Numerous factors that can influence the proliferation and differentiation in vitro of cells at various stages of hematopoiesis have been identified, but the mechanisms used by stromal cells to regulate the cycling status of the most primitive human hematopoietic cells are still poorly understood. Previous studies of long-term cultures (LTC) of human marrow have suggested that cytokine-induced variations in stromal cell production of one or more stimulators and inhibitors of hematopoiesis may be important. To identify the specific regulators involved, we performed Northern analyses on RNA extracted from human marrow LTC adherent layers, or stromal cell types derived from or related to those present in the adherent layer. These analyses showed marked increases in interleukin-1β (IL-1β), IL-6, and granulocyte colony-stimulating factor (G-CSF) mRNA levels within 8 hours after treatments that lead to the activation within 2 days of primitive hematopoietic progenitors in such cultures. Increases in granulocyte-macrophage (GM)-CSF and M-CSF mRNA were also sometimes seen. Bioassays using cell lines responsive to G-CSF, GM-CSF, and IL-6 showed significant elevation in growth factor levels 24 hours after IL-1β stimulation. Neither IL-3 nor IL-4 mRNA was detectable at any time. In contrast, transforming growth factor-β (TGF-β) mRNA and nanogram levels of TGF-β bioactivity in the medium were detected at all times in established LTC, and these levels were not consistently altered by any of the manipulations that stimulated hematopoietic growth factor production and primitive progenitor cycling. We also found that addition of anti-TGF-β antibody could prolong or reactivate primitive progenitor proliferation when added to previously stimulated or quiescent cultures, respectively. Together, these results indicate a dominant negative regulatory role of endogenously produced TGF-β in unperturbed LTC, with activation of primitive hematopoietic cells being achieved by mechanisms that stimulate stromal cells to produce G-CSF, GM-CSF, and IL-6. Given the similarities between the LTC system and the marrow microenvironment, it seems likely that the control of human stem cell activation in vivo may involve similar variations in the production of these factors by stromal cells.

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T he most primitive cells in the hematopoietic system represent a quiescent reserve population with the potential for generating very large numbers of mature blood cells at a much faster rate than is normally required. In vivo, this high proliferative potential is only expressed after various perturbations, as for example following transplantation of a small number of cells into a host whose hematopoietic system has been ablated by irradiation or chemotherapy. In this setting even single stem cells in a transplant can eventually reconstitute and maintain the hematopoietic system has been ablated by irradiation or chemotherapy. In this setting even single stem cells in a transplant can eventually reconstitute and maintain the entire lymphoid and myeloid system for many months. However, the endogenous mechanism(s) that promote or restrict the output of mature progeny from these transplantable hematopoietic stem cells in vivo are still largely unknown. One approach to the investigation of this area has focused on defining the in vitro responsiveness of very primitive hematopoietic cells whose behavior can be monitored following exogenous factor addition. Both in vivo and in vitro colony assays for progenitors of high proliferative and multi-lineage potential exist and, while it seems likely that most of the cells detected by these assays do not have totipotent long-term repopulating potential, they are thought to be closely related to such repopulating cells and to share an ability to alternate between a quiescent and a cycling state on demand. Recent in vitro factor addition experiments have shown that several growth factors, including interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and IL-3, can stimulate clonogenic hematopoietic progenitors with a high proliferative potential. Similarly, both transforming growth factor-β (TGF-β) and macrophage-inflammatory protein-1α (MIP-1α) have been shown to have an ability to selectively inhibit the proliferation of such cells.

To determine which of these factors might be involved in the mechanisms by which human hematopoietic cells are physiologically regulated within the marrow, we have analyzed their production and role in hematopoietically active long-term human marrow cultures (LTC). These cultures may be viewed as a model of the marrow microenvironment because they support the maintenance of high proliferative potential clonogenic progenitors for many weeks in the absence of exogenous hematopoietic growth factor addition if certain stromal cells are present. In addition, it has been found that the primitive hematopoietic progenitors in the adherent layer of established cultures only proliferate when medium containing fresh horse serum (HS) or one of a variety of indirect acting mesenchymal cell stimulators, such as IL-1β or platelet-derived growth factor (PDGF), is added. Because the effect of such treatments is short-lived, within a week the primitive hematopoietic progeni-
tors in the adherent layer return to a quiescent state, although they can be repeatedly stimulated to proliferate by the same treatments. This unique proliferative behavior appears to be under the control of thestromal cells in the adherent layer because quiescence is only established when the two cell types are in close proximity. Therefore, we have proposed that both the support and turnover of very primitive hematopoietic cells in the marrow might be regulated by close-range interactions with mesenchymal stromal cell types that, according to their own state of activation, differentially vary their production of factors with positive and negative effects on primitive hematopoietic cells.

Recently, mRNA for granulocyte-macrophage (GM)-CSF has been demonstrated in extracts of LTC of murine marrow, and a variety of human mesenchymal cell types related to the lineages represented in human marrow LTC adherent layers have been shown to increase their expression of GM-CSF, G-CSF, and IL-6 after exposure to IL-1 or PDGF. In addition, it has been shown that substantial amounts of GM-CSF can be extracted from the extracellular matrix in the adherent layer of human LTC. Induction of one or more of these factors would thus seem a likely part of the mechanism underlying the observed stimulation of primitive progenitors following stromal cell activation. Because the addition of exogenous TGF-β to human LTC can prevent the stimulation of high proliferative potential clonogenic cells, it also seemed possible that endogenous production of TGF-β by marrow stromal cells might contribute to the downregulation of proliferation of primitive hematopoietic cells in unperturbed LTC. To examine these possibilities, we undertook time-course studies of the mRNA and bioactivity levels of these and other factors in the adherent layer of LTC and performed anti-TGF-β antibody neutralization studies.

**MATERIALS AND METHODS**

**Cells and reagents.** Marrow was obtained in heparinized medium from informed and consenting donors of allogeneic transplants, and HUVEC cells were cloned by trypsinization and endothelial cells isolated independently in this laboratory by SV-40 transformation of three different human marrow cell cultures. HUVEC-C cells are human umbilical cord vein endothelial cells originally obtained from the American Type Tissue Collection (ATCC; Rockville, MD). Purified porcine TGF-β, and an affinity-purified rabbit antiporcine TGF-β, antibody preparation were purchased from Collaborative Research (Bedford, MA), and purified TGF-α from Bachem (Torrance, CA), and purified human epidermal growth factor (EGF) from Boehringer Mannheim (Montreal, Quebec, Canada). Purified recombinant human IL-1β was kindly provided by Biogen (Boston, MA).

**Cultures.** LTC were established in 60-mm tissue culture dishes (or appropriately scaled up in 150-cm² flasks) from unprocessed marrow aspirate cells, maintained, stimulated, harvested by trypsinization, and assayed in methylincellulose for high proliferative potential erythroid and granulopoietic progenitors (referred to as primitive burst-forming unit-erythroid [BFU-E] and colony-forming unit-GM [CFU-GM]) using procedures that are routine in this laboratory and that have been described in detail previously. Standard LTC medium consists of an enriched α medium plus 12.5% HS, 12.5% fetal calf serum (FCS), 10⁻⁴ mol/L 2-mercaptoethanol, and 10⁻³ mol/L hydrocortisone sodium succinate. For RNA studies of adherent layer cells, nonadherent cells were simply decanted and the adherent layer was then scraped off with a rubber policeman, pelleted, and RNA extracted as described below. Marrow “fibroblasts” were obtained by repeated trypsinization and subculturing of marrow cells as soon as they reached confluence in tissue culture dishes containing α medium plus 15% FCS. HUV-EC-C cells were maintained in F12K medium supplemented with endothelial cell growth factor (Collaborative Research, Bedford, MA) and 15% FCS as recommended by ATCC. Thymidine suicide assays. Suspended LTC adherent layer cells were washed and incubated for 20 minutes at 37°C in medium with or without high specific activity [3H]-thymidine before plating in methylcellulose and subsequent derivation of [3H]-thymidine suicide (% kill) values from colony counts in the two groups, exactly as in previous studies of this type. Northern analyses. Total cellular RNA was isolated using the guanidinium isothiocyanate procedure. RNA was separated by electrophoresis through 1.2% agarose gels containing 5% formaldehyde and transferred to Zetaprobe nylon membranes (Bio-Rad Laboratories, Richmond, CA) by centrifugation. Filters were hybridized and washed at high stringency as described. The following gel-purified DNA fragments were used to synthesize probes: the 1,050-bp EcoRI fragment of αβ1 containing most of a human TGF-β cDNA; the 900-bp XhoI fragment of pXMIL3 containing the complete coding region of a human IL-3 cDNA; the 900-bp insert of the pg2.15 human IL-6 partial cDNA; the 5' 450-bp fragment of a human GM-CSF cDNA cloned in our laboratory with the same sequence as previously reported; the 200-bp HindIII-PvuII fragment of a human IL-1β cDNA; the 900-bp XhoI fragment of pCDIL containing a complete IL-4 cDNA; the 1,050-bp BglII-PstI fragment of a human M-CSF cDNA; and the 1,200-bp PstI fragment of a chicken β-actin cDNA. Probes were labeled by hexamer priming as previously described. Bioactivity assays. TGF-β bioactivity was assayed by induction of anchorage-independent growth by NRK fibroblasts using a slight modification of the method described by Asoo et al in which methylcellulose was substituted for agar to provide a semisolid matrix. Briefly, NRK cells were suspended in a 0.8% methylcellulose culture medium containing 10% FCS and 2.5 ng/mL EGF and either TGF-β standard as a control, or test material, to give a final concentration of 3,500 cells/mL. One-milliliter aliquots were then plated in 35-mm Petri dishes (Greiner, Nütingen, Germany) which fail to support any anchorage-dependent fibroblast growth even in liquid medium. Cultures were incubated for 14 days in a humidified atmosphere of 5% CO₂ in air before scoring of colonies containing 20 or more cells. Results shown are for test conditioned media harvested as described in the text and stored at -20°C. Occasional samples were also subjected to acidification (pH 2.0) by addition of HCl and then adjustment of the pH to 7.0 by the further addition of NaOH as described to assess potential TGF-β precursor activity. The level of TGF-β detected in these instances was not different than preacidification levels (data not shown). Culture supernatants were also assayed for growth factor bioactivities by quantitation of [3H]-thymidine incorporation into appropriate responsive cells: AML-193 (for GM-CSF), NFS-605 (for G-CSF),
RESULTS

Cyclical expression of stimulatory growth factors and constitutive production of TGF-β in human marrow LTC and related stromal cell types. Northern blot analyses were performed on RNA extracted from the adherent layer of established LTC initiated with normal human marrow and maintained by weekly half-medium changes according to standard procedures. After 4 or 5 weeks, cultures were either subjected to the usual half-medium change (ie, addition of fresh HS) or to a mock medium change (ie, old medium returned to each culture) with or without the addition of 0.4 ng/mL of IL-1β or 5 ng/mL of PDGF. Eight or 24 hours later, the medium was collected for bioactivity measurements and the adherent layers were harvested for RNA extraction. Figure 1 shows the results of two representative experiments. Low but detectable levels of M-CSF and sometimes IL-6 mRNA were demonstrable in mock-fed cultures, whereas transcripts for IL-1β and GM-CSF could not be detected. The most pronounced effects seen after cytokine addition were IL-1β-induced increases in IL-1β, IL-6, and G-CSF mRNA levels. These levels were maximal 8 hours after addition of IL-1β, although still detectable after 24 hours. IL-1β induction of low levels of GM-CSF and M-CSF mRNA was also sometimes observed (Fig 1) by Northern analysis. GM-CSF mRNA increases could be more readily demonstrated by RNase protection using uniformly labeled probes (data not shown). Addition of HS also caused an early increase in IL-1β and IL-6 mRNA levels, but the degree of induction was less marked than that seen following the addition of IL-1β. Low-level induction of IL-1β and IL-6 mRNA was observed in a minority of cultures (data not shown) after PDGF addition. Message levels for IL-3 and IL-4 remained below the level of detection regardless of how the cultures were treated (data not shown).

As predicted by the RNA data, bioassays of LTC media removed at the 24-hour harvest time point showed a significant elevation in growth factor levels in cultures to which IL-1β was added (Table 1). Any alterations in production of these factors that may have been stimulated by HS or PDGF were insufficient to be detectable by this approach (data not shown). Thus, treatments that transiently stimulate primitive BFU-E and CFU-GM in the adherent layer to be in S-phase 2 to 3 days later (ie, addition of HS, IL-1β, and PDGF) also cause a rapid increase in mRNA levels of IL-1β, IL-6, G-CSF, GM-CSF, and M-CSF in adherent layer cells, the most pronounced of which are reflected in increased levels of bioactivity in the surrounding medium.

In contrast, although TGF-β mRNA was evident in adherent layer cells of all cultures analyzed, the mRNA levels detected in any given experiment were invariant (by comparison with β-actin or β-tubulin mRNA levels) regardless of how the cultures were manipulated (Fig 1), including cultures to which 50 ng/mL of TGF-α or 5 ng/mL TGF-β plus 0.4 ng/mL of IL-1β were added (data not shown). Addition of TGF-α, like HS, IL-1β, and PDGF, stimulates primitive progenitor cycling.21 TGF-β plus IL-1β does not prevent the accumulation of hematopoietic growth factor mRNA induced by IL-1β alone (data not shown), but does prevent the associated stimulation of primitive progenitor cycling.21 As shown in Fig 2, TGF-β bioactivity could be detected when diluted in fresh LTC medium and was consistently present at 1 ng/mL to 5 ng/mL in media removed from established cultures (Table 2). As predicted by the TGF-β mRNA results, there was no evidence of an increase or decrease in TGF-β production by any of the

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**Table 1**

<table>
<thead>
<tr>
<th>Time of RNA Harvest (hrs)</th>
<th>Control</th>
<th>HS</th>
<th>IL-1β</th>
<th>PDGF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>TGF-β</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
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<tr>
<td>24</td>
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</tbody>
</table>

**Fig 1.** Northern blot analysis of growth factor mRNA levels in adherent layer cells of two representative 4-week-old (LTC nos. 311 and 312). Cells were harvested at the times indicated (in hours) above each lane after a mock medium change (control), a regular half-medium change (HS), or addition of IL-1β (0.4 ng/mL) or PDGF (5 ng/mL). Membranes were sequentially hybridized to probes for the growth factors indicated and finally rehybridized (bottom panel) to a probe for β-actin-RNA to test the uniformity of RNA loaded.

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and B93 (for IL-6), recognizing that all of these cell lines show minor responses to various other factors. Recombinant human GM-CSF (kindly provided by Biogen), G-CSF (purchased from Amersham, Oakville, Ontario, Canada) and IL-6 (purchased from R & D Systems) were used as standards for bioactivity measurements.
GROWTH FACTOR CONTROL OF HEMATOPOIESIS IN LTC

Table 1. Growth Factor Bioactivities in LTC Media One Day After Addition of 0.4 ng/mL of IL-1β

<table>
<thead>
<tr>
<th>Cell Line (growth factor)</th>
<th>Before IL-1β (ng/mL)</th>
<th>After IL-1β (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFS 60 (G-CSF)</td>
<td>6 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>AML-193 (GM-CSF)</td>
<td>1 ± 0</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>B9 (IL-6)</td>
<td>2 ± 1</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>

Bioassays were performed on media harvested from three different LTC, each initiated with a different normal marrow sample. Values shown are the mean ± SEM in nanograms per milliliter based on the assumption that the predominant response is to the growth factor indicated. IL-1β was added to 4-week-old LTC together with a mock medium change 7 days after the previous 3-week regular half-medium change.

various LTC treatments evaluated. Thus, sufficient TGF-β appears to be produced by cells in established human marrow LTC to achieve a concentration in the medium similar to the amount of TGF-β, i.e., 5 ng/mL, that when added exogenously overrides the primitive progenitor-activating effect of factors such as HS or IL-1β.

To determine whether the growth factor production observed is likely to be a property of the stromal cell components of the adherent layer, which have been shown to include fibroblast, endothelial and adipose cell types, we analyzed RNA from subcultured primary bone marrow fibroblasts, from a number of independently isolated SV-40 immortalized human marrow stromal cell lines (each of which are phenotypically indistinguishable from each other and related stromal cells in human marrow LTC adherent layers), and from human umbilical vein endothelial cells. Addition of IL-1β to subcultured bone marrow fibroblasts and SV40 immortalized marrow stromal cell lines induced a rapid and marked increase in IL-6 mRNA levels (Fig 3) analogous to that observed in primary LTC adherent layers. GM-CSF mRNA levels were also elevated after IL-1β stimulation of both primary and SV40-immortalized human marrow fibroblasts as detected by either Northern analysis (Fig 3) or RNase protection assays (data not shown).

Table 2. TGF-β Bioactivity in Media Conditioned for 24 Hours by Cultures Treated With Various Feeding Procedures

<table>
<thead>
<tr>
<th>Culture</th>
<th>Treatment*</th>
<th>TGF-β (ng/mL)</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTC</td>
<td>Control</td>
<td>6.2 ± 2.9</td>
<td>6</td>
</tr>
<tr>
<td>LTC</td>
<td>HS</td>
<td>8.4 ± 4.3</td>
<td>3</td>
</tr>
<tr>
<td>LTC</td>
<td>PDGF</td>
<td>8.1 ± 4.0</td>
<td>3</td>
</tr>
<tr>
<td>LTC</td>
<td>IL-1β</td>
<td>8.3 ± 6.4</td>
<td>6</td>
</tr>
<tr>
<td>LTC</td>
<td>IL-1β + TGF-β</td>
<td>8.8 ± 0.8</td>
<td>3</td>
</tr>
<tr>
<td>CFU-ST16</td>
<td>Control</td>
<td>3.6 ± 1.3</td>
<td>2</td>
</tr>
<tr>
<td>CFU-ST16</td>
<td>IL-1β</td>
<td>7.4 ± 1.4</td>
<td>2</td>
</tr>
</tbody>
</table>

TGF-β was assayed by induction of anchorage-independent growth by NRK fibroblasts as described in Materials and Methods. Values shown are the mean ± SEM of measurements of media harvested from different LTC, each initiated with a different marrow sample, or from different experiments with CFU-ST16 cells as indicated by the number of samples.

*Control, mock medium change; HS, routine replacement of half of the old medium with fresh LTC medium containing HS. IL-1β added to give a final concentration of 0.4 ng/mL; TGF-β added to give a final concentration of 5 ng/mL.

However, G-CSF mRNA was induced only in the SV40-immortalized fibroblasts. Neither IL-6, G-CSF, nor GM-CSF mRNA was detected in either IL-1β-stimulated or unstimulated HUV-EC cells. In contrast, TGF-β mRNA was readily detected in all cells tested and was not affected by exposure of cells to IL-1β (Fig 3). TGF-β bioassays performed on media conditioned by one of the marrow

![Fig 2](image.png) Fig 2. A representative standard dose-response curve for TGF-β stimulation of anchorage-independent colony formation by NRK cells in methylocellulose in the presence (■) or absence (□) of 10% fresh LTC medium.

![Fig 3](image.png) Fig 3. Northern blot analysis of growth factor mRNA levels in subcultured bone marrow fibroblasts (BM Fib), an SV40-immortalized marrow stromal line (CFU-ST16), or human umbilical vein endothelial cells (HUV-EC-C). Cells were harvested for analysis 12 hours after a mock medium change (control) or addition of IL-1β (0.4 ng/mL). (Top panel) Hybridization to probes for the growth factors indicated. (Bottom panel) The same membrane rehybridized to a probe for β-actin mRNA.
stromal cell lines (CFU-ST16) for 24 hours with or without IL-1β stimulation showed levels comparable with those observed in LTC media, and these were slightly increased by IL-1β stimulation of the cells (Table 2).

Functional role of endogenous TGF-β in human marrow LTC. To test the potential role of endogenously derived TGF-β in regulating primitive hematopoietic progenitor proliferation in LTC, a series of experiments using neutralizing anti-TGF-β antibody were next undertaken. The two experimental protocols followed are shown schematically in Fig 4. In the first, anti-TGF-β antibody was added 3 days after a regular half-medium change. At this time primitive BFU-E and CFU-GM in the adherent layer are maximally cycling, but then normally return to a quiescent state within another 4 days. As shown in Fig 5, the effect of adding anti-TGF-β antibody according to this protocol was to maintain the majority of primitive BFU-E and CFU-GM in the adherent layer in S-phase when antibody concentrations of 25 μg/mL or more were used, i.e., amounts sufficient to neutralize at least 2.5 ng/mL of TGF-β according to data provided by the supplier. The effect of adding anti-TGF-β antibody in this type of experiment was consistent in all eight experiments performed, was specific, and could be reversed by the simultaneous addition of a competitive concentration of TGF-β (Table 3). These results suggest that neutralization of sufficient endogenous TGF-β in recently activated cultures can maintain the ratio of directing positive factors to TGF-β in favor of the former, thereby allowing the primitive progenitors to continue to proliferate.

In the second protocol (Fig 4), addition of anti-TGF-β antibody was delayed until 7 days after the previous half-medium change, i.e., at the time when another half-medium change would normally be performed. At this time primitive BFU-E and CFU-GM in the adherent layer are quiescent. The purpose of this design was to determine whether neutralization of TGF-β in such cultures could be sufficient to allow these quiescent progenitors to be reactivated, thereby testing whether the effects achieved in the first protocol might be dependent, not only on the absolute concentration of TGF-β present in the cultures (Table 2), but also on the level of positive stimulators of primitive progenitor proliferation present at the same time. The results of each of a total of six experiments performed using the second anti-TGF-β addition protocol are shown individually in Table 4. In three of these experiments (arbitrarily listed first), neutralization of endogenous TGF-β resulted in the reentry into S-phase of the primitive BFU-E and CFU-GM that had already become quiescent before the addition of anti-TGF-β antibody. In the other three experiments (listed last), these same progenitor types remained quiescent, suggesting inadequate levels of positive stimulators to counter residual levels of TGF-β and/or other possible inhibitors in these particular cultures.

**DISCUSSION**

Cells with a mesenchymal phenotype sharing features of fibroblasts and endothelial cells constitute a major component of the adherent layer that forms in LTC initiated with human marrow. Such cells are also present as fixed (noncirculating) elements in vivo, where they have been implicated for many years as playing a primary role in locally regulating hematopoietic cell turnover in the marrow. Additional evidence for this has come from studies of

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Table 3. Anti-TGF-β Antibody Allows Prolongation of Primitive Progenitor Cycling in the Adherent Layer of LTCs Activated Three Days Previously (Protocol 1)

<table>
<thead>
<tr>
<th>Addition</th>
<th>% Kill by 3H-Thymidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 ± 11 (2)†</td>
</tr>
<tr>
<td>Anti-TGF-β (50 μg/mL)</td>
<td>51 ± 4 (8)</td>
</tr>
<tr>
<td>Anti-TGF-β (50 μg/mL) + TGF-β (5 ng/mL)</td>
<td>0 ± 3 (5)</td>
</tr>
<tr>
<td>Control antibody (50 μg/mL)†</td>
<td>0 ± 3 (5)</td>
</tr>
</tbody>
</table>

*Primitive BFU-E, BFU-E producing colonies of >8 clusters of erythroblasts; Primitive CFU-GM, CFU-GM producing colonies of >500 granulocytes and macrophages.

†Number of experiments shown in parentheses.

‡Affinity-purified Ig from normal rabbit serum.
mice bearing mutant alleles at the Steel (Sl) locus. The macrocytic anemia and defective stem cell function seen in these mice are due to a deficient hematopoietic microenvironment that can only be repaired by the transfer of whole tissue fragments. Very recently the molecular basis of this deficiency has been characterized as an abnormality in the production of a multi-lineage growth factor that is produced by fibroblasts and that may exist in a membrane-bound form. Fibroblasts and endothelial cells also produce several other growth factors active on hematopoietic cells.

Further evidence that the physiologic regulation of hematopoiesis is achieved through the control of locally effective growth factors has been obtained from studies of the LTC system. These studies have shown that hematopoiesis cannot be sustained in LTC lacking a stromal cell-containing adherent layer or containing a defective adherent layer of Sl/Sf origin. Another function of the stromal cells in the adherent layer is to return adjacent (but not nonadherent) primitive hematopoietic cells to a quiescent state in unperturbed cultures.

In this report we have presented evidence that the downregulating activity of the stromal cells in LTC may be attributed at least in part to their constitutive production and release of bioactive TGF-β. TGF-β was a probable candidate for such a role as it had previously been shown to act directly, reversibly, and selectively to arrest or prevent the proliferation in vitro of the type of primitive hematopoietic cells that are normally quiescent in the marrow in vivo.

In LTC initiated with murine marrow, myelopoiesis could account for differential responses of developmentally distinct stages of hematopoietic cell differentiation, with earlier stages favoring quiescence (high sensitivity to TGF-β, low sensitivity to positive regulators) and later stages favoring proliferation (lower sensitivity to TGF-β and increased sensitivity to positive regulators). Appropriate changes in extracellular factor levels would, nevertheless, allow all stages of hematopoietic cell development to be altered by changing circumstances.

ACKNOWLEDGMENT

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Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. II. Analysis of positive and negative regulators produced by stromal cells within the adherent layer

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