathews, Joan Vaughan, Wylie Vale, and John Yu

The regulatory control of human erythropoiesis through a purified protein, activin A, was examined. Previous studies using mixed populations of bone marrow cells suggested that activin A has an indirect effect on cellular proliferation and DNA synthesis of erythroid progenitors through the mediation of accessory cells. In present studies, the cultures of purified erythroid progenitors were used to examine the effect of activin A on globin gene expression. Human erythroid burst-forming units (BFU-E) were partially purified from peripheral blood, and after 8 days of culture the cells generated consisted mainly of erythroid colony-forming units (CFU-E). It was found that the subsequent 7-day cultures of these purified progenitors yielded similar numbers and size distributions of erythroid colonies, regardless of the presence of activin A in the cultures. In addition, these erythroid progenitor cells were responsive, in terms of stimulation of DNA synthesis, to the addition of erythropoietin, but not to treatment by activin A. Therefore, once the erythroid progenitors are depleted of accessory cells, activin A has little effect on both the proliferation and the DNA synthesis of these progenitors. However, when these purified erythroid progenitors were cultured in the presence of activin A, the levels of all α, β, and γ globin transcripts and hemoglobins were significantly increased. In addition, disuccinimidyl suberate was found to chemically cross-link 125I-activin A to cell surface binding proteins (45 to 54 kDa) in both purified erythroid progenitors and K562 cells. The labeling of these binding proteins was specifically inhibited by the presence of unlabelled activin A, but not transforming growth factor-β. These results suggest that, in addition to its indirect effect on DNA synthesis and cellular proliferation of erythroid progenitors, activin A directly affects the levels of globin mRNAs and hemoglobins in developing human erythroid cells through its specific surface binding receptors.

© 1992 by The American Society of Hematology.

ACTIVIN A WAS first purified to homogeneity from gonadal fluids and shown to stimulate the secretion of pituitary follicle-stimulating hormone.1 It was found that activin A could modulate the colony formation of erythroid burst-forming units (BFU-E) and erythroid colony-forming units (CFU-E) in human bone marrow culture2 and could also induce human K562 and mouse Friend cells to become positively stained by benzidine.3,4 Recently, a protein that exhibited this inducing activity toward Friend cells was isolated from human THP-1 cells and found to be identical with activin A, because it was encoded by the same mRNA as that of activin A.4 In addition, it was found that this protein was expressed at least at the RNA level in the bone marrows of rats.2 These data all suggest a possible role of activin A in the regulatory control of erythropoiesis.2 However, mechanisms of the action of activin A on the process of erythropoiesis remain largely unknown.

It was reported that activin A increases the proliferative state of erythroid progenitors from both bone marrow and peripheral blood,4 and that this effect on erythroid cell proliferation is exerted indirectly, i.e., through mediation of both monocytes and T lymphocytes.5,6 It was also suggested that activin A may drive immature erythroid precursors to form hemoglobinized cells, thereby rendering the colonies recognizable as erythroid colonies.3,7,8 Conceivably, activin A may have effects on both cellular proliferation and erythroid differentiation. In the accompanying report,9 activin A was found to induce accumulation of hemoglobins and stimulate the many globin transcripts in K562 cells. Unlike our previous studies using mixed populations of hematopoietic cells,10-12 the present investigations address the issues of the effects of activin A on highly purified preparations of human erythroid progenitors. In contrast to the studies reported in the accompanying article that used the K562 cell line as a model,9 the culture of partially purified human erythroid progenitors in present studies provides a system more closely approximating human erythropoiesis in vitro. It was found that activin A directly affects the levels of globin mRNAs and hemoglobins in developing human erythroid cells. It was also found that both purified erythroid progenitors and K562 cells possess specific binding proteins on their surface. Therefore, activin A may directly interact with its specific surface receptors, and regulate the expression of globin genes, independent of accessory cells.

MATERIALS AND METHODS

Purification of activin A. Ten liters of porcine follicular fluid was subjected to several steps of purification procedures including preparative cation exchange chromatography, gel filtration, and reverse-phase high performance liquid chromatography (HPLC), as previously described.1 The preparation was characterized as a β,βA homodimer (i.e., activin A) with the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in reducing and nonreducing conditions.3 The structure of the purified protein was also characterized with partial amino acid sequence.

From the Department of Molecular and Experimental Medicine, The Scripps Research Institute; the Department of Pediatrics, University of California at San Diego; and the Clayton Foundation Laboratories for Peptide Biology, The Salk Institute for Biological Studies, La Jolla, CA.


Supported by Grants DK40218 and MOI-RR833 from National Institutes of Health and the Clayton Foundation, California Division. W.V. is a Clayton Foundation Investigator. This is publication no. 7045-MEM from The Scripps Research Institute.

Address reprint requests to John Yu, MD, PhD, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 N Torrey Pines Rd, La Jolla, CA 92037.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1992 by The American Society of Hematology.

0006-4971/92/7903-0008$3.00/0
analysis and Western immunoblotting. It reacted specifically with antibodies directed against \( \beta_3 \) but not \( \alpha \) or \( \beta_1 \) subunits. Stock solution of activin A at 1 \( \mu \)g/mL contained HPLC buffer vehicle with 0.1% bovine serum albumin (BSA), 2.8% acetonitrile, and 0.004% trifluoroacetic acid. In addition, recombinant human activin A was purified from spent culture medium of mammalian kidney cells after transfection with a plasmid containing the entire coding region of the \( \beta_3 \)-subunit of human inhibin under the control of a cytomegalovirus (CMV) promoter (generous gift from Dr R. Schwall, Genentech, Inc, South San Francisco, CA).

Purification of erythroid progenitors. The experimental design is presented in Fig. 1. Human peripheral blood was obtained from normal adult volunteers who had previously signed consent forms approved by the Human Subjects Committee at Scripps Clinic. Purification of erythroid progenitors was performed as described by Sawada et al with slight modifications. Briefly, peripheral blood was collected and centrifuged over a Ficoll-Paque density gradient (specific gravity 1.077 g/mL; Pharmacia Fine Chemicals, Piscataway, NJ) at 400g for 25 minutes at room temperature. The mononuclear cells at the interface were collected, washed two times with \( \alpha \)-MEM containing 2% heat-inactivated fetal calf serum (FCS) and resuspended in the 50% Iscove’s modified Dulbecco’s medium (IMDM)/50% \( \alpha \)-MEM. These cells were incubated with 1-aminooxyisothiocyanurium bromide-treated sheep erythrocytes, and nonrosetted cells were separated over Ficoll-Paque at 400g for 15 minutes at 24°C. Afterwards, the cells were washed twice, and cells expressing surface Ig were depleted by panning for 80 minutes at 4°C with dishes that had been previously coated with affinity-purified goat antihuman IgG specific for the F(ab')2, fragment (Accurate Corp, Westbury, NY). The nonadherent cells were collected and incubated overnight in polystyrene flasks at 37°C in a 5% CO\(_2\) atmosphere with 20% FCS and 10% giant cell tumor-conditioned medium (generous gift of Dr James K. Brennan, University of Rochester, Rochester, NY). The nonadherent cells were suspended at 7 \( \times 10^7 \) cells/mL and incubated on ice in a mixture of four monoclonal antibodies (MoAbs): 25 \( \mu \)L of CD11b/OKM*1 (20 \( \mu \)g/mL), 25 \( \mu \)L of CD2/OKT*11 (10 \( \mu \)g/mL) (Ortho Diagnostic Systems, Inc, Raritan, NJ), plus 50 \( \mu \)L of CD45R/MY11 and CD16/MY23 hybridoma supernatants. After 60 minutes, the cells were washed three times and then incubated at 4°C for 90 minutes on plastic tissue culture dishes that had been coated with affinity-purified goat antimurine IgG (Boehringer Mannheim Biochem, Indianapolis, IN). The antibody-negative, nonadherent cells were then collected for use as the enriched BFU-E. These BFU-E were cultured at 2 \( \times 10^6\) cells/mL in 35-mm Petri dishes in a 1-\( \mu \)L mixture containing 0.8% methylcellulose in IMDM, 30% FCS, 1% deionized BSA, 0.1 \( \mu \)mol/L L-α-biotinoglycerol, 50 U/mL of penicillin, 50 \( \mu \)g/mL of streptomycin, 10% giant cell tumor-conditioned medium, and 2 U of recombinant human erythropoietin (10,000 U/mg protein; generous gift from AMGen Biologicals, Thousand Oaks, CA). The dishes were incubated at 37°C in a humidified incubator flushed with 5% CO\(_2\) for a total of 8 days.

After culture in methylcellulose, the cells were collected and washed twice in excess \( \alpha \)-MEM. The adherent cells were removed as described, and a 2-ML cell suspension of the nonadherent cells was overlaid on 2 mL of Ficoll-Paque. After centrifugation at 400g for 15 minutes at 24°C, the interface cells were collected, washed three times, and resuspended in IMDM containing 2% FCS. Subsequently, these purified erythroid progenitors were cultured for another 7 days at 1 \( \times 10^5\) cells/mL in a mixture similar to BFU-E cultures except without giant cell tumor-conditioned medium. To quantitate purification of erythroid colony-forming cells, similar cultures were performed in parallel, with a reduced seeding density of 100 to 1,000 cells per plate. The CFU-E colonies were identified by using the classical criteria for colonies composed of 8 to 64 hemoglobinized cells.

Culture for erythroid progenitors and CFU-granulocyte/monocyte (CFU-GM). Culture of erythroid progenitor cells was performed as described by Iscove et al with slight modification. Culture of CFU-GM was performed in 0.3% agar as described. Incorporation of radioactive thymidine. Purified erythroid progenitors were washed twice with 50% IMDM/50% \( \alpha \)-MEM mixture containing 20% FCS and then resuspended in the same medium at 1 \( \times 10^5\) cells/mL. Afterward, 100 \( \mu \)L of cell suspension was pipetted to 96-well microtiter plates in quadruplicate. Then, 50 \( \mu \)L of various amounts of erythropoietin or activin was added to each well. The plates were incubated in a CO\(_2\) incubator at 37°C. After overnight incubation, 20 \( \mu \)L of \( ^{3}P \) thymidine at 80 \( \mu \)Ci/mL was added and the mixtures were incubated for another 2 to 4 hours. The incorporation of radioactive thymidine into DNA was measured by collecting the cells on a glass fiber strip with the use of an automatic cell harvester, washing the filters with water, drying the filter, and determining the radioactivity by liquid scintillation counting, as previously described.

RNA isolation. Total RNA was prepared by the guanidinium isothiocyanate method. The 7-day cultures of purified erythroid progenitors were washed three times with phosphate-buffered saline (PBS), and the cells were lysed in 4 mol/L guanidinium isothiocyanate, 20 mmol/L sodium acetate, pH 5.2 containing 0.1 mmol/L dithiothreitol and 0.5% N-lauryl sarcosine ( Sarkosyl;
The genomic DNA probes for the S, nuclease analyses were as follows: (1) α, the 1.0-kb Pst I-Hind III 5' fragment of human α-globin gene; (2) β, the 1.9-kb Bam HI 5' β fragment; and (3) γ, the 1.7-kb Eco Rl-Bam HI 5' A fragment. These DNA clones, obtained from Dr T. J. Ley (Washington University Medical Center, St Louis, MO), were grown in Escherichia coli JM 101 and the plasmid DNA was isolated by standard procedures. The globin genomic fragments were prepared by appropriate restriction digestion and purified as described in the accompanying report. After centrifugation in CsCl, Sigma, St Louis, MO). Then, RNA was prepared as described in Materials and Methods. The cells generated from these cultures were further purified and are referred to as "purified erythroid progenitors" in the present studies. When these purified progenitor cells were analyzed for their cellular compositions after Wright-Giemsa stain, 68.8% of cells were the "early erythroid cells" according to the criteria defined by Sawada et al. The remaining cells were proerythroblasts, erythroblasts, and other nonerythroid cells (about 9.0%, 17.0%, and 5.3%, respectively). The degree of purification up to this stage was consistent with the previous report. To study the effect of activin A on cellular proliferation, the preparations of these purified progenitor cells were cultured for another 7 days in the presence or absence of activin A (Fig 1), under conditions favoring growth of erythroid colonies. Under these conditions, the cloning efficiency of these purified progenitor cells was 74.2 ± 0.8 colonies per 100 nucleated cells plated (a value derived from three experiments with eight culture
plates each). Approximately 77.6% ± 8.7% of these colonies in the cultures were the classically defined CFU-E colonies, while the other colonies were smaller, but also with erythroid phenotypes (presumably derived from erythroblasts and other precursors more mature than the day 7 CFU-E). Therefore, the preparations of purified erythroid progenitors used in the present studies contained mostly the erythroid progenitor cells at a stage of maturation similar to "CFU-E."

Previously, we found that the addition of activin A in human bone marrow cultures significantly potentiated the proliferation of erythroid progenitors, up to 200% to 300%. Other studies further suggested that the potentiation effect of activin A might be attributed to an increase in the proliferative state of BFU-E and CFU-E in the bone marrow cultures and this was mediated through accessory cells such as T lymphocytes and monocytes. To study the direct effect of activin A on proliferation, preparations of purified erythroid progenitors were cultured in methylcellulose for 7 days with or without activin A (Fig 1). Then, erythroid colonies arising from these cultures were examined in terms of the size distribution (by scoring the number of cells per colony) and the number of erythroid colonies. It was found that the size distribution of these colonies was similar between samples prepared from cultures incubated in the absence and presence of activin A (Fig 2). In other words, activin A did not affect the size distribution of erythroid colonies when incubated with purified erythroid progenitors. In addition, cultures of these purified erythroid progenitors in the presence and absence of activin A yielded the same numbers of erythroid colonies as those from the control cultures (102.2% ± 2.1% relative to controls). In contrast, when unfractionated mononuclear cells from peripheral blood were used (in place of the purified erythroid progenitors) in the cultures, there were approximately twofold increases in the numbers of erythroid colonies in the cultures containing activin A. Therefore, this study using purified erythroid progenitors supports the suggestion that activin A does not enhance proliferation of erythroid progenitors, when the latter are depleted of accessory cells before activin treatment.

The effect of activin A on the DNA synthesis of the purified erythroid progenitors was also investigated (Fig 3). Previous experiments using unfractionated marrow cells had to resort the "cell suicide" method to assess DNA synthesis activity of the erythroid progenitors, because of the low frequency of progenitor cells present in the samples. However, these results indicated that the proportions of DNA-synthesizing erythroid progenitors were increased by activin A only in the presence of accessory cells. In other words, these experiments predicted that there was no direct effect of activin A on the DNA synthesis of the purified erythroid progenitors. This is confirmed in Fig 3, in which preparations of purified erythroid progenitors in suspension cultures responded to incubation of cells with erythropoietin in a dose-responsive manner, but did not respond to incubation with activin A up to 150 ng/mL. The erythropoietin-incubated cells exhibited an increase in the levels of incorporation of radioactive thymidine (Fig 3). In addition, the addition of activin A did not potentiate the stimulatory effects on DNA synthesis by erythropoietin (results not shown) if both additives were included. It was concluded that activin A cannot stimulate DNA synthesis of erythroid progenitors, but erythropoietin does.

**Effect of activin A on globin transcripts in developing erythroid cells.** To study the effects of activin A on globin gene expression, the erythroid colonies arising from purified erythroid progenitors after culture for 7 days in the presence and absence of activin A (Fig 1) were examined in the following experiments. The differential counts of these cells from the colonies of the activin-treated and untreated samples on Wright-Giemsa staining did not shown much..
Fig 3. Effect of activin A or erythropoietin on thymidine incorporation into purified erythroid progenitor cells. Human erythroid progenitors were purified as described in Fig 2 and in Materials and Methods. These progenitor cells were then cultured overnight in suspension cultures in the presence of various concentrations of activin A (-A+) or erythropoietin (--O--). Thymidine incorporation assay was performed as described.

Fig 4. S1 nuclease analyses of globin mRNA in erythroid colonies obtained from 7-day cultures of purified erythroid progenitors. The purified erythroid progenitors were prepared and cultured for 7 days in the absence (-) and in the presence (+) of activin A as presented in Fig 1. In (A), 1 μg of cellular RNA from these control and activin A-treated samples was hybridized with the specific globin probes and digested with S1 nuclease as described in Materials and Methods. The protected probe fragments were electrophoresed on 8% denaturing sequencing gels, and autoradiography was performed at -70°C. The probe fragment protected by α, β, and γ globin mRNAs is shown (180 nt, 209 nt, and 209 nt, respectively). The organization of the probes is shown in (B). Exons are represented as black boxes, introns as open boxes, and 5' and 3' flanking sequences as a thin line. The position of a HindIII and a BamHI site in exon 2 of globin genes is indicated. In (C), slot-blot hybridization with the probe for glyceraldehyde-3 phosphate dehydrogenase cDNA confirmed that there were equal amounts of RNA in the control (-) and activin A-treated (+) samples.
cells was examined by comparing the relative abundance of these specific globin fragments in the autoradiographs. As shown in Fig 4A, the specific 180-nt fragments for α-globin and the 209 nt for β-like globin family increased with treatment of purified erythroid progenitors with activin A. Figure 4C confirmed that approximately equal amounts of RNA were used for S₁ nuclease analyses in samples treated with or without activin A. Thus, it was concluded that the abundance of specific globin transcripts for α, β, and γ globins increased in developing erythroid cells when treated with activin A.

Effect on hemoglobin accumulation in erythroid cells. To determine the effect of activin A on hemoglobin content in the developing erythroid cells, immunochemical analysis was performed. Amounts of hemoglobins were determined using affinity-purified polyclonal antibodies against human hemoglobins. Based on the slot-blot immunoassays of the serially diluted cell extracts prepared from control and activin-treated samples, there was increase of hemoglobin contents above control levels after activin A treatment of the purified erythroid progenitors (Fig 5). By comparing with a standard curve of purified hemoglobin, it was estimated that there were approximately 15 to 20 μg of hemoglobins per 10⁶ cells in the samples treated with 5 ng/mL of activin A, an average of 74% ± 14% increase over the control values. Meanwhile, the extent of increase in globin mRNAs in these erythroid cells at this concentration of activin A, as analyzed with slot-blot assay (picture not shown), was estimated to be 88% ± 5% above the controls. Therefore, these findings indicate that activin A induces hemoglobin expression in developing erythroid cells, similar to its effect on the increase of hemoglobin accumulation in K562 cells.

Specificity of activin binding sites in purified erythroid progenitors. In the following experiments, a radioreceptor assay using iodinated recombinant activin A as a ligand and purified erythroid progenitors as a receptor source was used. Recombinant activin A was radioiodinated to a high specific activity with maintenance of bioactivity by the chloramine-T method (Mathews LS, et al, manuscript submitted). K562 cells and human erythroid progenitors, both known to differentiate in response to activin A, were found to possess high-affinity ¹²⁵I-activin receptors; and the results were similar to that previously reported for K562. These receptors were further found to be specific because erythropoietin, insulin, and transferrin do not compete for binding (data not shown). To examine the nature of the activin A binding components on the purified erythroid progenitors and K562 cells, disuccinimidyl suberate was used to chemically cross-link ¹²⁵I-activin A to the activin binding proteins on the surface of these cells. Triton X-100 extracts of cells affinity-labeled at 22°C were displayed by gel electrophoresis and autoradiography (Fig 6). Both K562 cells and human erythroid progenitors yielded affinity-labeled products of approximately 73 to 75 and 80 to 82 Kd as estimated by electrophoresis on reducing gels (Fig 6, lanes 3 and 4), corresponding to receptor proteins of approximately 45 to 47 and 52 to 54 Kd. The labeling of these binding proteins was specifically inhibited by the presence of a low concentration of unlabeled activin A (10 nmol/L) during the incubation with ¹²⁵I-activin A (Fig 6, lanes 1 and 5). In contrast, transforming growth factor-β (TGF-β), which is structurally related to activin A, did not inhibit the labeling of these activin A binding species (Fig 6, lane 2). These studies confirm that there are specific receptors for activin A present on these two cell types. It was also found that these receptors for activin A disappear after erythroid progenitors differentiated to become late erythroblasts (data not shown).

DISCUSSION

Human peripheral blood contains BFU-E and is relatively free from the presence of other erythroid progenitors such as CFU-E. Because of the low frequency of these BFU-E in the blood, they were partially purified and cultured in vitro for 8 days to develop into cells, comparable with CFU-E as far as the stages of maturation are con-
Concerned. These purified erythroid progenitor cells were used in the present studies to investigate the functions of activin A. Previously, it was reported that activin A had a potentiating effect on cellular proliferation in vitro bone marrow cultures. This potentiation effect was restricted mostly to erythroid lineage cells because activin A increases colony formation of both CFU-E and BFU-E but not CFU-GM colonies. In addition, the effect of activin A on cellular proliferation of erythroid colonies is exerted indirectly through mediation of both monocytes and T lymphocytes. Addition of monocytes or T lymphocytes to their respective depleted subpopulations of mononuclear cells reconstituted the enhancing effect of activin A on colony formation of CFU-E and BFU-E. Unlike these previous studies using mixed populations of hematopoietic cells, the present investigations analyzed the effects of activin A on highly purified preparations of human erythroid progenitors. It was shown that once the erythroid progenitors were depleted of accessory cells, incubation with activin A had little effect on either the number or the size distribution of the erythroid colonies derived from these purified preparations.

Previous experiments also showed that activin A increased the proportion of DNA-synthesizing erythroid progenitors in the unfractionated samples from both bone marrow and peripheral blood. Present studies indicated that the purified erythroid progenitors, depleted of accessory cells, were responsive, in terms of the increase in DNA synthesis, toward the addition of erythropoietin in a dose-responsive manner, but not to the treatment of activin A. Thus, these findings are consistent with the suggestion that the effect of activin A on erythroid DNA synthesis only occurs with the presence of accessory cells, eg, monocytes and T lymphocytes. Therefore, the ability of activin A to cause both proliferation of erythroid progenitors and stimulation of their DNA synthesis is not a direct effect on erythroid progenitors, but an indirect effect exerted through mediation of accessory cells.

In addition to this indirect effect, present studies have shown an apparently direct effect of activin A, which causes accumulation of globin transcripts and hemoglobin in developing erythroid cells, in the absence of accessory cells. Using S1 nuclease and immunochemical analyses, it was shown that there was significant increase in both globin transcripts and hemoglobins for the activin-treated cultures of the purified erythroid progenitors. This effect on erythroid progenitors was similar to that described for an erythroid cell line, K562, in inducing the accumulation of globin transcripts and hemoglobins. The vast majority of the cells developed from the purified preparations of erythroid progenitors in these studies are hemoglobinized erythroblasts with only negligible amount of nonerythroid contamination. Thus, it is unlikely that the difference in the globin contents between activin-treated and nontreated samples was due to a difference between the cell populations. Because activin A does not increase the numbers and the size distributions of erythroid colonies in the cultures of purified erythroid progenitors, this inducer is apparently affecting the levels of globin mRNA and hemoglobin production in developing human erythroid cells on a single-cell basis. This effect of activin A on the expression of globin genes among developing erythroid progenitors is consistent with the accompanying report indicating an apparent differentiation-inducing effect of activin A on both K562 and mouse Friend cells. We previously estimated that for the responsive K562 cells, activin A is about twice as effective as hemin in inducing the accumulation of hemoglobins in these cells on a single-cell basis. It was also found that activin A caused accumulation of globin transcripts and increased their rate of transcription in K562 cells. In addition, activin A could stimulate the transcriptional activity of an ε globin gene promoter in a transient
transfection assay. Recently, a protein that exhibited a potent differentiation-inducing activity toward mouse Friend cells was isolated and found to be activin A, because it was encoded by the same mRNA as that of the β-subunit of activin A. Thus, these data support a possible role of activin A in regulation of erythroid differentiation.

The addition of activin A potentiated the proliferation and differentiation of erythroid progenitors even at a maximum dosage of erythropoietin. It was also shown that both purified erythroid progenitors and K562 cells possess high-affinity receptors for activin A, which could be cross-linked with disuccinimidyl suberate to form complexes of approximately 75 and 82 Kd. The 82-Kd complex corresponds to the activin A receptor, which has recently been cloned from mouse ACT20 cells and suggested to be a protein serine kinase. A cross-linked complex of approximately 65 Kd has also been observed in other activin-responsive cells of rodent origin (Mathews LS, et al, manuscript submitted); the 75-Kd complex is assumed to be the human analogue of that protein. Apparently these activin A receptors are distinct from receptors for TGF-β and erythropoietin. Therefore, it is likely that activin A can exert its effects in the absence of erythropoietin. This lack of dependence on the presence of erythropoietin for activin A to manifest its effects on erythroid cells can be demonstrated with erythroleukemic cell lines such as K562 and mouse Friend cells. The latter are immortalized cell lines and no longer require erythropoietin for maintenance. However, activities of activin A cannot be studied separately from erythropoietin in primary cultures of human erythroid progenitors because of their continued requirement of erythropoietin for growth and maintenance. Whether activin A can exert its effects in vivo, independent of erythropoietin, awaits further studies.

The complexity of hematopoietic control is becoming apparent as a number of highly purifiable or recombinant hematopoietic growth factors become available. Many of these factors have at least two different effects: stimulation of the proliferation of progenitors and induction of differentiation in the more mature cells. Similarly, we have found that the potentiation effects of activin A on human erythropoiesis are also twofold (summarized in Fig 7). First, activin A has a direct effect on differentiation of erythroid progenitor cells by causing increase in globin mRNA and globin production. This effect might be elicited after direct interaction of activin A with its surface binding protein(s) in the erythroid progenitor cells. Secondly, it has an indirect effect on the proliferative state and DNA synthesis of erythroid progenitors and thus erythroid colony formation, which is mediated through both monocytes and T lymphocytes. In turn, these accessory cells may produce an unidentified cytokine or a combination of cytokines, whose synergistic interactions finally lead to the observed effects on the proliferation of erythroid progenitors indirectly.

The physiologic significance of activin A in hematopoietic control is thus suggested, but remains to be established. Chemical and biologic characterization of the activin A family has recently been reviewed. Activin A was initially recognized as a gonadal protein hormone that modulates follicle-stimulating hormone production by the anterior pituitary gland. Activin A, consisting of two β-subunits, has significant homology with TGF-β in primary amino acid sequence and distribution of cysteine residues. All of these findings lead to the following question: how do we envisage the control of hematopoiesis by activin A in bone marrow? By analogy to TGF-β, activin A may be considered to be a multifunctional regulator with distinct activities, depending on the cell types of target tissues. There is now evidence that marrow stromal cells can produce activin A, thus supporting some roles of activin A in hematopoiesis. Using bioassay and Western blots, we have demonstrated the presence of activin A in conditioned medium of monocytes and stromal cells, which has a potent differentiation-inducing activity toward K562 cells, and which is recognized by anti-activin A antibodies. Therefore, we propose that activin A could be produced locally in normal bone marrow and its paracrine action may fine-tune the regulation of erythropoiesis within the “microenvironment” of bone marrow.

ACKNOWLEDGMENT

We are most grateful to Dr Curt I. Civin at the Johns Hopkins Oncology Center (Baltimore, MD) for providing CD45R/Myel and CD16/Myel hybridoma supernatants. We would like to thank Judith Preston for secretarial support in the preparation of this manuscript.

REFERENCES


3. Eto Y, Tsuji T, Takezawa M, Takano S, Yokogawa Y, Shibai
H: Purification and characterization of erythroid differentiation factor (EDF) isolated from human leukemia cell line THP-1. Biochim Biophys Res Commun 142:1095, 1987


Effect of activin A on globin gene expression in purified human erythroid progenitors

L Shao, NL Jr Frigon, AL Young, AL Yu, LS Mathews, J Vaughan, W Vale and J Yu