High Proliferative Potential-Quiescent cells: a working model to study primitive quiescent hematopoietic cells

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SUMMARY

Human adult hematopoietic stem cells are mostly quiescent or slow cycling. We have previously demonstrated that blocking of transforming growth factor-β1 (TGF-β1) is able to activate, in the presence of cytokines, primitive quiescent hematopoietic multipotent progenitors which could not grow in a two week semi-solid culture assay (short term culture). We have also shown that anti-TGF-β1 can up-modulate c-KIT, the receptor of the stem cell factor (steel factor). To elucidate whether TGF-β1 plays a central role in controlling the quiescence of hematopoietic primitive cells, it was necessary to demonstrate, as detailed in this study, that: (1) whatever the cytokine combination tested, addition of anti-TGF-β1 releases from quiescence multipotent progenitors with a significantly higher hematopoietic potential than those activated by cytokines alone. (2) Other important cytokine receptors controlling the most primitive hematopoietic cells such as FLT3 and the IL6 receptor (IL6-R) are down-modulated by TGF-β1 but rapidly up-modulated by anti-TGF-β1. (3) Anti-TGF-β1-sensitive multipotent and high proliferative potential progenitors express these cytokine receptors at a low level (FLT3low and IL6-Rlow).

According to these results, we propose the working model of ‘High Proliferative Potential-Quiescent cells’ to refer to these primitive hematopoietic multipotent progenitors that are highly sensitive to the growth inhibitory effect of TGF-β1. This model could be valid not only to study the human hematopoietic quiescent progenitors but also for other somatic stem cell systems.

Key words: Anti-TGF-β1, Cytokine receptor, Hematopoietic progenitor cell activation

INTRODUCTION

Hematopoietic stem cells (HSC) are considered to be quiescent or slow cycling. This quiescence may be controlled by inhibitors such as transforming growth factor-β1 (TGF-β1) (Ottman and Pelus, 1988; Hatzfeld et al., 1991; Keller et al., 1992; Sitnicka et al., 1996). TGF-β1 is produced by the stromal microenvironment (paracrine TGF-β1) or by the hematopoietic progenitors themselves (para- and autocrine TGF-β1).

Using antisense oligonucleotides, we demonstrated that inhibition of TGF-β1 production could release umbilical cord blood (UCB) or bone marrow (BM) multipotent progenitors from quiescence (Hatzfeld et al., 1991; Cardoso et al., 1993; Li et al., 1994).

However, quiescence might also result from the absence of a specific growth stimulation. Although many cytokines were good candidates (Leary et al., 1988; Ikebuchi et al., 1987; Wong et al., 1988; Metcalf and Nicola, 1991; Carow et al., 1991), none of those we studied were able to release multipotent progenitors from TGF-β1-dependent quiescence in short term cultures (Cardoso et al., 1993; Li et al., 1994).

We demonstrated previously that c-KIT receptor was down-modulated by TGF-β1 and up-modulated by anti-TGF-β1 on human hematopoietic progenitors (Sansilvestri et al., 1995). This mechanism may contribute to render these cells unresponsive to KIT-ligand (steel factor). However, it is unclear whether this mechanism is of more general importance for the regulation of other receptors controlling human primitive hematopoietic progenitors.

More recently, basic fibroblast growth factor (bFGF) was shown to partially abolish TGF-β1 growth inhibition (Gabrilove et al., 1993). Another cytokine, FLT3-ligand (FL), has been shown to be a powerful cytokine accelerating the cell cycle and amplifying both myeloid and lymphoid progenitors (Lyman et al., 1994; Small et al., 1994; Brommeyer et al., 1995; Hirayama et al., 1995; Muench et al., 1995; Jacobsen et al., 1996; Ohishi et al., 1996; Shah et al., 1996; Yonemura et al., 1997), particularly in long term culture initiating cell (LTC-IC) assay (Petzer et al., 1996). IL6 also has been shown to activate early progenitors (Leary et al., 1988). Concerning the receptors of these latter cytokines, cell response to FL is mediated through the class III tyrosine kinase.
receptor FLT3/FLK2 (FLT3; CD135) (Rosnet et al., 1993). IL6 signaling occurs through two glycoproteins which can interact and form an heteromeric complex. The IL6 receptor (gp80 or α chain) specifically binds IL6 and then associates with a subunit common to other cytokine receptors, a glycoprotein of 130 kDa (gp130 or β chain) which triggers the signal transduction (Hibi et al., 1990; Murakami et al., 1993).

We have therefore investigated: (1) whether FL and bFGF counteract TGF-β1 growth inhibition in short term culture and mimic anti-TGF-β1 antibody effect; (2) whether FLT3 and IL6 receptors are modulated by TGF-β1 in a similar way to c-KIT; (3) whether UCB CD34+ progenitors could be separated into different subpopulations on the basis of their FLT3 and IL6-R level and whether they differentially respond to anti-TGF-β1.

This approach allowed us to better clarify the mechanism by which anti-TGF-β1 activates in short term culture a quiescent subpopulation of multipotent highly proliferative progenitors and to better characterize their phenotype.

MATERIALS AND METHODS

CD34+ cell preparation

UCB samples were collected immediately after delivery using the method of Brossard et al. (1990). Informed consent was obtained before sample collection. Early CD34++ or CD34 high cells were purified as previously described (Hatzfeld et al., 1994).

Cell sorting

CD34+CD38- cells were sorted using the following procedure: CD34+ cells were suspended in PBS/BSA (0.2%) and incubated with an anti-CD34 fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) (8G12 clone; Becton Dickinson, San Jose, CA) and anti-CD34 fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) (8G12 clone; Becton Dickinson) for 30 minutes at 4°C and washed twice as previously described (Li et al., 1994). Non-specific FITC- and PE-biotin/FITC-avidin as previously described. The cells were also centrifuged before sample collection. Early CD34++ or CD34 high cells were purified as previously described (Hatzfeld et al., 1994).

Receptor modulation studies

CD34+ cells were plated at 2×10^6 cells/ml in 96-well tissue culture plates (Falcon) in 200 µl liquid CSF medium. Depending on the experimental conditions, anti-TGF-β1 or FL and bFGF combinations were also added.

Liquid cultures

Clonal culture studies

Liquid SB+ medium (StemBio Research, Villejuif) containing IL3, IL6, SF, GM-CSF and Epo. FL, bFGF or anti-TGF-β1 were added to this control medium.

Clonal culture studies

For clonal studies, the cells were counted in each well under an inverted Wilovert microscope at 5 days and with a Malassez chamber at 18 days. The type of clones was determined at day 18 as already described (Fauser and Messner, 1978, 1979; Zhou et al., 1988) and in many cases, confirmed by phenotypic analyses and staining.

Phenotypic analysis and staining

Cells were harvested from the 96-well plates. They were labelled with FITC-anti-CD14 (TUK4 clone; Dako, Denmark), FITC-anti-CD66 (80H3 clone; Immunotech, France), PE-anti-glycoporin A (D2.10 clone; Immunotech), PE-anti-CD41 (SB12 clone; Dako), non specific FITC-IgG1 (Dako) and PE-IgG1 (Dako), with anti-FLT3 mAbs or IL6-biotin/FITC-avidin as previously described. The cells were analyzed on a Vantage flow cytometer. The cells were also centrifuged in a Cytospin centrifuge (Shandon Elliot, PA) and stained with a RAL555 kit (PolyLabo, France). Slides were examined microscopically (IM 35, Zeiss, Germany).

In situ hybridization analyses

The complete procedure is based on the methods described by Angerer et al. (1987) and has been detailed extensively in a previous report (Panterne et al., 1993). At the specified times, cells were centrifuged (500 rpm, 7 minutes, 5×10^3 to 10^4 cells/Denhardt-treated slide) on a Cytospin (Shandon Elliot, Pittsburg, PA). Cells were then fixed and slides were stored in 70% ethanol at 4°C until used for in situ hybridization. The Flt3 probe corresponding to the extracellular domain of the receptor was a generous gift from Dr O. Rosnet (Marseille, France). It was a 1 kb human Flt3 Smal-BamHI cDNA insert in pBS-SK+ vector. Antisense RNA probe was obtained using T7 RNA polymerase after linearization with BamHI and sense RNA probe using T3 RNA polymerase after linearization with HindIII. The IL6-Rα probe corresponding to the α chain was a generous gift from Drs T. Hirano and T. Kishimoto (Osaka, Japan). It was a 2.2 kb human
IL6-Rtx HindIII-XbaI cDNA insert in pBBI 76 vector. Antisense RNA probe was obtained using Sp6 RNA polymerase after linearization with HindIII and sense RNA probe using T7 RNA polymerase after linearization with XbaI. The labelled probes were synthesized using the Riboprobe Gemini II Core System (Promega, Madison, WI) with [γ-32P]UTP (Amersham, Buckinghamshire, UK) as specified by the manufacturer. The RNAs were purified by phenol extraction and ethanol precipitation after an alkaline hydrolysis. The specific activity of the labelled probe was approximately 1.3×10⁹ cpm/µg, 1×10⁹ cpm were applied per slide. Non-specific binding was prevented by a sufficient concentration of Escherichia coli tRNA (500 µg/ml). After 10 days exposure, the slides were developed and stained with Giemsa (Merck). Grains were counted using autoradiography software on a SAMBA image analyser (Alcatel TITN Answear, Meylan, France). Each cell on the reaction slides containing at least 10 grains was scored as a ‘positive cell’.

Statistical analysis
The paired Student’s t-test was applied to determine significance of differences between means for each condition.

RESULTS
The effects of FL, bFGF, and anti-TGF-β1 on primitive hematopoietic progenitors
In previous studies we have demonstrated that anti-TGF-β1 could activate primitive quiescent progenitors when added to various cytokine combinations either with or without SF (Hatzfeld et al., 1991; Cardoso et al., 1993; Li et al., 1994). Table 1 shows this effect on UCB CD34+ cells with an improved combination of cytokines: in semi-solid control medium (CM) optimized for IL3, IL6, G-CSF, GM-CSF, SF and Epo, the number of large mixed colonies (CFU-Mix) was significantly increased by anti-TGF-β1. The percentage of CFU-Mix stimulated by anti-TGF-β1 is significantly higher than total CFC activated by the same reagent (P=0.0001 versus P=0.001). The increase in total CFC is mainly due to HPP-Q-Mix and HPP-Q-GM.

To study whether FL and/or bFGF could substitute for anti-

Table 1. Effect of anti-TGF-β1 on CFU and CFU-Mix in semi-solid culture of UCB CD34+ cells: ‘HPP-Q’ test

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>CM</th>
<th>CM+anti-TGF-β1</th>
<th>Δ CFU-Mix</th>
<th>Total CFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.5</td>
<td>11.0</td>
<td>5.5</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>4.0</td>
<td>2.5</td>
<td>17</td>
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<tr>
<td>3</td>
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<td>2.5</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>3.5</td>
<td>3.0</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>11.5</td>
<td>5.5</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td>7.0</td>
<td>12.0</td>
<td>5.0</td>
<td>66</td>
</tr>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>3.5</td>
<td>7.5</td>
<td>4.0</td>
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<tr>
<td>9</td>
<td>2.0</td>
<td>5.5</td>
<td>3.5</td>
<td>31</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>8.0</td>
<td>2.5</td>
<td>67</td>
</tr>
</tbody>
</table>

Mean± s.d. 3.3±2.5 6.7±3.7 3.8±1.4 42±21 52±25

400 UCB CD34+ cells were cultured in duplicate or in quadruplicate in semi-solid medium (IL-3/IL-6/G-CSF/GM-CSF/SF/Epo) for 18 days with or without anti-TGF-β1. These results are means ± s.d. (n=10). Significance is evaluated by the paired Student’s t-test.

Table 2. Effect of FL/bFGF and anti-TGF-β1 on CFU-Mix frequency in clonal liquid culture

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>CFU-Mix with &gt;10⁵ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control medium (CM)</td>
<td>4 2 1</td>
</tr>
<tr>
<td>+ anti-TGF-β1</td>
<td>10 7 3</td>
</tr>
<tr>
<td>+ FL/bFGF</td>
<td>1 4 1</td>
</tr>
<tr>
<td>+ FL/bFGF + anti-TGF-β1</td>
<td>8 8 4</td>
</tr>
</tbody>
</table>

UCB CD34+CD38low cells were sorted by FACS to obtain one cell per well. 300 wells were prepared for each condition in experiments 1 and 2, 240 wells in experiment 3. The percentage of positive wells after 24 hours was 65, 43 and 44% for experiment 1, 2 and 3 respectively. The phenotype of clones was determined after 18 days of culture by inverted microscope observation, cytological staining and FACS analysis. The P value, calculated by the paired Student’s t-test, compared the effects of anti-TGF-β1 addition to the CM (*) and CM+FL/bFGF conditions (‡).
viability of cells remained constant at each time point in the three culture conditions, demonstrating that no cell death or division was induced by TGF-β1 or anti-TGF-β1 for the first 48 hours of culture (data not shown). Fig. 2 represents a detailed flow cytometry analysis after 36 hours of treatment with TGF-β1 or anti-TGF-β1 and illustrates the separation of CD34+ cells into FLT3low and FLT3high subpopulations. Fig. 3 summarizes the kinetics of FLT3 expression during the first 48 hours of culture. TGF-β1 decreased FLT3 expression at 24, 36 and 48 hours of incubation (P<0.01, <0.05 and <0.01, respectively). Conversely, anti-TGF-β1 increased FLT3 expression at the same time points (P<0.01, <0.01 and =0.06, respectively). As illustrated in Fig. 4, a similar modulation of Flt3 mRNA was observed by in situ hybridization.

Modulation of IL6 receptor by TGF-β1 or anti-TGF-β1
We further studied whether the IL6-R was modulated by TGF-β1 or anti-TGF-β1 in a way similar to FLT3. Two subpopulations of CD34+ progenitors were defined by flow cytometry on the basis of their capacity to bind IL6: the first, ‘IL6-Rhigh’, had a high capacity to bind IL6 (23.5±17.4%, n=6) and the second, ‘IL6-Rlow’, had a low capacity to bind IL6. CD34+ cells were grown for 48 hours in the presence of TGF-β1, anti-TGF-β1 or in medium alone and analyzed for IL6-R expression at both mRNA and protein level. Fig. 5 presents detailed flow cytometry analyses of IL6-R expression at both mRNA and protein level. Fig. 5 presents detailed flow cytometry analyses of IL6-R. As demonstrated for FLT3, we observed that IL6-R is down-modulated by TGF-β1 (P<0.05) but can be significantly up-modulated by anti-TGF-β1 (P<0.05) at the protein level. A similar modulation of IL6-R α chain mRNA was observed by in situ hybridization, as illustrated by the representative labelling profiles shown in Fig. 6.

Functional assay of CD34+FLT3low/high and CD34+IL6-Rlow/high subpopulations: ‘HPP-Q assay’
All these results lead us to propose the use of anti-TGF-β1 stimulation in a rapid in vitro assay to detect quiescent
progenitors which we call ‘High Proliferative Potential-Quiescent’ cells (‘HPP-Q’ cells). The ‘HPP-Q’ assay that we develop consists of a comparison between a control medium and an identical medium to which anti-TGF-β1 is added. The control medium reveals easily activated progenitors as does the normal mixed colony assay. In addition to these progenitors, the anti-TGF-β1 medium reveals quiescent progenitors which can be quantified by difference between the colony number observed in the two cultures.

In this study, the CD34+ population was separated into subpopulations on the basis of FLT3 and IL6-R expression level in order to perform functional studies. CD34+FLT3low and CD34+FLT3high, CD34+IL6-Rlow and CD34+IL6-Rhigh subpopulations were sorted by FACS and separately submitted to the HPP-Q assay (Table 3). We observed that most of the quiescent CFU-Mix activated by anti-TGF-β1 (‘HPP-Q-Mix’ progenitors) were included in the FLT3low and IL6-Rlow subpopulations: approximately 90% were FLT3low and 80% were IL6-Rlow. Because these two subpopulations were isolated from the same CD34+ population, we can estimate that at least 70% of the HPP-Q-Mix cells were both FLT3low and IL6-Rlow.

**DISCUSSION**

In previous studies, we demonstrated the ability of anti-TGF-β: (a) to reveal quiescent CFU-Mix and CFU-GM in a short term mixed colony assay (Hatzfeld et al., 1991), and (b) to up-modulate c-KIT in the CD34+Kitlow subpopulation (Sansilvestri et al., 1995). However, we did not demonstrate whether a low expression of this receptor correlates with an undifferentiated and quiescent state of primitive hematopoietic progenitors. Above all, we did not demonstrate that the anti-TGF-β1 effect could be extrapolated to other receptors playing a major role in the control of primitive progenitors. In this study, we demonstrate that anti-TGF-β1 up-modulates in a few hours various cytokine receptors such as FLT3 and IL6-R as well as the transferrin receptor (Trf-R) (unpublished results). A schematic design of this mechanism is proposed in Fig. 7. This up-modulation promotes the development and maturation of a subpopulation enriched with primitive quiescent progenitors in a 2-3 week culture assay.

We have first compared the ability of anti-TGF-β1 with that of FL and bFGF to activate subpopulations of hematopoietic progenitors, which remain quiescent in semi-solid or liquid medium containing optimized concentrations of IL3, IL6, GM-CSF, GM-CSF, SF and Epo. In semi-solid medium, anti-TGF-β1 induced primitive progenitor activation as measured by the enumeration of mixed colonies. Larger mixed colonies were observed when plates were not overcrowded. However, this phenomenon was more significant and could be better detailed by studying clonal liquid culture over more than two weeks.

During the first 5 days of clonal liquid culture, anti-TGF-β1 did not allow such a rapid progression of cell proliferation as FL and bFGF (Fig. 1a). In contrast, when we observed the culture at 18 days (Fig. 1b), anti-TGF-β1 activated significantly more clones containing over 10^5 cells. Among these clones, anti-TGF-β1 most significantly increased not only the two or three lineage CFU-Mix but also the more primitive CFU-GEMM containing erythroid, megakaryocytic and myeloid cells (Table 2). Similar results were obtained in semi-solid medium (data not shown) showing that FL and bFGF cannot substitute for anti-TGF-β1.

These results are in agreement with previous reports concerning anti-TGF-β1 (Hatzfeld et al., 1991; Cardoso et al., 1993; Li et al., 1994), FL- (Lyman et al., 1994; Small et al., 1994; Broxmeyer et al., 1995; Hirayama et al., 1995; Muench et al., 1995; Jacobsen et al., 1996; Ohishi et al., 1996; Shah et al., 1996; Yonemura et al., 1997) and bFGF-dependent (Gabianelli et al., 1990; Huang and Terstappen, 1994) activations in short term culture assays. A recent study demonstrated that bFGF could not completely reverse TGF-β1 inhibition of human bone marrow myeloid progenitors.

**Fig. 3.** FLT3 receptor modulation by TGF-β1 or anti-TGF-β1 on UCB CD34+ progenitors. Purified UCB CD34+ cells were cultured in control medium (●), in the presence of TGF-β1 ( ● ) or anti-TGF-β1 ( ▲ ). FLT3 receptors were detected by FACS every 12 hours on at least 10,000 cells for each condition. The number of FLT3high cells found in each culture condition was divided by the number of FLT3high cells present in control medium. This represents means ± s.d. calculated from five independent experiments.

**Table 3. Functional test of the CD34+FLT3low/high and CD34+IL6-Rlow/high subpopulations: ‘HPP-Q’ test**

<table>
<thead>
<tr>
<th>Subpopulation:</th>
<th>CFU-Mix</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FLT3low</td>
</tr>
<tr>
<td>Control (CM)</td>
<td>2.4±2.2</td>
</tr>
<tr>
<td>CM+anti-TGF-β1</td>
<td>8.7±3.2</td>
</tr>
<tr>
<td>Δ=HPP-Q</td>
<td>6.3±1.1</td>
</tr>
</tbody>
</table>

400 sorted UCB CD34+FLT3low, CD34+FLT3high, CD34+IL6-Rlow and CD34+IL6-Rhigh cells were cultured in semi-solid medium with or without anti-TGF-β1 in duplicate in 3 independent experiments. The difference (Δ) between the mean of CFU-Mix in control and anti-TGF-β1 conditions was calculated for each subpopulation and each experiment.
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It is interesting to note that the effect of anti-TGF-β1 is similar whether cells are pretreated for only 6 hours before plating in semi-solid medium or whether the anti-TGF-β1 is maintained or added repeatedly throughout the culture (data not shown).

Taken together, these results suggest that anti-TGF-β1 acts as a competence factor for quiescent progenitors, as defined by the studies of Pledger et al. (1977) and Stiles et al. (1979). Anti-TGF-β1 allows the rapid expression of cytokine receptors in quiescent cells which can then respond to the cytokines present in the culture medium. We define HPP-Q cells as primitive progenitors for which very low concentrations of TGF-β1 can down-modulate receptors controlling their cycling status.

When a quiescent stem/progenitor cell is activated, it maintains for a few divisions its immature phenotype and its high proliferative potential. For example, there are LTC-IC which are quiescent and others which are not. Similarly, we have evidence that even after activation, HPP-Q cells maintain for at least one division their ability to return to a quiescent state in response to physiological concentrations of TGF-β1. On the contrary, when these cells start to differentiate, they loose their high sensitivity to low concentrations of TGF-β1.

It is important to note that a low but significant expression of Flt3 and IL6-R mRNA is detected in cells cultured in the control medium or in the presence of TGF-β1 (Figs 4 and 6). Moreover, we know that the fluorescent activated cell sorter cannot detect a low level of receptors. This is the reason why the sorted subpopulations containing HPP-Q cells are not called ‘negative’ but ‘low’ for the expression of FLT3 and IL6-R.

Fig. 4. Flt3 mRNA modulation by TGF-β1 or anti-TGF-β1 on UCB CD34+ progenitors. UCB CD34+ cells were grown in liquid culture for 24 hours in the following conditions: (A) Control Medium (CM); (B) CM + 10 μg/ml anti-TGF-β1 blocking antibody and (C) CM + 5 ng/ml TGF-β1. Cells were harvested after 24 hours and treated by in situ hybridization with an antisense 35S-labelled riboprobe corresponding to the extracellular domain of FLT3. Grain labelling was counted by semi-automatic image analysis. We scored as ‘positive’ cells containing more than 10 grains, in comparison to a negative control (D) hybridized with a sense 35S-labelled riboprobe. The percentage of positive cells is indicated as well as the mean of grains per cell for 100 cells. These labelling profiles are representative from similar results of three independent experiments where cell samples were analyzed in duplicate.

Fig. 5. IL6 receptor modulation by TGF-β1 or anti-TGF-β1 on UCB CD34+ progenitors. UCB CD34+ cells were cultured with 1 ng/ml TGF-β1, 10 μg/ml anti-TGF-β1 or in medium alone (CM). These cells were harvested after 48 hours of culture, labelled with biotin-IL6/FITC-avidin and analyzed by FACS. The percentage of CD34+IL6-Rhigh cells is calculated from samples corresponding to at least 10,000 cells. This represents one typical experiment out of three.
R (Table 3). A low level of FLT3 expression is not sufficient for a rapid short term expansion of HPP-Q cells. However, it could be sufficient to explain why a 10-day pretreatment by FL amplifies slow cycling LTC-IC (Petzer et al., 1996). It is not clear whether this pretreatment amplifies cycling or quiescent LTC-IC as both subpopulations exist. Determination of the overlap between the LTC-IC and HPP-Q populations would help address this issue.

The working concept of High Proliferative Potential Quiescent cells or HPP-Q refers to highly proliferative progenitors which can be activated within hours after neutralization of autocrine or endogenous TGF-ß. This working concept relies on many studies performed on molecular controls of cell cycle inhibitors. Indeed, G1-S transition inhibitors such as Rb or p15 have been demonstrated to be under TGF-ß1 control in various systems (Reynisdottir et al., 1995) including hematopoietic cells (Hatzfeld et al., 1991). However, it does not mean that the primitive quiescent cells are not regulated by other negative factors or cannot be revealed by other in vitro methods such as the LTC-IC (Sutherland et al., 1990), CAFC (Breems et al., 1994) and Blast assays (Nakahata and Ogawa, 1982). Other mechanisms might also control quiescent progenitors in vivo as well (Larochelle et al., 1996; Baum et al., 1992; Zanjani et al., 1992).

It is possible that, in the future, the use of new cytokines and improved culture systems will allow the detection of still more primitive progenitors such as lympho-myeloid progenitors in short term culture. However, we expect that most of them will be quiescent. We may hypothesize that they will express low levels of FLT3, IL6-R, Trf-R, and perhaps other new receptors also down-modulated by TGF-ß1. In this case, the addition of anti-TGF-ß1 would still improve in short term culture the efficiency of the new combination of cytokines to release from quiescence these progenitors.

Does TGF-ß1 play a physiological role in vivo regulating the quiescence of stem cells? In TGF-ß1 knockout mice, defective hematopoiesis has been described (Dickinson et al., 1995). However, the implication of TGF-ß1 in the development of many other tissues renders these results difficult to interpret. Clinical studies are more informative by providing various cases where tumorigenic progenitors which produce autocrine TGF-ß have a selective advantage due to a loss of TGF-ß receptors. The most commonly held model for TGF-ß1 signalling proposes that TGF-ß1 first binds to TGF-ß receptor type II (TGF-ßRII), which is a constitutively active kinase and that this complex secondly recruits TGF-ß receptor type I (TGF-ßRI), causing its phosphorylation and signal propagation to downstream substrates (Wrana et al., 1994). We have detected both TGF-ßRI and TGF-ßRII in various immature subpopulations of normal human hematopoietic progenitors, confirming the possible regulatory role of TGF-ß1 in the early stages of hematopoiesis. The importance of TGF-ß1 in the control of human hematopoiesis has recently been better demonstrated in vivo by studying disregulated systems such as cancer cells. Production of active TGF-ß1 by leukemic cells has been reported to inhibit the growth of normal hematopoietic cells in the bone marrow microenvironment. A selective advantage was given to the tumor cells by the loss of TGF-ßRI or TGF-ßRII. This is one mechanism by which leukemic cells acquire resistance to growth inhibition in the development of lymphoproliferative malignancies (Le Bousse-Kerdiles et al., 1996; DeCoteau et al., 1997). It is interesting to note that a loss of functional anti-TGF-ß1 activates HPP-Q cells

Fig. 6. IL6 Receptor α chain mRNA modulation by TGF-ß1 or anti-TGF-ß1 on UCB CD34+ progenitors. UCB CD34+ cells were grown in liquid culture for 48 hours in the following conditions: (A) control medium (CM); (B) CM + 10 μg/ml anti-TGF-ß1 blocking antibody and (C) CM + 1 ng/ml TGF-ß1 added at the onset of the cultures. Cells were harvested after 48 hours and treated by in situ hybridization with an antisense 35S-labelled riboprobe corresponding to the α chain of the IL6 receptor (IL6-Rα). (D) cells cultured in CM and hybridized with an IL6-Rα sense 35S-labelled riboprobe. Grain labelling was counted by semi-automatic image analysis. We scored ‘positive’ cells containing more than 10 grains. The percentage of positive cells is indicated as well as the mean of grains per cell for 100 cells. These labelling profiles are representative from results of three similar independent experiments where cell samples were analyzed in duplicate.

Fig. 7. A model of quiescent hematopoietic progenitor cell activation.
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