original paper
Haematologica 1995; 80:5-12

INTERLEUKIN-11 (IL-11) AND IL-9 COUNTERACT THE INHIBITORY ACTIVITY OF TRANSFORMING GROWTH FACTOR β3 (TGF-β3) ON HUMAN PRIMITIVE HEMATOPOIETIC PROGENITOR CELLS

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ABSTRACT

Background. **TGF-β3 has been proven to be a potent suppressor of human hematopoietic progenitor cells and its effects on hematopoiesis are only inhibitory.**

Methods. **In this paper we investigated the antiproliferative activity of TGF-β3 on highly purified bone marrow (BM) CD34$^+$ cells and more immature CD34$^+$/4-hydroperoxycyclophosphamide (4-HC) resistant cells. Primitive hematopoietic progenitors were stimulated by early acting stimulatory factors such as SCF, IL-11, IL-9 and the intermediate-late acting growth factors IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), alone and in combination.**

Results. **The addition of TGF-β3 to cultures of CD34$^+$ cells containing IL-11, IL-9 or SCF alone resulted in 86% or more inhibition of total colony formation. Conversely, IL-3 and GM-CSF-stimulated colony growth was inhibited by 57% and 58%, respectively ($p < 0.02$). IL-11 and IL-9 acted synergistically or additively with IL-3 and GM-CSF on the clonogenic growth of BFU-E derived from CD34$^+$ cells, in both the presence and absence of TGF-β3. Co-incubation of CD34$^+$ cells with 2 synergistic factors (e.g. IL-11 and SCF or IL-9 and SCF), with or without TGF-β3, resulted in the enhancement of both CFU-GM and BFU-E growth. The percentage of CD34$^+$ cells inhibited by TGF-β3 was significantly reduced when IL-11 or IL-9, but not SCF, was added to the other cytokines (e.g. IL-11 and IL-3-stimulated cultures were inhibited by 42%, compared to 57% and 90% for the CSF alone; $p< 0.05$). Similarly, the addition of IL-11 or IL-9 to SCF decreased the suppressive activity of TGF-β3 (e.g. IL-11 and SCF in combination were inhibited by 52.4%, compared to 90% or more when the same cytokines were used separately; $p< 0.001$). These effects were mainly observed on CD34$^+$-derived BFU-E although IL-9 appeared to override TGFβ-3 on both CFU-GM and BFU-E. When tested on CD34$^+$/4-HC resistant progenitors, IL-11, IL-9 and SCF increased the number of clonogenic cells responsive to IL-3 and GM-CSF. However, TGF-β3 demonstrated a greater inhibitory activity on earlier cells than on the more mature CD34$^+$ cell fraction, and none of the study cytokines completely abrogated the activity of TGF-β3.**

Conclusions. **These data confirm that TGF-β3 exerts its suppressive effect on hematopoietic progenitor cells according to the differentiation state of the target cells and the presence of other cytokines interacting with the cells. The permissive growth factors IL-11 and IL-9 seem to be able to partially counteract the negative regulation of TGF-β3.**

Key words: IL-11, IL-9, SCF, TGF-ß3, stem cells

The TGF-β family of proteins has been shown to influence the growth and differentiation of many cell types.[1] Five isoforms of TGF-β (TGF-β1-5), which are encoded by different genes, have been identified.[2] In particular, early reports demonstrated that the TGF-β1 and TGF-β2 isoforms are bimodal regulators of murine and human hematopoietic

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Acknowledgments: this research was supported by MURST 40% and 60%.
Received August 30, 1994; accepted October 13, 1994.

progenitor cells, and that their activity depends upon the differentiation state of the target cells and the presence of growth factors.[3] Conversely, TGF-β3 has been proven to be a more potent suppressor of human BM precursors than TGF-β1 and 2, and its effects on hematopoiesis are only inhibitory.[3]

Studies in our laboratory have shown that TGF-β3 exerts its activity on normal and leukemic cells by lengthening or arresting the G1 phase of the cell cycle;[4] this activity serves to protect normal hematopoietic progenitor cells from the dose-limiting toxicity of alkylating agents.[5] However, previous investigations of the regulatory activity of TGF-β3 on human BM progenitor cells were carried out using either low density mononuclear cells (MNC)[3] or highly enriched hematopoietic precursors stimulated with intermediate-late acting growth factors.[4] In both cases the experimental model may not be fully representative of the early BM cell compartment.

SCF (c-kit ligand) is a stromal cell-produced cytokine that has been shown to have a pivotal role in regulating early hematopoiesis.[6,7] SCF has demonstrated minimal stimulating activity by itself; however, in combination with EPO, G-CSF, GM-CSF and IL-3 it promotes the proliferation of very primitive $CD34^+$ lin^- cells.[8] IL-9 is a novel stimulatory factor originally found in the conditioned medium from a human T-cell leukemia virus-1 transformed T-cell line.[9] Recent evidence demonstrated that IL-9 as a single agent did not support colony formation, while it synergized with SCF, IL-3 and GM-CSF to induce the growth of early pluripotent hematopoietic progenitor cells characterized as $CD34^+$ $CD33^-$ $HLA\text{-}DR^-$.[10]

IL-11 is another microenviroment-derived CSF that appears to belong to the class of cytokines (*synergistic factors* such as SCF, IL-6 and G-CSF) capable of recruiting primitive hematopoietic progenitors from their quiescent status so that they are then able to respond to intermediate-late colony stimulating factors (CSFs).[11] Moreover, IL-11 in combination with SCF stimulates the proliferation of early murine and human hematopoietic cells.[12,13]

In the present study, we examine the suppressive activity of TGF-β3 on highly purified $CD34^+$ cells and more immature $CD34^+$/4-HC resistant precursors. Primitive hematopoietic progenitor cells were stimulated with early acting growth factors (i.e. IL-11, IL-9 and SCF) as well as with IL-3 and GM-CSF, alone and together.

The results of our experiments show that TGF-β3 inhibits predominantly the proliferation of more immature progenitors and the presence of IL-11 or IL-9 in culture seems to be important for reversing the negative regulation of TGF-β3.

Materials and Methods

Recombinant hematopoietic CSFs

The following human recombinant growth factors were used in this study: IL-11, IL-9 and IL-3 were supplied by Genetics Institute (Cambridge, MA) and were used at concentrations of 10 U/mL, 10 U/mL and 50 ng/mL, respectively.

IL-11 had a specific activity of 2.44×10^6 U/mL. IL-9 was derived from the conditioned medium of CHO cells. Its biological activity, by the MO7e proliferation assay was 2.4×10^5 U/mL. SCF and GM-CSF were provided by Amgen (Thousand Oaks, CA) and were added to the cultures at concentrations of 100 ng/mL and 1000 U/mL, respectively. Epo (Dompé Biotec, Milan, Italy) was used at a concentration of 2 U/mL. CSF concentrations were selected after review of our previous experience.[10,13] They were diluted and stored as previously described.[10,13]

TGF-β3

Recombinant human TGF-β3 (Oncogene Science, Manhasset, New York) was purified from conditioned media of Chinese hamster ovary transfected cells.[14] It was used at a concentration of 1 nM, which was selected according to the results of a previous study[5] and a preliminary dose-response curve on the colony growth inhibition of normal $CD34^+$ cells stimulated by phytohemagglutinin-leukocyte conditioned medium (PHA-LCM) (data not shown).

4-hydroperoxycyclophosphamide

4-HC is an active cyclophosphamide derivative which abrogates, in a dose dependent manner, the growth of committed hematopoietic progenitor cells while sparing more immature precursors.[15] 4-HC powder (Pergamid, Nova Pharmaceutical Co., Baltimore, MD) was diluted immediately before use with warm PBS and used in all the experiments at a concentration of 100 μg/mL.

BM cells and enrichment of hematopoietic progenitors

BM was aspirated from the posterior iliac crest of healthy volunteers who gave written informed consent. The MNC fraction, collected after Ficoll-Hypaque gradient (1.077 g/cm^3), was split in two cellular suspensions: one was diluted in Iscove's modified Dulbecco's medium (IMDM) with 20% of fetal calf serum (FCS) (Sera Lab, Crawley Down, Sussex, UK) and kept refrigerated, whereas the other was submitted to 4-HC treatment. In brief, normal BM cells were adjusted to a concentration of 2×10^7/mL and incubated for 30 min at 37°C with 4-HC. The cells were then put in ice for 5 min and washed twice in IMDM with 2% FCS to remove the drug.[5]

Enriched CD34$^+$ cells were subsequently obtained from both the 4-HC-treated and untreated cell suspension using the Ceprate LC Kit (CellPro, Bothell, WA).[5,13] Briefly, MNC were diluted in 1% bovine serum albumin (BSA) (Sigma Chem, St. Louis, MO) in phosphate-buffered saline (PBS), resuspended at a concentration of 1×10^8 cells/mL and incubated with 40 μg/mL of biotinylated 12.8 monoclonal antibody directed against the CD34 antigen. Cells were then washed and loaded into the Ceprate LC kit sample chamber at a flow rate of 1 mL/min. Labeled CD34$^+$ cells were then collected by manually squeezing the column containing the gel bed.[13] After separation, BM cells were evaluated by flow cytometry to determine the CD34$^+$ cell content and assayed for their growth in semisolid medium. The purity of the enriched CD34$^+$ cell fraction was determined by restaining the cells with the anti-CD34 monoclonal antibody IgG2a-FITC, HPCA-2 (Becton-Dickinson, Mountain View, CA, USA). Avidin-biotin cellular immunoabsorption gave a cell suspension representing 1.2±0.4% of the starting MNC fraction. The percentage of CD34$^+$ cells was always greater than 90%.

Colony assay

Five thousand CD34$^+$ cells or 5,000 to 10,000 CD34$^+$/4-HC resistant cells were plated in duplicate in colture medium consisting of 1 mL of IMDM supplemented with 24% FCS (Sera Lab), 0.8% BSA (Sigma), 10^{-4}M 2-mercaptoethanol (Sigma), 2 U rh Epo (Dompé Biotec) and bovine hemin 0.2 mM. Methylcellulose final concentration was 1.32%. CSFs were added as reported above. CFU-GM, BFU-E and CFU-MIX colonies were scored after 14 days of incubation at 37°C in a fully humidified 5% CO_2 atmosphere.[5,13]

Statistical analysis

All experiments were performed, unless otherwise indicated, three or more times and the mean±standard error of the mean (SEM) of each experiment was calculated. The results were analyzed with the paired non parametric Wilcoxon rank sum test.

Results

Inhibition of CD34$^+$ cell colony formation by TGF-β3

Table 1 shows the TGF-β3-mediated inhibition of enriched CD34$^+$ cells stimulated with IL-11, SCF, IL-9, IL-3 and GM-CSF used alone. All the tested CSFs resulted in significant colony formation in the presence of EPO. The low number of CFU-MIX observed in our experiments, especially in the presence of TGF-β3, did not allow any meaningful statistical analysis. Therefore, only the number of CFU-GM and BFU-E is reported.

TGF-β3 almost completely inhibited the colony growth of highly purified CD34$^+$ cells induced by IL-11, IL-9, EPO and SCF. In contrast, IL-3- and GM-CSF-stimulated colony formation was inhibited by 57 and 58%, respectively ($p < 0.02$). As reported previously,[13] the

Table 1. Colony formation of enriched CD34+ cells.

CSF	TGF-β3	±	colonies/10⁴ cells CFU-GM	BFU-E	TOTAL
IL-11		–	10±3.5	105±21.5	115±11.5
IL-11		+	2.5±1.8	9±0.9	11.5±1
IL-3		–	81.6±54.7	212±90	293.6±70
IL-3		+	16.6±13.6	110±37	126.6±40
GM-CSF		–	78.3±43.8	180±56	258±50
GM-CSF		+	38.3±25.4	70±35	108±32
SCF		–	22.5±12.4	200±26	225.5±20
SCF		+	2.5±1.8	12.5±2	15±6
IL-9		–	12.5±1.8	111±48.9	123.5±32
IL-9		+	0	15± 7	15±4
EPO		–	0	82± 55	82±55
EPO		+	0	2.5± 5	2.5±5

Mean number (±SEM) of CFU-C-derived colonies developed in duplicate cultures from 5 normal subjects. The cells were plated in the presence of 2 U/mL of EPO with or without 1 nM of TGF-β3. Control cultures (medium alone) did not generate colonies.

addition of IL-11 or IL-9 to IL-3 and GM-CSF augmented synergistically or additively the clonogenic efficiency of the BFU-E derived from CD34+ cells (Table 2). Co-incubation of the same cellular population with 2 early acting CSFs (e.g. IL-11 and SCF or IL-9 and SCF) resulted in the enhancement of both CFU-GM and BFU-E growth. A similar pattern also was observed in the presence of TGF-β3, mainly when IL-11 and IL-9 were combined with SCF. TGF-β3 inhibited total colony growth of CD34+ cells in response to IL-11 and SCF in combination by 52.4%, as compared to 90% or more when the same cytokines were used alone ($p < 0.001$). It is noteworthy that BFU-E were inhibited by 48.4%, whereas CFU-GM colony count decreased by 83% (Table 2). Similar results were obtained when IL-11 was combined with IL-3 or GM-CSF (e.g. the IL-11-IL-3 combination was inhibited by 42% as compared to 57% and 90% for individual cytokines; $p< 0.05$). In this case too mean BFU-E inhibition was lower than that reported for CFU-GM (38.5 and 63.7%, respectively). Moreover, mean erythroid progenitor cell inhibition was 56.2% in the presence of SCF and IL-9, while CFU-GM were suppressed by 97% (Table 2). However, CFU-GM colony formation was not inhibited by TGF-3 when IL-9 was associated with GM-CSF. Conversely, SCF increased the number of BFU-E induced by IL-3 and GM-CSF, but it did not overcome the suppressive activity of TGF-β3 (Table 2).

Effect of TGF-β3 on CD 34+/4-HC resistant cells

In subsequent experiments we evaluated the stimulatory effect of the study cytokines on primitive CD34+/4-HC resistant cells in the presence and absence of TGF-β3. *In vitro* treatment with 4-HC allows selection of a subset of CD34+ cells that retain some of the properties of the putative hematopoietic stem cell.[15]

TGF-β3 almost completely suppressed the growth of clonogenic CD34+/4-HC resistant cells induced by single cytokines (Table 3). It exerted much greater inhibitory activity on these cells than on the CD34 cell fraction, even in the presence of various two-factor combinations. For instance, clonogenic CD34+/4-HC

Table 2. Colony formation of enriched CD34+ cells in response to cytokines in combination.

CSF	TGF-β3	±	colonies/10⁴ cells CFU-GM	BFU-E	total
IL-11, IL-3		–	73.3±45	481±185	554.3±92
IL-11, IL-3		+	26.6±13	296±124	322.6±76
IL-11, GM-CSF		–	86.6±49	483±242	569±112
IL-11, GM-CSF		+	65±49	162±83	227±59
IL-11, SCF		–	58.7±25.8	432±115	490.7±74
IL-11, SCF		+	10±5.8	223.7±100	233.7±51
SCF, IL-3		–	81.6±40	450±93	531.6±32
SCF, IL-3		+	18.3±12	150±74.5	168.3±48
SCF, GM-CSF		–	112.3±42	360±108	472.3±80
SCF, GM-CSF		+	50±24	140±66	190±32
IL-9, IL-3		–	103±41	510±145	613±80
IL-9, IL-3		+	55±17	243±76	298±26
IL-9, GM-CSF		–	60±15	380±135	440±62
IL-9, GM-CSF		+	61.6±18	175±31	236.6±20
IL-9, SCF		–	83.7±25	521±132	604.7±60
IL-9, SCF		+	2.5±2	228±113	230.5±71

The results presented in this table are from the same set of experiments as Table 1. For further details see the legend to Table 1.

Table 3. Effect of TGF-β3 on CSF-stimulated growth of CD34+/4-HC resistant cells.

			colonies / 10^4 cells		
CSF	TGF-β3	±	CFU-GM	BFU-E	TOTAL
IL-11		–	1± 0.1	9.2±3.5	10.2±1
IL-11		+	0	0.9±0.3	0.9±0.3
IL-3		–	9.2±3	28.2±5.5	37.4±4
IL-3		+	0.8±0.3	3.4±1.4	4.2±1
GM-CSF		–	5.5±0.3	18.5±7.7	24±4
GM-CSF		+	1.5±1	1.5±0.5	3±1
SCF		–	8.2±2.4	35.2±11	43.4±6
SCF		+	1.7±1.2	3±1.1	4.7±2
IL-9		–	0.5±0.1	18.1±3	18.6±2
IL-9		+	0	1.1±0.8	1.1±0.8
EPO		–	0	2±05	2±05
EPO		+	0	0.5± 0.3	0.5±0.3

Mean number (±SEM) of CD34+/4-HC resistant-derived colonies developed in duplicate cultures from 5 normal individuals. BM cells were plated in the presence of 2 U/mL of EPO with or without TGF-β3. Early hematopoietic cells did not generate CFU-C in the absence of CSFs.

resistant cells stimulated by IL-11 and IL-3 were inhibited by approximately 80% by TGF-β3, as compared to 42% of CD34+ cells (Tables 2 and 4). The inhibitory activity of TGF-β3 did not significantly differ between CFU-GM and BFU-E. In this set of experiments, IL-11 acted synergistically or in an additive manner with IL-3, GM-CSF and especially SCF, with and without TGF-β3, on both CFU-GM and BFU-E (Table 4). In addition, IL-9 augmented the number of BFU-E responsive to SCF and IL-3 and the number of CFU-GM grown in the presence of SCF and GM-CSF. However, neither IL-11 nor IL-9 was able to completely reverse the suppressive effect of TGF-β3. Taken together, these results indicate that a subset of very primitive BM progenitor cells incubated with TGF-β3 is still responsive to IL-11 or IL-9, although the inhibitory effects of TGF-3 appear to be strongly differentiation stage related.

Discussion

Recent studies have shown that the TGF-β family regulates the proliferation of normal hematopoietic and leukemic progenitor cells.[2] The mechanism by which TGF-β inhibits cell proliferation is still unclear. However, several potential modes of action have been suggested, including down modulation of CSF receptors,[16] interaction with the underphosphorylated form of the retinoblastoma gene product in late G1 phase,[17] alteration of gene expression.[18] TGF-β-mediated growth inhibition seems to be reversible and preferentially directed toward primitive hematopoietic precursors whereas the more mature unipotent CFU-granulocyte (CFU-G), -monocyte (CFU-M), -eosinophil (CFU-E) are not affected.[19] In this regard, it has been shown that a subset of primitive murine hematopoietic cells produces active TGF-β1 by an autocrine mechanism,[20] suggesting that the stromal component of BM may not be the main part of the TGF-β inhibitory loop that maintains the quiescent status of hematopoietic stem cells.[21]

TGF-β1 and 2 isoforms have shown both inhibitory and stimulatory activity on hemopoiesis, while TGF-β3 has been found to be a more potent inhibitor than TGF-β1 or 2 of IL-3-induced day 7 and day 14 CFU-GM and CFU-Mix. Moreover, it does not enhance the growth of day 7 GM-CSF-stimulated CFU-GM.[3] Whereas several studies on the regulatory activity of TGF-β1, 2 have been performed at the level of the most immature progenitor cells, early reports on the growth inhibition of TGF-β3 have been limited to colony formation of committed precursors stimulated with intermediate-late CSFs.[3,4]

In this study we asked whether the antiproliferative activity of TGF-β3 could be demonstrated on highly enriched hematopoietic progenitors at various stages of differentiation and whether co-incubation with early acting growth factors, rather than IL-3 and GM-CSF, could modulate the suppressive effect of TGF-β3. The results presented here extend the observation that TGF-β3 inhibits early hematopoiesis according to the maturation state of the target cells and the presence of different cytokines in the culture medium. In fact, TGF-β3 showed greater inhibitory activity on more immature CD34+/4-HC resistant progenitor cells than on

Table 4. Colony growth of CD 34/ 4-HC resistant cells in response to cytokines in combination.

		colonies/10^4 cells		
CSF	TGF-β3 ±	CFU-GM	BFU-E	TOTAL
IL-11, IL-3	-	18.2±1.9	44.2±12	62.4±7
IL-11, IL-3	+	4.7±2	7.5±2.6	12.2±4
IL-11, GM-CSF	-	10±6	25±4.9	35±5
IL-11, GM-CSF	+	2.1±0.8	1.9±0.3	4±0.5
IL-11, SCF	-	12.2±5.2	61±12.7	73.2±8
IL-11, SCF	+	1±0.7	12.5±2.6	13.5±2
SCF, IL-3	-	16±2.4	35±6	51±4
SCF, IL-3	+	1.5±0.2	2.4±0.6	3.9±0.5
SCF, GM-CSF	-	2.1±0.7	37±5	39.1±3
SCF, GM-CSF	+	2.5±0.7	3±1	5.5±1
IL-9, IL-3	-	1.7±0.6	42.5±0.7	44.2±1
IL-9, IL-3	+	0.6±0.3	4.7±0.5	5.3±1
IL-9, GM-CSF	-	13±2	19.7±1.8	32.7±2
IL-9, GM-CSF	+	6.2±3.7	4.2±1.3	10.4±4
IL-9, SCF	-	13.2±5	87±3.9	100.2± 3
IL-9, SCF	+	6.2±0.1	16.5± 0.3	22.7± 0.5

The results reported in this Table are from the same set of experiments as Table 3. For further details see the legend to Table 3.

$CD34^+$ precursors. Moreover, colony formation from both cell populations stimulated by SCF and GM-CSF or IL-3, in the presence of EPO, was greatly reduced, confirming that these combinations of factors stimulate more primitive progenitors than GM-CSF or IL-3 alone (Tables 1 and 2).[8] Our results are consistent with previous studies which have shown the ability of TGF-β to abrogate the colony growth induced by SCF in mouse or in a human model.[19,21]

Conversely, IL-11 partially opposed the inhibitory activity of TGF-β3 on $CD34^+$ cells when combined with SCF, IL-3 and GM-CSF (see *Results* and Table 2). This effect was exerted mainly on erythroid progenitor cells since the percent decrease of CFU-GM was 83% for IL-11 and SCF in combination, and did not differ significantly from that of the same stimulatory factors used separately. Similar results were reported when IL-9, a cytokine which exerts burst promoting activity on erythoid precursors, was used as a growth promoting factor. Moreover, IL-9 appeared to abrogate completely, or reduce markedly, the inhibitory effects of TGF-β3 on GM-CSF- and IL-3-mediated CFU-GM, respectively. This finding is consistent with our previous study showing that IL-9 stimulates the proliferation of multipotent and granulocyte-macrophage progenitor cells rather than just subpopulations of early BFU-E.[10] However, it remains to be determined whether the increase in the number of CFU-GM comes exclusively from the synergistic effect of the two cytokines, or from an overriding effect of IL-9 on TGF-β3. Our results suggest that a combination of both effects may be operative. It should be pointed out that the addition of IL-9 did not augment the number of CFU-GM mediated by GM-CSF in the absence of TGF-β3, suggesting a direct effect of the cytokine on TGF-β3 (Table 2).

Because IL-11 and IL-9 induce the proliferation of both very primitive progenitor cells and committed precursors,[10,13] we then asked whether there is a portion of the earliest hematopoietic cells responsive to these growth factors that is resistant to the effects of TGF-β3. Our results demonstrate that IL-11 and IL-9 enhance synergistically or in an additive manner the clonogenic efficiency of $CD34^+$/4-HC resistant cells incubated with IL-3 and SCF, and IL-3, GM-CSF and SCF, respectively, but they are not able to reverse the suppressive effect of TGF-β3 completely. Thus, it may be that IL-11 and IL-9 counteract TGF-β3 mainly at the level of committed $CD34^+$ progenitor cells and, to a lesser extent, at the level of pluripotent hematopoietic precursors. It has already been reported that synergistic factors such as G-CSF or IL-6 can influence the inhibitory effects of TGF-β1 and 2 on murine primitive progenitors in serum-containing and serum-free cultures.[23] When we tested TGF-β3 in serum-depleted conditions, we observed a more pronounced suppressive effect on both $CD34^+$ and $CD34^+$/4-HC resistant cell populations (data not shown). This finding is consistent with the high binding affinity of TGF-β to serum α2 macroglobulin, which prevents TGF-β from binding to its receptor on the cell membrane.[24] The partial reversal of TGF-

β1-mediated suppression was also shown by fibroblast growth factor on human hematopoietic cells.[25]

Although we have demonstrated the opposing activity of IL-11, IL-9 and TGF-β3 on the proliferation of early BM cells, the mechanisms by which these factors interact remain to be elucidated. In this regard, a recent work demonstrates that TGF-β regulates the responsiveness of murine hematopoietic progenitors to SCF through a decrease in c-kit mRNA stability that leads to decreased cell-surface expression.[26] Experiments designed to investigate whether IL-9 reverses TGF-β3-mediated downregulation of GM-CSF and IL-3 receptors are underway. Our results could reflect a direct action of the study cytokines on the progenitor cells. However, the presence of accessory cells or mature myeloid cells cannot be completely ruled out. Therefore the use of single-cell culture systems would be important for elucidating this issue. A partial abrogation of the inhibitory effect of TGF-β3 by IL-11 may explain the stimulation of myelopoiesis obtained in IL-11-containing long-term BM cultures.[27] Similarly, SCF, which is a major amplifier of *in vivo* colony growth[28] but has shown much lower modulation of the TGF-β3 suppressive effect, induces a minimal enhancement of the growth and differentiation of human hematopoietic progenitor cells in long-term BM cultures.[29,30]

In summary, we report here for the first time that TGF-β3 inhibits predominantly the proliferation of human immature hematopoietic progenitor cells, and cytokines such as IL-11 and IL-9 seem to be able to oppose the negative regulation of TGF-β3 on $CD34^+$ cells. Given the potential role of TGF-β3 in the protection of normal stem cells from damage caused by radiation or *in vitro* and *in vivo* cytotoxic agents,[5] our results may be relevant to its clinical use.

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