Influence of Steel Factor on Hemoglobin Synthesis in Sickle Cell Disease

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A new hematopoietic growth factor (Steel factor) has been identified which stimulates erythroid proliferation both in vitro and in vivo. We evaluated the influence of recombinant Steel factor on hemoglobin synthesis in peripheral blood (PB) BFU-E–derived cells from normal donors by radioimmunoassay (RIA) and compared it with stimulation with GM-CSF and interleukin-3 (IL-3). Only Steel factor stimulated a significant increase in BFU-E–derived colony size and a significant increase in fetal hemoglobin (HbF) in BFU-E–derived erythroblasts from 0.49% ± 0.27% to 6.33% ± 1.11% in serum-deprived media and from 1.88% ± 0.24% to 11.17% ± 0.91% in serum. To determine whether this influence on hemoglobinization also occurred in sickle cell disease, we studied 13 patients with sickle cell disease. In serum-deprived conditions, there was a significant increase in the number and size of BFU-E–derived colonies with Steel factor that was dose-dependent. In addition, the proportion of HbF in progenitor-derived cells increased by 66% from 4.1% ± 0.6% to 6.8% ± 1.2% with Steel factor. In serum-containing conditions studied in 12 patients, the increase in percentage of HbF was even greater, from 10.7% ± 0.9% in control cultures to 22.5% ± 2.6% with Steel factor. These increases in percentage of HbF were significant and dose-dependent. An increase in percentage of HbF was observed in erythroblasts harvested on day 11, 14, and 18 of culture. A decrease in mean picograms of total Hb per cell after coculture with Steel factor was noted, suggesting that growth kinetics influenced complete hemoglobinization. In serum-deprived conditions, picograms of HbF per cell was not affected by Steel factor, and in serum-containing conditions that augment in vitro HbF production it was enhanced. Thus, Steel factor stimulated a significant increase in percentage of HbF in erythroid cells from normal donors and patients with SCA in vitro.

DURING MAMMALIAN development, the genes in the β-globin gene cluster are sequentially expressed in a stage-specific manner.[1] Because the β-globin genes are reciprocally expressed with γ globin, the severe β hemoglobinopathies, including sickle cell anemia (SCA), could be clinically ameliorated if adequate production of γ globin could be maintained to perform the function of the abnormal β-globin genes.[1] Determination of physiologic and environmental factors that interact to regulate γ- or β-globin chain synthesis is important for the design of strategies to alter globin chain production. Expression of γ-globin genes can be manipulated in vivo and in vitro with 5-azacytidine,[2] hydroxyurea,[3-5] recombinant erythropoietin (Epo),[6] and sodium butyrate.[7-9] However, potential problems of bone marrow (BM) toxicity and teratogenicity, especially in pediatric patients, complicate the use of some of these agents. The hematopoietic growth factor GM-CSF has been reported to induce an increase in fetal hemoglobin (HbF) synthesis in serum-free conditions up to the level observed in serum-containing cultures,[10] although Migliaccio et al could not demonstrate an influence of GM-CSF or interleukin-3 (IL-3) on fetal globin synthesis in serum-deprived cultures.[11] In addition, the presence of adherent cells,[12] activin,[13] and fetal calf serum (FCS)[10,11,14,15] can enhance HbF synthesis in culture, whereas γ-interferon (γ-IFN)[16] and a factor or factors in fetal sheep serum[17] inhibits synthesis of HbF relative to HbA.

Recently, a novel hematopoietic growth factor alternatively called mast cell growth factor (MGF), stem cell factor (SCF), and *kit* ligand (KL), and referred to here as Steel factor, was identified.[18-20] When combined with other growth factors in culture, Steel factor significantly increases both the number and size of colonies when rodent or human BM is stimulated with each factor alone.[19,21-22] Steel factor is the protein product of the murine Steel locus,[20,23,24] mutations in which lead to several developmental abnormalities, including severe macrocytic anemia.[25] Based on the phenotype of Steel mutant mice[25] and effects of Steel factor in culture,[19,21-22] this new protein appears to be a potent hematopoietic growth factor that plays a normal role in regulating growth and development of early hematopoietic stem cells. We analyzed the ability of Steel factor to stimulate HbF production in progenitor-derived cells from normal individuals and from patients with sickle cell disease. We show here that Steel factor induces a dramatic increase in the size of erythroid colonies (BFU-E) and a significant increase in percentage of HbF.

MATERIALS AND METHODS

Peripheral blood (PB) samples. Blood samples were obtained from normal donors at The Milton S. Hershey Medical Center and from patients with sickle cell disease followed in the hematology clinic of the Hospital for Sick Children (Toronto, Canada) under protocols approved by both institutions' Human Subject's Review Committee. SCA patients were not studied if they had undergone a blood transfusion in the previous 3 months or had experienced a sickle cell crisis or active infection in the 7 days before assay. All

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Table 1. Influence of Steel Factor on BFU-E Proliferation and Hemoglobin Accumulation in Normal Donors

Condition	No. of BFU-E–Derived Colonies	No. of Cells × 10^4/ BFU-E–Derived Colony	Total Hb (pg/cell)	HbF (pg/cell)	HbF (%)
Serum-deprived					
C	84 ± 38	1.3 ± 0.2	29.8 ± 1.1	0.14 ± 0.08	0.49 ± 0.27
SI	128 ± 34	9.4 ± 1.5*	8.8 ± 2.5*	0.50 ± 0.07*	6.33 ± 1.11*
GM-CSF	125 ± 34	1.8 ± 0.4	28.6 ± 1.7	0.21 ± 0.09	0.75 ± 0.33
IL-3	199 ± 53	1.8 ± 0.3	32.3 ± 2.2	0.23 ± 0.07	0.68 ± 0.18
Serum					
C	69 ± 3	1.2 ± 0.3	25.1 ± 4.4	0.49 ± 0.14	1.88 ± 0.24
SI	118 ± 6*	8.3 ± 0.5*	13.7 ± 0.9	1.53 ± 0.17*	11.17 ± 0.91*
GM-CSF	130 ± 17	1.5 ± 0.3	28.4 ± 2.9	0.93 ± 0.23	3.33 ± 0.88
IL-3	239 ± 53	1.5 ± 0.6	36.0 ± 3.2	0.91 ± 0.40	2.78 ± 1.48

Mean number of BFU-E ± SEM per 1 × 10^5 partially purified PBMNC plated with IMDM (C), 100 ng/mL Steel factor (SI), 100 ng/mL GM-CSF or 100 ng/mL IL-3 in media containing serum or in serum-deprived media. Mean number of cells × 10^4 ± SEM per BFU-E–derived colony harvested on day 14 of culture. Percentage of HbF ± SEM calculated from measurement of mean picograms of total Hb and HbF/cell by radioligand assay. Three donors were studied.

*Significant change from control ($P < .05$).

SCA patients were aged more than 3 years. Samples were drawn in 10% acid-citrate-dextrose or heparin and shipped at 4°C. PB HbF was measured by alkali denaturation. All cultures were performed within 30 hours.

Preparation of BFU-E–derived cells. PB mononuclear cells (MNC) were separated on Ficoll-Paque (Pharmacia, Piscataway, NJ). In cultures of blood from patients with sickle cell disease, no further enrichment was performed owing to the small quantity of blood available. Adult blood BFU-E from normal donors was partially purified by 2-aminoethylisothio-uronium bromide hydrobromide (AET)-treated sheep RBC rosetting and adherence to plastic. For serum-containing cultures, MNC were cultured in 0.9% methylcellulose media containing 30% FCS, 9.0 mg/mL deionized bovine serum albumin (BSA) (Cohn fraction V, Sigma, St Louis, MO), 1.4 × 10^{-4} mol/L β-mercaptoethanol, 2 U/mL Epo (recombinant Epo > 10,000 U/mg, Amgen, Thousand Oaks, CA), 0 to 100 ng/mL recombinant murine Steel factor,[26] 0 to 100 ng/mL recombinant human GM-CSF (Immunex, Seattle, WA), or 0 to 100 ng/mL recombinant human IL-3 (Immunex). Doses were on plateau for maximal stimulation of colony growth.

Serum-derived cultures were prepared with 1% deionized BSA 300 μg/mL iron-saturated transferrin (Boehringer-Mannheim, Indianapolis, IN), 10 μg/mL insulin (Sigma), 10^{-4} mol/L β-mercaptoethanol, 2 × 10^{-4} mol/L BSA-absorbed cholesterol (Sigma), Iscove's modified Dulbecco's medium (IMDM), and 0.9% methyl cellulose with 2 U/mL Epo and 0 to 100 ng/mL Steel as described previously.[27] Because BSA may be contaminated with trace levels of serum proteins, the medium is termed serum-deprived.[27]

Cells were plated at 1.5 × 10^4 (partially purified normal donor) or at 1 to 2 × 10^5 cells/mL (SCA), and cultures were incubated in humidifed 4% CO_2 at 37°C. Erythroid colonies were then counted and harvested at different days of culture (day 11, 14, or 18).

Analysis of Hb production by radioligand immunoassay. Thirty to 50 BFU-E–derived colonies were harvested and pooled, and the average number of erythroid cells per colony was determined. When samples were plucked in duplicate, the number of cells per colony differed from the average count by ±5% to 20%. A radioligand assay of total Hb or HbF in picograms per BFU-E–derived cell was performed as previously described.[28-30] Adult and HbF standards were prepared from adult blood, cord blood, or sheep blood samples by CCl_4 lysis of washed cells. The adult and cord blood samples were used to construct standard curves and were diluted with 0.5 mg/mL sheep Hb. Sonicated cell extracts derived from erythroid colonies were diluted with 0.5 mg/mL sheep Hb. Linear standard curves were consistently obtained, and the Hb in 2,000 to 5,000 cells was routinely assayed. Reproducibil-

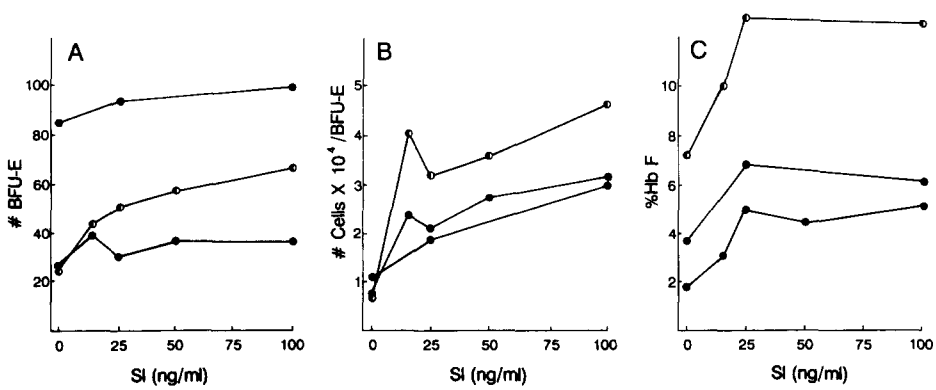

Fig 1. Dose-response curve after stimulation of BFU-E from three patients with Steel factor in serum-deprived conditions. (A) Number of PB BFU-E–derived colonies per 2 × 10^5 cells plated with 0, 12.5, 25, 50, or 100 ng/mL Steel factor. (B) Number of cells × 10^4 per BFU-E–derived colony cultured with 0 to 100 ng/mL Steel factor. (C) Percentage of HbF in BFU-E–derived cells cultured with 0 to 100 ng/mL Steel factor. Two patients with SCA (-●-) and one patient with sickle cell/β thalassemia (-◐-) were studied. Hb content was measured by radioligand assay.

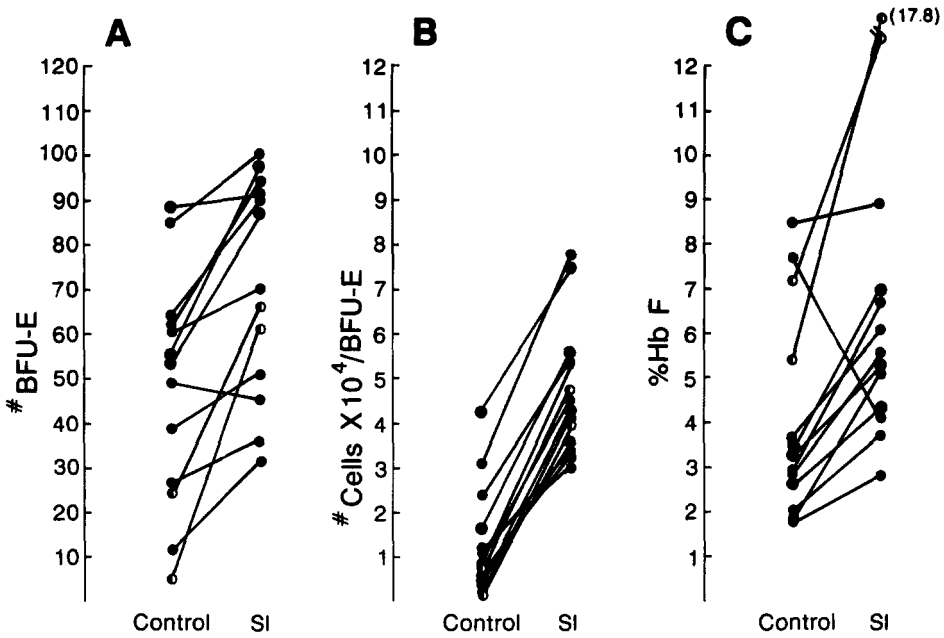

Fig 2. Influence of Steel factor on BFU-E proliferation and HbF levels in sickle cell disease in serum-deprived conditions. (A) Mean number of PB BFU-E–derived colonies per 2 × 10^5 cells plated with 0, 50 (one patient), or 100 ng/mL Steel ($P < .001$). (B) Mean number of cells × 10^4 per BFU-E–derived colony cultured with or without Steel ($P < .001$). (C) Percentage of HbF in BFU-E–derived cells cocultured with or without Steel factor ($P < .02$). Eleven patients with SCA (-●-) and two patients with sickle cell/thalassemia (-◐-) were studied. Hb content was measured by radioligand assay.

ity of this assay has been described previously,[29] but duplicate dishes performed on samples from one individual generally yielded results within 10% of the mean. The potential for HbF production in a population of progenitor-derived cells represents the product of the two independent mechanisms that regulate the circulating HbF level: the HbF produced per F cell and the number of F cells produced per progenitor cell. The latter cannot be accurately measured owing to assay insensitivity that prevents detection of small amounts of HbF in single cells. Therefore, for the purposes of this assay, we assume that all erythroblasts in progenitor-derived erythroid colonies produced in vitro are F cells. The HbF program estimated by this in vitro assay is of total F production, without loss of any population due to selective survival.

Analysis of globin synthetic ratios and Hb accumulation in BFU-E–derived erythroblasts by urea-Triton X-100 gel. Globin biosynthesis was assayed in erythroblasts harvested on day 13 of culture and labeled overnight with 25 μCi ^{3}H-leucine in leucine-free minimal essential media, 10% FCS, with and without 100 ng/mL Steel factor. Cell lysates were electrophoresed on acid-Triton-urea gels, which were then autoradiographed. Globin chain synthesis was quantitated by laser densitometry and computer analysis as previously described.[16]

RESULTS

Influence of recombinant Steel factor on Hb accumulation in normal donor BFU-E–derived cells. The influence of Steel factor on Hb synthesis was studied in serum-deprived or serum-containing conditions using partially purified peripheral blood BFU-E from three normal donors. Coculture with Steel factor was compared with stimulation with IMDM, GM-CSF, or IL-3. Serum-derived culture conditions were studied to determine the influence of Steel factor with low background of HbF expression.[11,14] Concentrations of factors were selected for plateau stimulation of colony growth (described below). Results are shown in Table 1. Enhancement of colony number was noted for all factors studied but did not reach statistical significance. The number of cells per BFU-E–derived colony was significantly stimulated by Steel factor under both culture conditions. In addition, the percentage of HbF per BFU-E–derived cell was significantly stimulated above control only in the presence of Steel factor in both serum-containing and serum-deprived media. This resulted from a significant increase in picograms of HbF per cell as well as a decrease in picograms of total Hb accumulation.

Influence of recombinant Steel factor on Hb accumulation in sickle cell disease in serum-deprived media. The influence of Steel factor on Hb synthesis was then examined in sickle cell disease to determine whether a similar effect occurred. The optimal concentration of Steel factor was established by plating PB progenitor cells from three sickle cell patients in semisolid culture conditions with varying concentrations

Table 2. Influence of Steel Factor on BFU-E Proliferation and Hb Accumulation in Sickle Cell Disease

Condition	No. of Patients	No. of BFU-E–Derived Colonies		No. of Cells × 10^4/BFU-E–Derived Colony		HbF (%)	
		C	SI	C	SI	C	SI
Serum-deprived	13	48 ± 7	71 ± 7*	1.3 ± 0.03	4.7 ± 0.4*	4.1 ± 0.6	6.8 ± 1.2*
Serum	12	48 ± 8	53 ± 9	2.5 ± 0.5	5.9 ± 0.9*	10.7 ± 0.9	22.5 ± 2.6*

Mean number of BFU-E ± SEM per 2 × 10^5 cells plated with 0 (C, control) or 100 ng/mL Steel factor (SI) in media containing serum or in serum-deprived media. One patient's cells were treated with 50 ng/mL Steel factor. Mean number of cells × 10^4 ± SEM per BFU-E–derived colony. Percentage of HbF ± SEM calculated from measurement of mean picograms of total Hb and HbF/cell by radioligand assay.

*Significant increase above control analyzed by paired t test ($P < .05$).

Table 3. Radioligand Assay of Hb Produced by Erythroid Progenitors in Serum-Deprived Media

Patient	Total Hb (pg/cell)	HbF (pg/cell)	HbF (%)	PB HbF (%)
1				
C	35.3 ± 0.2	0.63 ± 0.14	1.8	9.6
SI	20.3 ± 0.2	0.56 ± 0.02	2.8	
2				
C	25.2 ± 0.6	0.46 ± 0.05	1.8	30.4
SI	28.0 ± 0.5	1.44 ± 0.08	5.1	
3				
C	29.0 ± 0.6	0.57 ± 0.08	2.0	3.6
SI	11.9 ± 0.3	0.44 ± 0.17	3.7	
4				
C	20.1 ± 0.2	0.51 ± 0.02	2.5	5.9
SI	12.7 ± 0.6	0.48	3.8	
5				
C	31.3 ± 1.0	0.89 ± 0.04	2.8	18.9
SI	19.5 ± 0.2	1.09 ± 0.02	5.6	
6				
C	38.4 ± 0.6	1.10 ± 0.03	2.9	4.3
SI	29.1 ± 0.6	1.94	6.7	
7				
C	31.3 ± 2.1	0.96 ± 0.12	3.1	2.8
SI	14.6 ± 0.4	0.70 ± 0.17	4.8	
8				
C	37.4 ± 0.1	1.32 ± 0.19	3.5	23.9
SI	23.0 ± 0.4	1.57 ± 0.13	6.8	
9				
C	16.5 ± 0.6	0.61 ± 0.08	3.7	13.7
SI	9.5 ± 0.1	0.58 ± 0.14	6.1	
10				
C*	16.4 ± 1.1	0.89 ± 0.21	5.4	22.8
SI	14.4 ± 0.4	2.57 ± 0.01	17.8	
11				
C*	26.6 ± 0.5	1.93 ± 0.20	7.2	22.6
SI	20.1 ± 0.1	2.53 ± 0.05	12.6	
12				
C	30.2 ± 0.4	2.34 ± 0.08	7.7	43.6
SI	11.1 ± 0.2	0.45 ± 0.02	4.1	
13				
C	35.2 ± 0.1	3.03 ± 0.04	8.6	42.0
SI	14.7 ± 0.1	1.31 ± 0.01	8.9	
Mean of 13 patients				
C	28.7 ± 2.1	1.17 ± 0.22	4.1 ± 0.6	
SI	17.6 ± 1.8†	1.20 ± 0.21	6.8 ± 1.2†	

Mean picograms ± SEM total Hb and HbF/BFU-E–derived cells measured by radioligand assay after culture of BFU-E from sickle cell disease patients for 14 days in serum-deprived media with or without Steel factor (SI, 50 or 100 ng/mL). Percentage of HbF was calculated. PB HbF levels [PB HbF (%)] were measured by alkali denaturation.

*Patient with HbS/β thalassemia.

†Significant change from control ($P < .05$).

of Steel protein (0, 12.5, 25, 50, or 100 ng/mL). Serum-deprived culture conditions were again studied to determine the influence of Steel factor with a low background of HbF expression.[11,14] Steel factor induced a marked increase in the size of BFU-E–derived colonies and in the percentage of HbF measured by radioligand assay, which plateaued at concentrations of 25 to 50 ng/mL (Fig 1).

PB samples from 13 patients with sickle cell disease were then cultured in serum-deprived methylcellulose media with Epo 2 U/mL with or without Steel factor 50 or 100 ng/mL for 14 days. Results are shown in Fig 2 and Tables 2 and 3. Culture with Steel factor caused a significant increase in the number of BFU-E–derived colonies and in the number of cells per BFU-E–derived colony (fourfold) (Fig 2, Table 2). Percentage of HbF significantly increased by 66%, from a mean of 4.1% ± 0.6% without Steel factor to 6.8% ± 1.2% with Steel factor (Fig 2, Table 2), owing to a significant decrease in total Hb (from a mean of 28.7 ± 2.1 to 17.6 ± 1.8 pg/cell) after stimulation with Steel factor, a decrease noted in 12 of 13 patients studied (Table 3). This decrease may reflect perturbation in total hemoglobinization associated with increased cell division. HbF content was not significantly affected, with a mean of 1.2 pg/cell in both control and Steel-stimulated cultures (Table 3), indicating a decrease in HbS production.

PB percentage of HbF for the 13 patients is shown in Table 3. Higher PB percentage of HbF correlated with higher BFU-E–derived control erythroblast HbF content ($r = .74$ by simple correlation), but not with the percentage increase in HbF in vitro in response to Steel factor ($r < .1$).

Influence of recombinant Steel factor in Hb accumulation in sickle cell disease in serum-containing media. Because in vitro activation of HbF is diminished in serum-deprived conditions,[11,14] the influence of Steel factor on Hb synthesis in the presence of serum was determined. Twelve patients with sickle cell disease were studied in serum-containing conditions. There was a significant increase in the number of cells per BFU-E–derived colony in response to culture with Steel factor (Fig 3, Table 2).Coculture with Steel factor also resulted in a significant increase in percentage of HbF from a mean of 10.7% ± 0.9% without Steel factor to 22.5% ± 2.6% with Steel factor (Fig 3, Table 2). Total Hb content was again decreased after Steel stimulation. Picograms of HbF per cell were significantly increased (Table 4). Baseline percentage of HbF and percentage of HbF stimulated by Steel factor were significantly greater than their respective values measured in serum-deprived conditions ($P < .01$).

Influence of recombinant Steel factor on Hb accumulation on different days of culture. To determine the influence of maturation on Hb accumulation, BFU-E–derived cells from patients with sickle cell disease were cultured in serum-containing media with or without Steel factor and harvested on day 11, 14, or 18 of culture. Results are shown in Fig 4. Number of BFU-E and colony size did not increase significantly during the culture period examined, although the number of cells per colony was greater with Steel factor than in control cultures on days 11 and 14 of culture. Both control cultures and those treated with Steel factor displayed a significant increase in total Hb accumulation from day 11 to day 18. Cultures stimulated with Steel factor had a mean of 6.2 pg/cell less Hb than control cultures at day 11; this difference persisted, with a mean of 6.6 pg/cell less than control at day 18. Picograms of HbF per cell in Steel-stimulated cultures were greater than control, with a significant difference noted on day 14. Significant increases in percentage of HbF were noted at all time points examined.

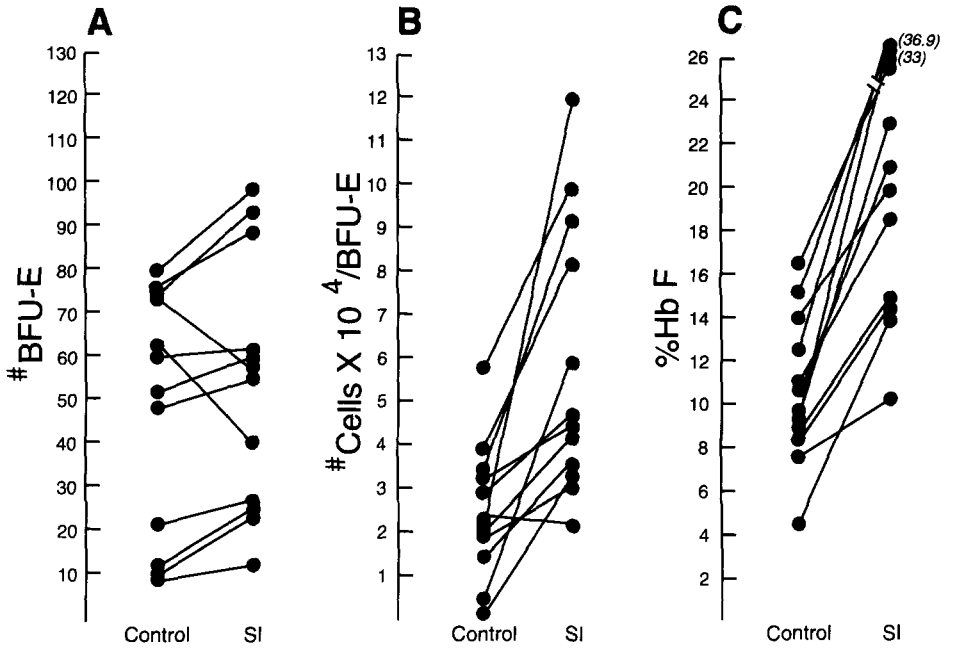

Fig 3. Influence of Steel factor on BFU-E proliferation and HbF levels in sickle cell disease in serum-containing media. (A) Mean number of PB BFU-E derived colonies per 2 × 10^5 cells plated with 0, 50 (one patient), or 100 ng/mL Steel factor (P = NS). (B) Mean number of cells × 10^4 per BFU-E–derived colony cultured with or without Steel factor (P < .01). (C) Percentage of HbF in BFU-E–derived cells cocultured with or without Steel factor (P < .001). Twelve patients with sickle cell disease were studied.

Effect of Steel factor on globin synthesis measured by ^{3}H-leucine incorporation. To determine whether an increase in HbF synthesis was also measured with ^{3}H-leucine incorporation, BFU-E from two patients with sickle cell disease were cocultured with or without Steel factor. Synthetic measurements performed on day 13 of culture showed a mean increase of 16.1% γ-globin in erythroblasts continuously exposed to Steel factor.

To determine how rapidly Steel factor might have this effect, BFU-E–derived erythroblasts from a third patient with sickle cell disease were removed from culture on day 13 and incubated without (control) or with Steel factor (100 ng/mL) only during the 16-hour overnight ^{3}H-leucine labeling. Results are shown in Fig 5. Addition of Steel factor produced a 25.7% increase in γ-globin synthesis within 16 hours: from 17.6% in control erythroblasts to 43.3% in Steel-stimulated cells. Both Gγ- and Aγ-globin expression was stimulated, as was observed in the other patients. Measurements of Hb accumulation in the same patient in cultures that had been incubated with or without Steel factor during 13 days of culture showed an increase in γ-globin from 14.0% to 23.3%.

DISCUSSION

The mechanisms through which HbF synthesis is regulated in adult life have been explored extensively. Environmental influences on HbF synthesis which have been identified include factors secreted by adherent cells,[12] sodium butyrate,[7-9] and GM-CSF,[10] which enhance HbF synthesis, and γ-IFN and a factor in fetal sheep serum,[17] which decrease it. Steel factor is a naturally occurring, novel hematopoietic growth factor recently cloned and purified and shown to be the protein product of the Steel locus in the mouse.[20,23,24] We demonstrated that Steel factor increased the percentage of HbF in the erythroid progenitors of normal donors and of patients with sickle cell disease.

In normal donors, picograms of HbF per cell and percentage of HbF were significantly stimulated in cultures with Steel factor. Although picograms of HbF per cell and percentage of HbF were increased somewhat by stimulation by GM-CSF and IL-3, results did not reach statistical significance. This result may differ from those previously reported[10,11] owing to different culture conditions or progenitor purity; the result might have reached significance with a much larger sample size.

The observation that Steel factor stimulates BFU-E–derived colony size and influences globin gene expression in a manner enhancing accumulation of γ and decreasing accumulation of β chains was confirmed in sickle cell disease as well. This observation is consistent with the report that treatment of baboons with Steel factor resulted in increased hematocrit but decreased mean corpuscular volume and Hb.[31,32] Although total Hb per cell decreased, mean HbF content per cell was not changed in serum-deprived conditions and increased in serum-containing conditions that promote γ-chain expression.[11,14] The mechanism of this effect is not yet clear. The period of incubation of colonies with Steel factor was extended to allow full maturation of erythroblasts within colonies (Fig 4). At all time points examined, a significant increase in percentage of HbF per erythroblast was noted. On day 11 of culture, erythroid colonies stimulated with Steel factor were larger but cells contained less Hb, a difference that persisted to day 18 of culture. The influence of Steel factor on cell proliferation and the assymetry in β- and γ-globin gene expression that occurs during RBC differentiation results in an early decrease in hemoglobinization which is not compensated for during later development. As suggested by the rapid effect on γ-synthesis (Fig 5), Steel factor, like 5-azaytidine, may also act by altering expression of the γ and β genes at the level of transcription.[33] Alternatively, Steel factor may promote growth and differentiation of progeni-

Table 4. Radioligand Assay of Hb Produced by Erythroid Progenitors in Serum-Containing Media

Patient	Total Hb (pg/cell)	HbF (pg/cell)	HbF (%)
1			
C	26.9 ± 0.2	1.23 ± 0.01	4.6
SI	19.5 ± 0.3	2.84 ± 0.04	14.6
2			
C	27.6 ± 0.0	2.19 ± 0.07	7.9
SI	22.4 ± 0.3	2.38 ± 0.07	10.6
3			
C	27.0 ± 0.2	2.30 ± 0.10	8.5
SI	12.5 ± 0.7	1.80 ± 0.10	14.4
4			
C	25.7 ± 0.8	2.26 ± 0.19	8.8
SI	17.5 ± 0.3	2.56 ± 0.13	14.6
5			
C	15.3 ± 0.6	1.42 ± 0.29	9.3
SI	11.6 ± 0.2	4.28 ± 0.35	36.9
6			
C	18.4	1.78 ± 0.10	9.7
SI	19.5 ± 1.7	4.52 ± 0.32	23.2
7			
C	21.5 ± 0.1	2.39 ± 0.18	11.1
SI	21.5 ± 0.8	3.99 ± 0.10	18.6
8			
C	21.5 ± 6.0	2.44 ± 0.46	11.4
SI	26.8 ± 0.4	5.64 ± 0.05	21.0
9			
C	21.6 ± 2.0	2.63 ± 0.13	12.2
SI	21.7 ± 4.0	7.25 ± 0.29	33.4
10			
C	22.3 ± 0.4	3.12 ± 0.01	14.0
SI	24.9 ± 0.4	5.07 ± 0.15	20.4
11			
C	27.0 ± 0.1	3.90 ± 0.3	14.4
SI	13.5 ± 0.6	4.80 ± 0.4	35.6
12			
C	21.7 ± 0.4	3.56 ± 0.37	16.4
SI	14.5 ± 0.2	3.84 ± 0.00	26.5
Mean of 12 patients			
C	23.0 ± 1.1	2.43 ± 0.23	10.7 ± 0.9
SI	18.8 ± 1.4*	4.08 ± 0.45*	22.5 ± 2.6*

Mean picograms ± SEM total Hb and HbF/BFU-E–derived cell measured by radioligand assay after culture of BFU-E from 12 sickle cell disease patients for 14 days in serum-containing media with 0, 50, or 100 ng/mL Steel factor. Percentage of HbF was calculated.

*Significant change from control by paired *t* test ($P < .05$).

tor cells that normally are present at low levels in vivo and are unable to grow in culture except in the presence of Steel factor.

Recent experiments by Papayannopoulou et al demonstrated the high frequency of c-*kit* receptor on human erythroid BFU-E, strongly supporting a direct mechanism for the effect of Steel factor.[34] Our observation of the effect of Steel factor on hemoglobinization of normal partially purified human blood erythroid progenitors is consistent with this data.

The increase in HbF synthesis relative to HbS was significantly greater in serum-containing cultures as com-

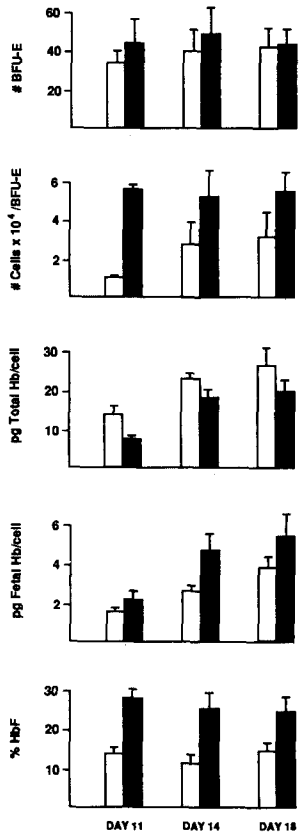

Fig 4. Effect of day of erythroblast maturation on Hb accumulation. Mean number of BFU-E ± SEM per 2 × 10^5 cells, plated with 0 (open bar) or 100 ng/mL Steel factor (solid bar) in media containing serum. Mean number of cells × 10^4 ± SEM per BFU-E–derived colony, mean picograms of total Hb and HbF per cell measured by radioligand assay and percentage of HbF per cell calculated from picogram measurements for colonies stimulated with and without Steel factor are also shown. Colonies were harvested at day 11, 14, or 18 after culture. Five patients with sickle cell disease were studied.

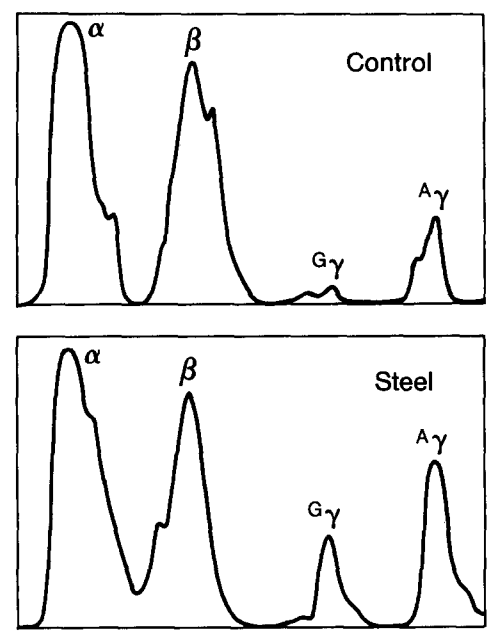

Fig 5. Densitometry scans of autoradiographs of radiolabeled globin chains from control and Steel-treated BFU-E–derived cells harvested on day 13 of culture from a patient with SCA. Addition of Steel factor only during overnight ^{3}H-leucine labeling resulted in a 25% increase in γ-globin synthesis. Both Gγ- and Aγ-globin synthesis were increased.

pared with serum-deprived cultures. Thus, two independent factors, FCS and Steel factor, both enhanced HbF levels. Other investigators have speculated whether FCS contains a HbF inducer or whether a negative regulator exists in serum-deprived conditions. They concluded that enhancement of erythroid maturation in serum-deprived conditions lowering the γ- and β-globin chain ratios is an unlikely explanation,[10,11] a concept supported by our data on colony size and number.

Clinical and experimental data demonstrate that increasing HbF to a critical level is of benefit in inhibiting polymerization of HbS.[35] In naturally occurring populations of SCA, levels of HbF greater than about 20% appear to ameliorate the clinical severity of disease.[36,37] Stimulation of HbF production by various mechanisms, using a number of chemotherapeutic agents such as 5-azacytidine and hydroxyurea, has therefore been attempted with moderate success,[2,38-40] but concerns regarding teratogenicity and carcinogenicity of such agents preclude their widespread use for this indication. A physiologic agent capable of stimulating HbF production would therefore be useful in treatment of sickle cell disease.

Whether Steel factor has the ability to stimulate HbF synthesis in vivo in SCA is an important question. By decreasing β-chain production while maintaining HbF, Steel factor may have therapeutic utility. In vitro, the magnitude of response in HbF levels to Steel factor is greatly influenced by environmental conditions, specifically by a factor in FCS to enhance HbF synthesis. However, even in serum-deprived conditions, the percentage of increase in HbF was more than 60% above control. Because Steel factor has a potent effect on erythroid proliferation, any enhancement in HbF to HbS ratios in vivo might be balanced by an increased RBC mass and viscosity which would adversely affect patients with SCA. Conversely, decreased mean corpuscular volume and Hb[31] together with the increase in percentage of HbF, might effectively compensate for the increased RBC mass. Another concern is that other cell types that express c-*kit*, the receptor for Steel factor, might be stimulated.[19,21-33,41,42] Nevertheless, Steel factor, in combination with other stimuli such as the unidentified serum factor(s), has the potential to increase the percentage of HbF significantly.

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