Activin A Suppresses Proliferation of Interleukin-3–Responsive Granulocyte-Macrophage Colony-Forming Progenitors and Stimulates Proliferation and Differentiation of Interleukin-3–Responsive Erythroid Burst-Forming Progenitors in the Peripheral Blood

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We examined the effects of activin A on the proliferation and differentiation of immature hematopoietic progenitors prepared from peripheral blood (PB) using methylcellulose and liquid-suspension culture. In a kinetic analysis, colony formation by PB granulocyte-macrophage colony-forming unit (CFU-GM) was delayed in a dose-dependent manner by the addition of activin A only when stimulated with interleukin-3 (IL-3), but not when stimulated with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), or stem cell factor (SCF) plus G-CSF. DNA-synthesizing CFU-GM was increased by IL-3, but this effect was abolished by activin A. In contrast, PB erythroid burst-forming unit (BFU-E) was accelerated by the addition of activin A only when exposed to IL-3 plus erythropoietin (Epo), but not when exposed to Epo or Epo plus SCF. DNA-synthesizing BFU-E was in-creased by IL-3 and activin A, alone and additively in combination. In a mixed culture of myeloid and erythroid progenitors, activin A increased the numbers of BFU-E and CFU-Mix colonies at concentrations of 1 and 10 ng/mL and decreased the number of CFU-GM colonies in a dose-dependent manner. However, in a liquid-suspension culture of erythroid progenitors, activin A decreased total cell count and the percentage of hemoglobin-containing cells only when cells were exposed to IL-3 plus Epo. These results indicate that activin A suppresses the proliferation of IL-3–responsive CFU-GM progenitors and stimulates the proliferation and differentiation of IL-3–responsive BFU-E progenitors, and suggest that activin A acts as a commitment factor of immature hematopoietic progenitors for erythroid differentiation.
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ACTIVIN A has been shown to enhance the release of follicle-stimulating hormone from the pituitary[1,2] and to modulate the proliferation and differentiation of erythroid progenitors in vitro.[3-9] Similarly, erythroid differentiation factor (EDF) isolated from the culture supernatant of phorbol ester-treated THP-1 cells was shown to induce differentiation of mouse Friend cells and human K-562 cells into hemoglobin (Hb)-containing cells.[10-12] Subsequent molecular genetic studies showed that EDF is identical to activin A.[12] Recently, using bone marrow (BM) progenitors in a semisolid culture system, it has been shown that activin A affects differentiation of erythroid progenitors, but has no influence on myeloid progenitors.[4-6] More recently, it was shown that activin A might act as a megakaryocyte potentiator.[13]

Circulating hematopoietic progenitors in the peripheral blood (PB) are considered to be more primitive than their counterparts in the BM.[14] Previous reports showed that interleukin-3 (IL-3) is the most potent stimulator of PB erythroid burst-forming units (BFU-E) and the only factor that can directly support the proliferation of PB granulocyte-macrophage colony-forming unit (CFU-GM).[15-17]

In the present study, we examined the effect of activin A on immature progenitors that were enriched from the peripheral mononuclear pool. Our findings are that activin A suppresses the proliferation of immature myeloid progenitors, and stimulates the differentiation and proliferation of immature erythroid progenitors.

MATERIALS AND METHODS

Cell preparation. Heparinized PB samples were collected from healthy adult volunteers after obtaining informed consent. Light-density PB mononuclear cells were isolated by Ficoll (Pharmacia LKB, Uppsala, Sweden)-Conray gradient centrifugation (specific gravity 1.077) at 350g for 30 minutes. PB hematopoietic progenitor cells were enriched from the mononuclear cells by the following procedure: adherent cells were removed after a 60-minute incubation in 100-mm plastic culture dishes; the nonadherent cell fraction was incubated in a nylon wool fiber column (Wakojunyaku Co, Tokyo, Japan) at 37°C for 60 minutes[18]; cells not adherent to the nylon wool

fibers were collected and depleted of T cells by rosette formation with neuraminidase-treated sheep erythrocytes at 4°C for 60 minutes and centrifugation in a Ficoll-Conray gradient (specific gravity 1.090). This procedure gave an average recovery of 1% of the total light-density mononuclear cells. The progenitor-enriched mononuclear cells (PEMNC) contained monocytes, T cells, and B cells at less than 2%, 4%, and 2%, respectively, of total cells as determined by flow cytometry with monoclonal antibodies My4/CD14, Leu1/CD5, and B1/CD20, respectively.

This study was approved by the Institutional Review Board of the University of Tokushima.

Colony-stimulating factors (CSFs). Recombinant human (rh) erythropoietin (Epo), rh-granulocyte-CSF (G-CSF),[19] and rh–IL-3[20] were provided by Kirin Brewery Co (Tokyo, Japan). rh-granulocyte-macrophage–CSF (GM-CSF)[21] was provided by Sumitomo Pharmaceutical Co (Hyogo, Japan). rh-stem cell factor (SCF)[22] and rh–IL-6 were purchased from Genzyme Co (Cambridge, MA). Optimal concentrations of all CSF were determined experimentally.

Colony assays in methylcellulose culture (MC). Colony-forming cells were assayed in MC as described by Iscove et al[23] with minor modification. In BFU-E–derived colony formation, 1×10^4 PEMNC were plated in 35-mm tissue-culture dishes in 1 mL of Iscove's modified Dulbecco's medium (IMDM) containing 1% methylcellulose, 20% fetal calf serum (FCS), 1% bovin serum albumin (BSA; Sigma Chemical Co, St Louis, MO), 450 ng/mL iron-saturated transferrin, 2 U of Epo, and 2.5 ng of IL-3 or 5 ng of SCF. In CFU-GM–derived colony formation, 2×10^4 PEMNC were plated in dishes containing 1% methylcellulose, 20% FCS, 1% BSA, and CSFs. CSFs were used

at the following doses: 500 U of G-CSF, 10 ng of IL-6, 500 U of GM-CSF, 2.5 ng of IL-3, and 5 ng of SCF. In mixed-colony culture, 2×10^4 PEMNC were cultured with 2 U of Epo, 500 U of G-CSF, and 2.5 ng of IL-3 or 5 ng of SCF. Various concentrations of rh-activin A (supplied by Ajinomoto Co, Kanagawa, Japan) were added to the culture dishes. The dishes were incubated at 37°C in a hu-midified atmosphere under 5% CO_2 and 5% O_2. BFU-E- and CFU-GM-derived colonies were counted every 2 or 3 days from day 9 to day 17 and CFU-Mix-derived colonies were counted on day 14 of the culture by an inverted microscope. Erythroid burst, myeloid, and mixed colonies were defined as colonies containing at least 100 he-moglobinized cells, more than 40 myeloid cells, and more than 200 cells of erythroid and myeloid lineages, respectively. Three dishes were counted at every time point in the experiment. Results are pre-sented as the mean number of colonies ± SD of triplicate cultures and are representative of several separate experiments.

In vitro suicide of progenitors. PEMNC, 1×10^6/mL, were preincubated in IMDM containing 10% FCS with 2.5 ng/mL of IL-3 and/or 10 ng/mL of activin A at 37°C for 2 hours in a humidified atmosphere under 5% CO_2 in air. After the preincubation, the cells were separated into two aliquots. ^{3}H-thymidine (40 μCi/mL) was added to one aliquot and withheld from the other as a control. Cell suspensions were incubated at 37°C for 45 minutes. The cells were then washed three times with IMDM and cultured in methylcellulose. The percentage of DNA-synthesizing progenitors was calculated from the equation:

$$\text{DNA-synthesizing cells (\%)} = \frac{X - Y}{X} \times 100$$

X, the number of colonies in culture without ^{3}H-thymidine incor-poration; Y, the number of colonies in culture with ^{3}H-thymidine incorporation.

Limiting dilution assay (LDA) of progenitors. The LDA culture was performed as previously described by Takaue et al[24] with minor modifications. PEMNC were resuspended in Dulbecco's minimum essential medium (DMEM) supplemented with 2.6×10^{-2} mol/L of NaHCO$_3$, 1.8×10^{-2} mol/L of HEPES, 3×10^{-5} mol/L of hypo-xanthine, 3×10^{-6} mol/L of thymidine, 1×10^{-3} mol/L of pyruvate, 5×10^{-5} mol/L of 2-mercaptoethanol, 1×10^{-4} mol/L of MEM

Table 1. Effect of Various CSF on Colony Formation of PB Progenitors

Stimulator (/mL)	No. of Colonies	
	Day 9	Day 16
CFU-GM/2 × 10⁴ cells		
G-CSF (500 U)	6 ± 2	14 ± 1
GM-CSF (500 U)	16 ± 1	21 ± 2
IL-3 (2.5 ng)	30 ± 2	35 ± 3
IL-3 (2.5 ng) + G-CSF (500 U)	37 ± 2	43 ± 2
IL-3 (2.5 ng) + IL-6 (10 ng)	35 ± 2	40 ± 1
SCF (5 ng) + G-CSF (500 U)	24 ± 3	31 ± 3
SCF (5 ng) + IL-3 (2.5 ng)	41 ± 4	47 ± 5
BFU-E/1 × 10⁴ cells		
Epo (2 U)	5 ± 1	30 ± 3
IL-3 (2.5 ng) + EPO (2 U)	82 ± 4	141 ± 7
SCF (5 ng) + EPO (2 U)	36 ± 4	68 ± 5

PEMNC were grown at concentrations of 2×10^4/mL for CFU-GM and 1×10^4/mL for BFU-E colony formation induced by various CSF. Colonies were scored on day 9 and 16 of culture. Results are presented as the mean number of colonies ± the SD of triplicate cultures and are repre-sentative of three separate experiments.
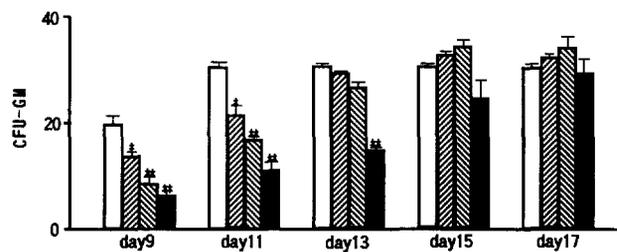

Fig 1. Effect of activin A on colony formation of IL-3–stimulated PB CFU-GM. PEMNC, 2×10^4/mL, were grown with 2.5 ng/mL of IL-3 in the absence (□) or presence of activin A at concentrations of 1 ng/mL (▨), 10 ng/mL (▩), and 100 ng/mL (■). CFU-GM colonies were counted every other day from day 9 to 17 of the culture. The mean ± the SD of triplicate determinations is shown. *P < .05; **P < .01

nonessential amino acids (GIBCO, Grand Island, NY), 15% FCS, 500 U/mL of G-CSF, and 2.5 ng/mL of IL-3 in the presence of various concentrations of activin A. The cell suspensions were seeded into 60-well microplates at cell concentrations of 50, 100, 150, 200, and 300 cells/10-μL well and incubated at 37°C in a humidified atmosphere under 5% CO_2 and 5% O_2, and the medium was replaced every other day. After 9 days of incubation, colonies were scored as positive or negative using an inverted microscope and based on a threshold number of cells (more than 40 cells) in the largest cluster in each well. The percentage of negative wells was determined at each cell dilution and the data were plotted as the log of the percentage of negative wells (Y axis) versus the number of cells plated per well (X axis). Statistical analysis was performed by chi-square minimi-zation of the relationship between the logarithmic percentage of non-responding wells and the number of cells plated.[25]

Liquid suspension culture of PB erythroid progenitors. PEMNC were resuspended in DMEM at a concentration of 1×10^5 cells/mL with 2 U/mL of Epo, 2.5 ng/mL of IL-3, and 10 ng/mL of activin A, alone or in combination. Each 1.0 mL of cell suspension was plated in a 24-well culture plate and incubated at 37°C under 5% CO_2 and 5% O_2. After 9 days of culture, the total cell number and the percentage of dianicidine-positive cells was determined.

Statistical analysis. Student's t-test was used to evaluate the sta-tistical significance of the generated data.

RESULTS

Effect of CSFs on colony formation of PB hematopoietic progenitors. Colony yields of hematopoietic progenitors in the PB were investigated using various CSFs as shown in Table 1. The ability of IL-3 to stimulate CFU-GM colony growth was superior to that of G-CSF or GM-CSF after 16 days. The number of colonies induced by IL-3 plus G-CSF was greater than that by SCF plus G-CSF and similar to that by IL-3 plus IL-6. Similarly, the number of BFU-E colonies induced by IL-3 plus Epo was greater than that by Epo alone or Epo plus SCF. Therefore, IL-3 is the most potent stimulator of colony formation of immature progenitors in the PB.

Effect of activin A on colony formation of PB CFU-GM. IL-3–stimulated CFU-GM colony formation was suppressed by activin A in a dose-dependent manner from days 9 through 11 (Fig 1). Activin A at 1, 10, and 100 ng/ mL decreased the number of colonies of IL-3–stimulated CFU-GM to 73%, 40%, and 33%, respectively, on day 9, but no significant effect on the colony formation could be ap-

preciated on days 15 and 17. Because the maximum number of CFU-GM colonies between incubation days 9 and 17 of the culture were virtually the same in the absence and presence of activin A, activin A appears to have delayed the colony growth of IL-3-stimulated CFU-GM. When 100 ng/mL of activin A was added on top of the methylcellulose culture every 3 days, the colony formation of IL-3-stimulated CFU-GM on day 16 of the culture remained to be suppressed on the level of day 11 (Table 2). However, CFU-GM colony formation was not affected by the addition of activin A when stimulated with G-CSF or GM-CSF. In the culture with IL-3 plus G-CSF, IL-3 plus IL-6, or SCF plus IL-3, activin A induced a suppressive effect on CFU-GM growth similar to that with IL-3 alone. In contrast, SCF plus G-CSF-stimulated CFU-GM colony formation was not influenced by the addition of activin A. In the LDA of CFU-GM induced by IL-3 plus G-CSF, clonal growth of progenitors with single-hit kinetics was observed (Fig 2), indicating that the colony formation of PEMNC was stimulated only by the addition of CSF and not by residual accessory cells. Activin A concentrations of 1, 10, and 50 ng/mL clearly decreased the percentage of colony-forming wells in a dose-dependent manner compared with activin A-absent cultures and abrogated clonal growth of progenitors with single-hit kinetics. These results indicate that activin A suppresses only the colony growth of CFU-GM that can respond to IL-3.

Effect of activin A on colony formation of PB BFU-E. IL-3 plus Epo-stimulated blood BFU-E colony formation on day 9 was increased by the addition of activin A at 1 and 10 ng/mL, but decreased at 100 ng/mL (Fig 3). BFU-E colony formation on day 17 was decreased by the addition of activin A at all concentrations in a dose-dependent manner. Colony size of BFU-E in activin A-containing cultures was observed to be notably smaller than in activin A-absent cultures. Colony growth of BFU-E induced by IL-3 plus Epo appeared to be accelerated by the addition of activin A. However, Epo-stimulated and SCF plus Epo-stimulated BFU-E colony formation were not influenced by the addition of activin A (Table 3). Activin A affected BFU-E colony growth only when stimulated with IL-3 plus Epo.

Effect of activin A on colony formation of progenitors in mixed culture. PEMNC were cultured with Epo, G-CSF, and IL-3 and the numbers of colonies of CFU-GM, BFU-E, and CFU-mix were counted in the same plate on day 14 (Fig 4). The number of BFU-E and CFU-mix colonies increased at 1 and 10 ng/mL of activin A but the number of CFU-GM colonies decreased in a dose-dependent manner. Total colony numbers were virtually unchanged by the addition of activin A.

Effects of activin A on DNA-synthesis in IL-3-stimulated PB progenitors. In the control culture without the administration of ^{3}H-thymidine, the number of BFU-E and CFU-GM increased to 120% and 130%, respectively, during a 2-hour preincubation with IL-3 as well as with IL-3 plus activin A. The preincubation with activin A alone had no significant effect on BFU-E and CFU-GM colony formation (data not shown).

Figure 5 shows the effect of IL-3 and activin A on DNA synthesis in progenitors by the treatment with ^{3}H-thymidine. The percentage of DNA-synthesizing BFU-E was increased

Table 2. Effect of Activin A on Colony Formation of PB CFU-GM

Stimulator (/mL)	Activin A (ng/mL)	Colonies (%)	
		Day 9	Day 16
G-CSF (500 U)	—	7 ± 2 (100)	15 ± 1 (100)
G-CSF (500 U)	10	6 ± 2 (86)	14 ± 1 (93)
GM-CSF (500 U)	—	11 ± 1 (100)	17 ± 1 (100)
GM-CSF (500 U)	10	14 ± 1 (127)	21 ± 3 (124)
IL-3 (2.5 ng)	—	30 ± 2 (100)	35 ± 3 (100)
IL-3 (2.5 ng)	1	22 ± 1 (73)*	31 ± 3 (89)
IL-3 (2.5 ng)	10	12 ± 0 (40)†	32 ± 5 (91)
IL-3 (2.5 ng)	100	10 ± 1 (33)†	30 ± 3 (86)
IL-3 (2.5 ng)	100‡	8 ± 0 (27)†	18 ± 2 (51)†
IL-3 (2.5 ng) + G-CSF (500 U)	—	34 ± 2 (100)	39 ± 5 (100)
IL-3 (2.5 ng) + G-CSF (500 U)	1	29 ± 3 (85)	35 ± 2 (90)
IL-3 (2.5 ng) + G-CSF (500 U)	10	26 ± 2 (76)*	39 ± 1 (100)
IL-3 (2.5 ng) + G-CSF (500 U)	100	19 ± 0 (56)†	33 ± 3 (85)
IL-3 (2.5 ng) + IL-6 (10 ng)	—	32 ± 2 (100)	38 ± 3 (100)
IL-3 (2.5 ng) + IL-6 (10 ng)	100	22 ± 2 (69)*	37 ± 4 (97)
SCF (5 ng) + G-CSF (500 U)	—	15 ± 1 (100)	21 ± 0 (100)
SCF (5 ng) + G-CSF (500 U)	1	18 ± 2 (120)	23 ± 2 (110)
SCF (5 ng) + G-CSF (500 U)	10	18 ± 1 (120)	22 ± 2 (105)
SCF (5 ng) + G-CSF (500 U)	100	15 ± 2 (100)	22 ± 3 (105)
SCF (5 ng) + IL-3 (2.5 ng)	—	38 ± 3 (100)	44 ± 2 (100)
SCF (5 ng) + IL-3 (2.5 ng)	100	21 ± 1 (55)†	41 ± 3 (93)

PEMNC were grown at 2 × 10⁴/mL in methylcellulose. Triplicate cultures were supplemented with various CSF in the presence or absence of increasing concentrations of activin A. Results are presented as the mean number of colonies ± the SD of triplicate cultures on days 9 and 16 and are representative of two separate experiments. Numbers within parenthesis represent the percentage of colony formation relative to control cultures without activin A. Data were analyzed statistically by Student's t-test.

* P < .05.

† P < .01.

‡ Activin A was added on top of the methylcellulose every 3 days.

by preincubation with IL-3 or activin A alone, and additively increased by preincubation with both IL-3 and activin A (Fig 5A). In contrast, DNA-synthesizing CFU-GM increased by preincubation with IL-3 alone, but not with activin A alone or with IL-3 plus activin A (Fig 5B). These results indicate that IL-3 stimulates resting BFU-E and CFU-GM to synthesize DNA and that activin A stimulates BFU-E for the DNA synthesis, yet suppresses IL-3-dependent DNA synthesis of CFU-GM.

Effect of activin A on proliferation of erythroid progenitors in liquid-suspension culture. Total cell numbers did not increase significantly when Epo or activin A was added to the culture, but marked increases were observed in all of the cultures in the presence of IL-3 (Fig 6). The addition of activin A decreased the number of dianicidine-positive cells to about one-third level in cultures of IL-3 plus Epo, but had no effect on the number of cells cultured with Epo alone. These results suggest that activin A promote the differentiation and suppress the proliferation of late erythroid progenitors, although it stimulates the proliferation of IL-3 plus Epo-responsive and immature BFU-E as indicated in methylcellulose culture.

DISCUSSION

Activin A is known to have a lineage-specific effect on erythroid progenitors,[3-6] but it has only recently been reported
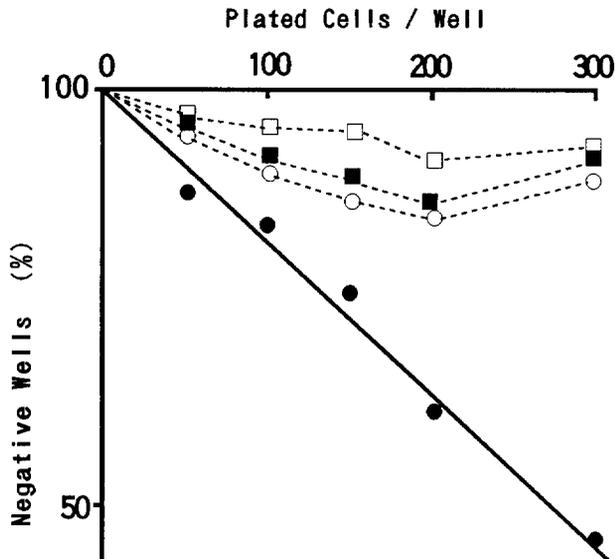
Plated Cells / Well

Fig 2. Effect of activin A on colony formation of IL-3 plus G-CSF–stimulated PB progenitors by limiting-dilution assay. PEMNC were grown with 2.5 ng/mL of IL-3 plus 500 U/mL of G-CSF in the absence (●) or presence of activin A at concentrations of 1 ng/mL (○), 10 ng/mL (■), and 50 ng/mL (□) by limiting-dilution assay. Clonal growth of progenitors with single-hit kinetics was observed with IL-3 plus G-CSF. The addition of activin A abrogated clonal growth of progenitors with single-hit kinetics and inhibited the colony formation of progenitors in a dose-dependent manner.

to act possibly as a megakaryocyte potentiator.[13] Here we showed that activin A also has a suppressive effect on the proliferation of immature myeloid progenitors. The kinetic and in vitro suicide studies showed that activin A delayed the colony growth of IL-3–responsive CFU-GM by the suppression of DNA synthesis. These findings mean that activin A inhibits the proliferation of immature myeloid progenitors by keeping the cell cycle in resting phase against the proliferation-promoting effect of IL-3. This suppressive effect was more clearly shown in liquid-suspension LDA and methylcellulose culture with delayed addition of activin A.
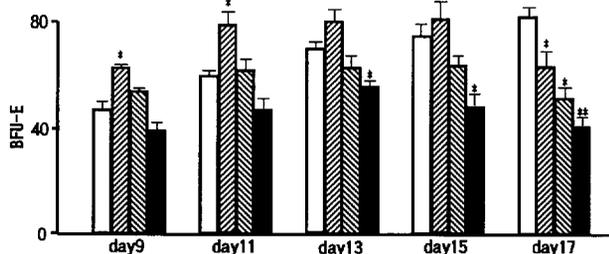

Fig 3. Effect of activin A on colony formation of IL-3 plus Epo-stimulated PB BFU-E. PEMNC, 1 × 10⁴/mL, were grown with 2.5 ng/mL of IL-3 plus 2 U/mL of Epo in the absence (□), or presence of activin A in methylcellulose at concentrations of 1 ng/mL (▨), 10 ng/mL (▩), and 100 ng/mL (■). BFU-E colonies were counted every other day from day 9 to 17. The mean of triplicate determinations is shown along with the respective SD. *P < .05, **P < .01

Table 3. Effect of Activin A on Colony Formation of PB BFU-E

Stimulator (/mL)	Activin A (ng/mL)	Colonies (%)	
		Day 9	Day 16
Epo (2 U)	—	8 ± 1 (100)	30 ± 3 (100)
Epo (2 U)	10	9 ± 1 (113)	32 ± 4 (107)
IL-3 (2.5 ng) + Epo (2 U)	—	48 ± 4 (100)	84 ± 5 (100)
IL-3 (2.5 ng) + Epo (2 U)	1	63 ± 2 (131)*	76 ± 6 (90)
IL-3 (2.5 ng) + Epo (2 U)	10	60 ± 1 (125)	67 ± 2 (80)*
IL-3 (2.5 ng) + Epo (2 U)	100	54 ± 8 (113)	54 ± 7 (64)*
SCF (5 ng) + Epo (2 U)	—	31 ± 4 (100)	50 ± 4 (100)
SCF (5 ng) + Epo (2 U)	1	31 ± 1 (100)	43 ± 2 (86)
SCF (5 ng) + Epo (2 U)	10	35 ± 2 (113)	51 ± 5 (102)
SCF (5 ng) + Epo (2 U)	100	31 ± 3 (100)	42 ± 0 (84)

PEMNC were grown at 1 × 10⁴/mL in methylcellulose. Methods and results are the same as in Table 2.
* P < .05.

In contrast, activin A accelerated the colony growth of IL-3 plus Epo-responsive BFU-E in the PB by increasing the number of DNA-synthesizing BFU-E either alone or additively with IL-3. But hemoglobin-containing cells cultured with IL-3 plus Epo were decreased by the addition of activin A in the liquid-suspension culture and the colony size of BFU-E also became smaller in methylcellulose culture. These results suggest that activin A stimulates the proliferation and differentiation of IL-3–responsive BFU-E that is predominantly present in the PB, but inhibits the proliferation of mature erythroid progenitors and promotes their differentiation to Hb-containing cells. Analysis of mixed colony cultures containing IL-3 also showed that activin A in the appropriate context increased BFU-E and CFU-Mix colonies but decreased CFU-GM colonies. CFU-GM colonies were decreased dose-dependently by the addition of activin A in the mixed culture, but not affected at concentrations of 1 and 10 ng/mL in the kinetic study on days 13 and 15. In contrast, BFU-
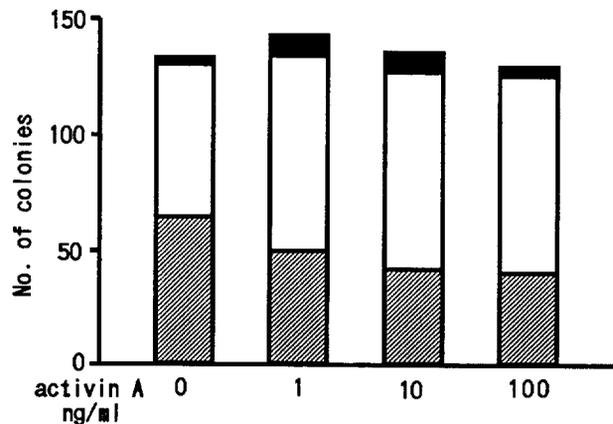

Fig 4. Effect of activin A on colony formation of PB progenitors in mixed culture. PEMNC, 2 × 10⁴/mL were grown with 2.5 ng/mL of IL-3, 2 U/mL of Epo, and 500 U/mL of G-CSF and various concentrations of activin A for 14 days. CFU-GM (▨), BFU-E (□), and CFU-Mix (■) colonies were counted within the same dishes. Results are shown as the mean of triplicate cultures.
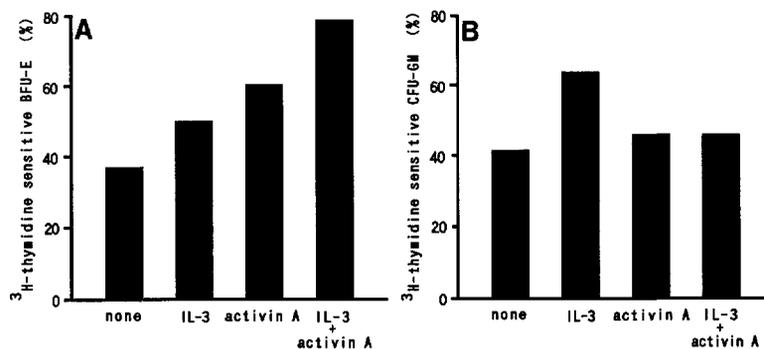
Fig 5. Effects of activin A on DNA-synthesizing hematopoietic progenitors. PEMNC were preincubated with 10 ng/mL of activin A and/or 2.5 ng/mL of IL-3. The values represent the percentage of BFU-E (A) and CFU-GM (B) that were killed by ³H-thymidine treatment.

E colonies were increased by the addition of activin A in the mixed culture, but slightly decreased in the kinetic study. IL-3, produced by T cells, but not by BM stromal cells,[26] stimulates proliferation of multipotent hematopoietic progenitors synergistically with G-CSF, IL-1 and/or IL-6.[27-29] Discrepancy between the mixed culture and the kinetic studies may be caused by the use of G-CSF in the mixed culture. The addition of G-CSF stimulates the proliferation of multipotent progenitors and then activin A may promote them to shift into erythroid progenitors with IL-3.

Several previous reports concluded that activin A could modulate directly or indirectly the proliferation of human BM erythroid and multipotential progenitors, but it could not affect BM myeloid progenitors.[3-6] We also have already reported that activin A indirectly enhances the colony formation of human BM BFU-E by stimulating adherent cells to produce soluble factors, but directly inhibits the colony formation of BM CFU-E and does not affect BM myeloid progenitors.[6] In our previous report, we could not detect the direct effect of activin A on BFU-E in the BM mononuclear cells that include many mature erythroid progenitors. In the present study, we used PB mononuclear cells depleted of adherent, T, and B cells as the source of hematopoietic progenitors to clarify the direct effects of activin A on immature
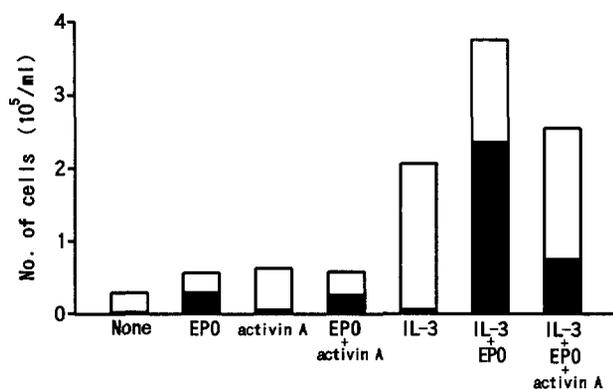

Fig 6. Effects of activin A on the proliferation of erythroid progenitors in liquid-suspension culture. PEMNC were grown with 2 U/mL of Epo, 2.5 ng/mL of IL-3, or 10 ng/mL of activin A or a combination of these agents. Closed columns (■) indicate the number of dianicidine-positive cells. The results are shown as the mean number of duplicate cultures.

progenitors, because circulating progenitors in the PB are considered to be more primitive and immature than their counterparts in the BM,[14] and IL-3 was reported to be the only factor that can directly support the proliferation of CFU-GM in the PB.[15] Discrepancy between previous reports and our present results may be mainly caused by the use of different target progenitors and different combination of CSF, especially IL-3. Shao et al[9] recently reported that activin A has little effect on both the proliferation and the DNA synthesis of CFU-E progenitors when these progenitors are depleted of accessory cells, but it directly affects the level of globin mRNAs and hemoglobins. In addition, Shiozaki et al[30] reported that the administration of activin A to anemic mice resulted in increasing erythroid progenitor level but not increased red blood cell count of PB. These reports suggest that activin A induces the differentiation of early to late erythroid progenitors, and inhibits the proliferation but promotes the differentiation of late erythroid progenitors. Moreover, several reports using the cell lines[10-12] showed that activin A induces the differentiation of K-562 and mouse Freind cells into Hb-containing cells and suppresses the proliferation of these cells. Such effects of activin A on the cell lines similarly support our in vitro effects on normal erythroid progenitors and in vivo effect on mice.

Activin A is a member of the transforming growth factor-β (TGF-β) superfamily that may exert a broad range of effects on growth, differentiation, and maturation in various cell types, including hematopoietic progenitors and embryonal cells.[31] TGF-β1 has been reported to have a potent suppressive effect on the growth of specific IL-3-responsive hematopoietic progenitors and a stimulatory effect on mature CFU-GM that respond to G-CSF and GM-CSF at low concentrations.[32-40] In our study using PB progenitors, TGF-β1 also had potent suppressive effects on immature progenitors stimulated with IL-3 and bidirectional effects on the growth of GM-CSF- and SCF plus G-CSF-stimulated myeloid progenitors, and TGF-β1 suppresses only the growth of erythroid progenitors (our unpublished data). These results suggest that TGF-β1 can act not only as an inhibitor, but also a stimulator, of hematopoiesis, and that these effects depend both on the concentration of TGF-β1 and on stage-related characteristics of progenitors. The results on activin A presented in this study also showed that concentration and stage-related effects apply to the effects of activin A on immature hematopoietic progenitors.

BM stromal cells produce various cytokines such as IL-1β, IL-6, SCF, GM-CSF, G-CSF, and M-CSF. Recent reports showed that both activin A and TGF-β1 mRNAs are detectable in BM tissue[41] and stromal cells,[26,42] and that nanogram concentrations of activin A and TGF-β1 are also found in the culture medium of stromal cells.[26,42] Moreover, Shiozaki et al[43] reported that the in vivo administration of follistatin, a specific binding protein for activin A that inhibits its activity, results in the decrease number of erythroid progenitors in mice. Hematopoiesis is positively and negatively regulated by various factors in the hematopoietic inductive microenvironment of the BM. Activin A is one of the constitutive regulators not only on erythropoiesis but also on myelopoiesis with other cytokines including IL-3 and must act as a commitment factor in erythroid differentiation of immature hematopoietic progenitors.

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