Further Studies on Growth Factor Production by the TC-1 Stromal Cell Line: Pre-B Stimulating Activity

By Timothy A. Woodward, Ian K. McNiece, Pamela L. Witte, Patrick Bender, Rowena Crittenden, Daniel S. Temeles, Brian E. Robinson, Gwen B. Baber, Donna H. Deacon, Peter C. Isakson, and Peter J. Quesenberry

Adherent murine stromal cells support long-term in vitro lymphopoiesis or myelopoiesis dependent on the culture conditions used. A cell line, TC-1, isolated from long-term liquid murine marrow cultures under conditions approaching those permissive for lymphoid growth, has been found to produce an activity that acts synergistically with interleukin-3 (IL-3) or colony-stimulating factor-1 (CSF-1) to stimulate in vitro myeloid colonies, but which has no intrinsic colony-stimulating activity. We report here the presence of multiple growth factors in conditioned medium (CM) from the TC-1 line, including granulocyte-macrophage colony-stimulating factor (GM-CSF) (bioassay with antibody block and messenger RNA [mRNA] analysis), granulocyte CSF (G-CSF) and IL-4 (factor-dependent cell line bioassay), and CSF-1 (radioimmunoassay, mRNA) along with a pre-B cell inducing activity, which appears separate from these CSFs and segregates with the myeloid synergizing activity through anion exchange, sizing, and Concanavalin A chromatography. Because these activities are not yet purified to homogeneity, their identity or lack of identity remains an open question. Assays of TC-1 CM or cellular mRNA analysis have given negative results for IL-1, IL-2, IL-3, IL-6, and IL-7. IL-6 does not stimulate pre-B cells in this assay. However, IL-4 and G-CSF do stimulate in vitro induction of pre-B cells from pre-B and B-cell-depleted Balb/C marrow and are present in CM by selective cell line assay. A monoclonal antibody to IL-4 that inhibited its pre-B inducing activity did not inhibit pre-B inducing activity of TC-1 CM. These data suggest the existence of a unique synergizing and pre-B inducing factor(s) in TC-1 CM. Given the known capacity of subliminal levels of growth factors to act synergistically, an alternate possibility is that these biologic phenomena represent the actions of low concentrations of growth factors acting synergistically and possibly associated with some core protein.

© 1990 by The American Society of Hematology.

We have previously reported that a murine stromal cell line produces high levels of colony-stimulating factor 1 (CSF-1) plus a myeloid synergizing activity. Other investigators have demonstrated the presence of a variety of growth factors, including the recently described B cell factors interleukin-6 (IL-6) and IL-7, in supernatants from different stromal cell lines. In addition, there are a growing number of examples of growth factors that show cross-lineage stimulation of both myeloid and lymphoid lineages (IL-1, IL-4, IL-5, IL-6, and IL-7) and that interact synergistically to augment various functions or proliferative effects. Thus, the possibility exists that any described bioactivity in a nonpurified mixture of molecules may represent the effects of mixtures of growth factors acting synergistically. In addition, since these synergisms may occur at low concentrations of growth factors, a negative result in the primary growth factor assay does not necessarily indicate that a synergistic biologic effect is not mediated by a specific growth factor. Thus, it is important to define the growth factor activities produced by a cell population and to consider the possibility of synergistic interactions between these growth factors.

Accordingly, we have continued our studies on growth factor production by the TC-1 cell line. Here we report the presence of a pre-B inducing activity and a number of known growth factors as detected by bioassay of conditioned medium or analysis of messenger RNA (mRNA) species by cDNA hybridization. In addition, we have defined IL-4 and granulocyte-CSF (G-CSF) as agents capable of inducing pre-B cells from murine marrow in liquid culture.

Materials and Methods

Bone marrow cells were obtained from femoral and tibial blowouts from 6- to 8-week-old female BALB/c mice (Dominion Labs, Dublin, VA), CBA/J mice 5 to 8 weeks old, and BDF-1 mice 11 to 16 weeks of age (Jackson Labs, Bar Harbor, ME). Cells were flushed from bones with RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 2 mmol/L L-glutamine, vitamins, 1 mmol/L sodium pyruvate, nonessential amino acids, 0.05 mmol/L 2-mercaptoethanol, penicillin, streptomycin, and 5% prescreened fetal calf serum (FCS).

Antibodies. Conditioned medium from rat hybridoma line 14.8 (ATCC, Rockville, MD), concentrated 10-fold on an Amicon XM-50 membrane (Amicon, Danvers, MA) was the source of 14.8 antibodies. Monoclonal antibody (MoAb) 14.8 recognizes epitopes on murine pre-B and B cells and some T lymphocytes. This MoAb recognizes the B220 surface glycoprotein. Affinity-purified goat anti-mouse immunoglobulin (Ig) and anti-rat Ig were obtained from Southern Biological Associates, Birmingham, AL. These antibodies were used for depleting B and pre-B cells by panning. A monoclonal rat anti-IL-4 antibody, affinity purified from conditioned medium (CM) from the hybridoma 11B11, was provided by Dr W.E. Paul (National Institutes of Health, Bethesda, MD), and a control rat MoAb was provided by Dr E. Vitieta (University of Texas Southwestern Medical School, Dallas). Tetramethyl rhodamine isothiocyanate (TRITC)-conjugated affinity purified goat anti-mouse μ antibodies and fluorescein isothiocyanate (FITC)-conjugated affinity purified goat anti-rat Ig and anti-mouse Ig were also obtained from Southern Biological Associates. All antibodies were used at saturating concentrations in antibody binding studies.
Growth factors. TC-1 preparations consisted of neat and partially purified CM from the TC-1 cell line grown in Fischer's medium and supplemented with 0.1% to 20% FCS with the 0.1% to 1% FCS levels also containing 1% Nutridoma supplement (Boehringer-Mannheim Biochemicals). Initial separations included anion exchange on DEAE cellulose, Concanavalin A chromatography, and size selection on G-100 Sephadex. Fractions showing synergy with CSF were found in the salt eluates (0.5 to 1.0 mol/L) from DEAE, in the α-methyl mannoside eluate from Concanavalin A and in the high molecular weight (greater than 80,000 dalton) fractions from G100 gel filtration. These same fractions were tested for pre-B inducing activity. Fractions that were active for CSF-1 synergy were evaluated for pre-B induction. In selected instances, neat or partially purified material were rendered free of CSF by chromatography on CSF-1 antibody columns as previously described. Pre-B cell stimulatory factor-1 or IL-4 preparations included supernatants from the EL-4 cell line eluted from 1B11-Sepharose as previously described or recombinant IL-4 and mock cos supernate controls (courtesy of Dr Donna Rennick, DNAX, Palo Alto, CA). Recombinant murine granulocyte-macrophage CSF (rmGM-CSF) was obtained from Dr J. DeLamarter, Biogen Research Corp, Geneva, Switzerland, and purified recombinant human G-CSF and recombinant human IL-6 from Dr Larry Souza, Amgen, Inc, Thousand Oaks, CA. Pure IL-3, as previously described, was provided by Dr Jim Ihle, Frederick Cancer Center, Frederick, MD. Levels of activity (or factors) tested for pre-B induction were expression positive from 18.05% of 14.8+ cells were scored to assess pre-B depletion. In different experiments, 14.8+ cells to less than 1%.

Cell depletion. Murine marrow was depleted of surface Ig positive and 14.8 positive cells by incubation of marrow cells at 20 x 10^6 cells/3 mL in Falcon 1001 Petri dishes (100 x 5 mm) (Thomas Scientific, Swedesboro, NJ). Briefly, one set of Petri dishes were first incubated with 50 μg of affinity purified goat anti-mouse Ig and another set of Petri dishes with 50 μg of affinity purified mouse anti-rat Ig, both in 0.5 mol/L Tris (hydroxymethyl) aminoethane buffer, pH 8.0, at 4°C for 1 hour. Plates were then washed three times with phosphate-buffered saline (PBS). The mouse anti-rat Ig plates were then incubated with 100 μg (10 x CM) of 14.8 antibody for 20 minutes at 4°C, swirled, and then incubated for an additional 30 minutes. Marrow cells (20 x 10^6 cells in 3 mL) were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes.

Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%. Different immunofluorescent antibodies were used to assess pre-B depletion: in different experiments, 14.8+, μ- or μ'/Ig of cytoplasmic light chain negative (see below) cells were scored to assess pre-B cell depletion. In 22 experiments, 84.5% of 14.8+ cells were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes. Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%.

Different immunofluorescent antibodies were used to assess pre-B depletion: in different experiments, 14.8+, μ- or μ'/Ig or cytoplasmic light chain negative (see below) cells were scored to assess pre-B cell depletion. In 22 experiments, 84.5% of 14.8+ cells were washed three times with phosphate-buffered saline (PBS). The mouse anti-rat Ig plates were then incubated with 100 μg (10 x CM) of 14.8 antibody for 20 minutes at 4°C, swirled, and then incubated for an additional 30 minutes. Marrow cells (20 x 10^6 cells in 3 mL) were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes. Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%.

Different immunofluorescent antibodies were used to assess pre-B depletion: in different experiments, 14.8+, μ- or μ'/Ig or cytoplasmic light chain negative (see below) cells were scored to assess pre-B cell depletion. In 22 experiments, 84.5% of 14.8+ cells were washed three times with phosphate-buffered saline (PBS). The mouse anti-rat Ig plates were then incubated with 100 μg (10 x CM) of 14.8 antibody for 20 minutes at 4°C, swirled, and then incubated for an additional 30 minutes. Marrow cells (20 x 10^6 cells in 3 mL) were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes. Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%.

Different immunofluorescent antibodies were used to assess pre-B depletion: in different experiments, 14.8+, μ- or μ'/Ig or cytoplasmic light chain negative (see below) cells were scored to assess pre-B cell depletion. In 22 experiments, 84.5% of 14.8+ cells were washed three times with phosphate-buffered saline (PBS). The mouse anti-rat Ig plates were then incubated with 100 μg (10 x CM) of 14.8 antibody for 20 minutes at 4°C, swirled, and then incubated for an additional 30 minutes. Marrow cells (20 x 10^6 cells in 3 mL) were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes. Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%.

Different immunofluorescent antibodies were used to assess pre-B depletion: in different experiments, 14.8+, μ- or μ'/Ig or cytoplasmic light chain negative (see below) cells were scored to assess pre-B cell depletion. In 22 experiments, 84.5% of 14.8+ cells were washed three times with phosphate-buffered saline (PBS). The mouse anti-rat Ig plates were then incubated with 100 μg (10 x CM) of 14.8 antibody for 20 minutes at 4°C, swirled, and then incubated for an additional 30 minutes. Marrow cells (20 x 10^6 cells in 3 mL) were first incubated on anti-Ig coated plates at 4°C for 70 minutes. Surface Ig nonadherent cells were washed gently from the plates and then incubated on two series of anti-14.8 Petri dishes as described above for the anti-mouse Ig Petri dishes. Different panning approaches were used (in attempts to improve panning efficiency) throughout the course of these studies. Variations on the panning procedures included using two or three 14.8+ pans, without an IgG pan, or using one IgG and three 14.8+ pans. In general, panning reduced FITC-goat anti-mouse Ig positive cells to less than 1%.
methyl mercuhydr oxide or formamide agarose gels. The gel was
capillary blotted onto zeta bind paper and further electrophotobotted
overnight. Poly A+ or whole RNA from EL-4 cells previously
exposed to phorbol myristate acetate or from WEHI-3 cells served as
positive controls for GM-CSF and IL-3 expression, respectively. The
hybridization probes were: (1) A 750-base pair (bp) fragment of the
murine GM-CSF cDNA29 (pGMS19 provided by Dr N. Gough, Ludwii Institute, Melbourne, Australia) prepared by BamHI and
EcoRI digestion of plasmid pJL4; (2) a full-length murine G-CSF
cDNA30 (provided by S. Nacata, Osaka, Japan) prepared by excision of the 1,400-bp fragment from the plasmid pMG2 using
EcoRI digestion of plasmid pJL4; (3) a full-length murine G-CSF
cDNA from pcDV1 using Neol and HindII133; and (6) a
1,633 bp insert of the IL-5 cDNA excised from pcDSR with BamHl.

RESULTS

CM from the TC-1 cell line reproducibly stimulated pre-B
cell formation over 18 hours of culture (Table 1). Similar
results were obtained at 42 hours of culture or if marrow
harvested from mice 3 or 8 days after intravenous administra-
tion of 150 mg/kg 5-fluorouracil was assayed (data not
shown). CM depleted of CSF-1 was fully active (Table 1).
CM partially purified for CSF-1 and IL-3 synergistic activity
by separation or DEAE cellulose, Concanavalin A, G-100
Sephadex, and anti-CSF chromatography induced pre-B
cells in liquid culture. Light density marrow cells depleted of
adherent, B, pre-B, and T cells, while showing higher
background pre-B levels, responded to TC-1 CM by generat-
ing high levels of pre-B cells (Table 2).

TC-1 contains GM-CSF, CSF-1 (Fig 1), IL-4, and proba-
bly G-CSF (Table 3). There is also present another B-cell
differentiating activity, termed B-cell stimulatory factor
(BSF-TC), which is physically separable from the pre-B and
CSF-1 synergizing activity described here.35 As noted above,
preparations free of CSF-1 had pre-B activity. Recombinant
GM-CSF has no pre-B inducing activity (117% ± 18% of
control in three experiments). Similarly, both IL-3, rhIL-6,
and CSF-1 were without significant pre-B inducing activity (single experiments). TC-1 CM was negative for ILs 1, 2, 3, 6, and 7 by thymocyte proliferation or selective cell line stimulation assays (Table 3). Extensive investigation for the presence of IL-3 mRNA probing whole or Poly A selected TC-1 RNA gave negative results. Negative results were also obtained with regard to the presence of IL-5, IL-6, or IL-7 mRNA.

Stimulation of pre-B cell generation from nonadherent cells from long-term lymphoid marrow cultures. In the presence of TC-1 CM, lymphocytes from Whitlock-Witte type cultures were stimulated to generate pre-B cells (detected by MoAb 14.8 or by expression of cytoplasmic $\mu$) (Table 4). There was a lesser effect on sIg$^+$ cells, and CM from the Whitlock-Witte cultures had marginal effects on pre-B cell generation. Pre-B generation from these nonadherent cells was quite variable but was consistently stimulated by TC-1 CM as compared with control cultures or cultures exposed to 50% CM from the Whitlock-Witte cultures. No differences were found between experiments using lymphocytes from Whitlock-Witte cultures that were unseparated or had been depleted of 14.8$^+$ cells by panning, and these were therefore pooled for presentation in Table 4. Supernates from clones of the TC-1 cell line (TC-1-C3 and TC-1-C11)$^*$ were also effective in stimulating an increase in 14.8$^+$ cell frequency (data not shown).

Effects of IL-4 on in vitro pre-B cell generation. Crude preparations of IL-4 or recombinant IL-4 have pre-B inducing activity in this assay system against both unseparated (Table 5) and separated adherent B- and T-cell-depleted marrow (Table 6). Results similar to those presented in Tables 5 and 6 against unseparated marrow were seen at 5,

### Table 2. Pre-B Induction by Partially Separated TC-1 Conditioned Media Tested Against Normal and Separated Marrow

<table>
<thead>
<tr>
<th>Marrow Group</th>
<th>$\mu^* \times 10^{-9}/10^6$ Cells Cultured</th>
<th>% Control</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Balb/C marrow</td>
<td>1.696 ± .2</td>
<td>357 ± 75</td>
<td>6</td>
</tr>
<tr>
<td>Light density, nonadherent T- and B-cell depleted marrow†</td>
<td>6.48 ± .6</td>
<td>219 ± 6</td>
<td>4</td>
</tr>
</tbody>
</table>

*CM with 5% FCS and then eluted from DEAE cellulose and Concanavalin A (see Materials and Methods).
†See Materials and Methods.

![Fig 1. TC-1 mRNA analysis for CSF-1 and GM-CSF. Northern blot of TC-1 RNA hybridized with cDNA probes for CSF-1 and GM-CSF (see Materials and Methods).](image)
Table 4. Stimulation of Pre-B Cell Generation by TC-1 CM

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Stimulation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.8 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>3.0 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>2.0 ± 10^-11</td>
</tr>
<tr>
<td>TC-1a</td>
<td>(1.3-12.9)</td>
</tr>
<tr>
<td></td>
<td>(1.0-9.0)</td>
</tr>
<tr>
<td></td>
<td>(1.3-4.6)</td>
</tr>
<tr>
<td>TC-1b</td>
<td>3.0 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>5.4 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>(1.5-3.5)</td>
</tr>
<tr>
<td></td>
<td>(1.2-15.4)</td>
</tr>
<tr>
<td></td>
<td>(1.0-1.8)</td>
</tr>
<tr>
<td>CM from Whitlock-Witte culture</td>
<td>1.1 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>1.3 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 10^-11</td>
</tr>
<tr>
<td></td>
<td>(1.0-1.2)</td>
</tr>
<tr>
<td></td>
<td>(1.0-1.9)</td>
</tr>
<tr>
<td></td>
<td>(1.0-2.6)</td>
</tr>
</tbody>
</table>

TC-1 a and b were CM from TC-1 cell line at 5% or 20% FCS. TC-1 CM were tested at 10%. Stimulation Index expressed as fold increase over control (media) values of particular cell types after hours of culture (see Materials and Methods). Results represent mean from a total of five experiments. Numbers in parentheses represent the ranges of individual experimental values.

100, and 400 U/mL rIL-4 using either $\mu^+$ or $\mu^-$ surface Ig, or cytoplasmic light chain negative criteria for pre-B cells. However, an MoAb to IL-4 that blocked pre-B induction by rIL-4 or EL-4 supernates did not block pre-B induction by TC-1 CM (Fig 2). This indicates that while IL-4 is a pre-B inducer in this assay system the pre-B inducer in TC-1 CM is probably not IL-4.

DISCUSSION

Dexter et al 47 and Whitlock and Witte 48 culture systems present models for regulation of myeloid and lymphoid differentiation, respectively. Adherent stromal cells are necessary in each system for ongoing cell production and appear to selectively support early hematopoietic stem cells. Explant Dexter stroma or cell lines derived from Dexter or Whitlock-Witte cultures appear capable of producing a variety of hemolymphopoietic growth factors, including CSF-1, G-CSF, GM-CSF, IL-6, IL-1, and IL-7, which may be involved in early stem cell regulation.19,40,44,49-55

The present studies indicate that a stromal cell line, TC-1, isolated from stromal cultures under conditions approaching lymphoid permissiveness, produces GM-CSF, CSF-1, G-CSF, IL-4, and a pre-B inducing activity. This cell line also produces a CSF-1 and IL-3 synergistic activity,2 and this synergistic activity segregates with a pre-B activity through three biochemical separative steps. The pre-B activity may or may not be identical with the synergistic activity but appears to be separate from CSF-1, GM-CSF, and G-CSF as determined by selective bioassay, antibody blocking, and differences in molecular size. G-CSF has pre-B inducing activity but, as opposed to the TC-1 pre-B activity, appears to act indirectly via accessory cells and has a much lower apparent molecular weight. Supernatants from the TC-1 cell

Table 5. IL-4 Induction of Pre-B Cells in Liquid Culture

<table>
<thead>
<tr>
<th>Group</th>
<th>$\mu^+ \times 10^3/10^6$ Cells Cultured</th>
<th>% Control</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.67 ± 0.6</td>
<td>187 ± 28</td>
<td>6</td>
</tr>
<tr>
<td>EL-4 BSF-1*</td>
<td>8.04 ± 0.7†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cos Control</td>
<td>3.25 ± 0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rIL-4 (BSF-1)</td>
<td>4.95 ± 0.6†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 U/mL†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. IL-4 Induction of Pre-B Cells From Murine Marrow

<table>
<thead>
<tr>
<th>Group</th>
<th>$\mu^+ \times 10^3/10^6$ Cells Cultured</th>
<th>% Control</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unseparated Balb/C marrow</td>
<td>1.97 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rIL-4 10 U/mL</td>
<td>6.56 ± 2.6</td>
<td>360 ± 42</td>
<td>4</td>
</tr>
<tr>
<td>Depleted Balb/C marrow†</td>
<td>6.48 ± 1</td>
<td>237 ± 30</td>
<td>2†</td>
</tr>
</tbody>
</table>

*Control media and not cos media were used in these experiments. In a total of 22 experiments, cos supernatants gave generally equivalent results to diluent media controls. Pre-B cell induction by cos supernatants as compared with control media was 110% ± 12% of control.
†Light density, nonadherent marrow sequentially depleted of B, pre-B, and T cells (see Materials and Methods).
‡Two experiments, four individual cultures.

Fig 2. Effect of MoAb against IL-4 (BSF-1) on pre-B stimulation by TC-1 CM or IL-4. The effect of anti--BSF-1 at 1 to 2 µg/mL on pre-B induction over 18 hours of culture (see Materials and Methods) expressed as a percent of rat IgG control.

From www.bloodjournal.org by guest on April 26, 2017. For personal use only.
STROMAL PRE-B STIMULATING ACTIVITY

Table 7. rhG-CSF Effect on Pre-B Cell Generation

<table>
<thead>
<tr>
<th>Media</th>
<th>Control</th>
<th>G-CSF 100 U/mL</th>
<th>% Control</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.40 ± .37</td>
<td>5.38 ± .4</td>
<td>177 ± 10*</td>
<td>39</td>
</tr>
</tbody>
</table>

*p < .01.

REFERENCES

12. Sanderson C: Eosinophil differentiation factors (IL-5). Presented at "Haemopoietic Growth and Differentiation" meeting, August 7-9, 1988, Melbourne, Australia.

LINE ARE NEGATIVE FOR IL-1, IL-2, IL-3, IL-6, AND IL-7 (Table 7), AND mRNA STUDIES ARE NEGATIVE FOR IL-3, IL-5, IL-6, AND IL-7. THE B-CELL ACTIVITY (BSF-TC) IS PRESENT IN TC-1 CM* BUT IS PHYSICALLY SEPARABLE FROM THE PRE-B ACTIVITY AND OF SMALL (APPROXIMATELY 15,000 DALTON) MOLECULAR WEIGHT.

These Data Suggest That TC-1 Cells Produce Unique Pre-B AND MYELOID SYNERGIZING ACTIVITIES, POSSIBLY RESIDENT IN THE SAME MOLECULE, AND THAT THESE ACTIVITIES MAY BE IMPORTANT FOR EARLY PROGENITOR/STEM CELL SUPPORT. FURTHER, THEY INDICATE THAT THE PRE-B AND MYELOID SYNERGIZING ACTIVITIES ARE HIGH MOLECULAR WEIGHT (GREATER THAN 120,000 DALTON) GLYCOPROTEINS (BINDING TO CONCONEVALIN A). Considering the growing body of information showing that synergy between growth factors is common[10,14,16-21] that low-dose synergy occurs,[21] and that GM-CSF + CSF-1, G-CSF + GM-CSF, G-CSF, AND CSF-1[19,20] ARE AMONG THE COMBINATIONS THAT SHOW POTENT MYELOID SYNERGY, ESPECIALLY ON HIGH PROLIFERATIVE POTENTIAL TYPE STEM CELLS, AN ALTERNATIVE POSSIBILITY IS THAT THE PRE-B AND MYELOID SYNERGIZING ACTIVITIES MAY REPRESENT COMBINATIONS OF GROWTH FACTORS ACTING AT VERY LOW CONCENTRATIONS (BELOW DETECTION IN THEIR PRIMARY ASSAY SYSTEM) AND BOUND TO A CORE PROTEIN. THIS LATTER FACT COULD ACCOUNT FOR THE APPARENT HIGH MOLECULAR WEIGHT OF THESE ACTIVITIES. RESOLUTION OF THESE TWO POSSIBILITIES WILL AVOID FINAL PURIFICATION TO HOMOGENEITY OF THE PRE-B AND MYELOID SYNERGIZING ACTIVITIES.

From www.bloodjournal.org by guest on April 26, 2017. For personal use only. 
46. Song Z, Thomas C, Innes D, Quesenberry P: A characterization of two clones isolated from the TC-1 murine marrow stromal cell line: Growth factor and retrovirus production and physical support of hemopoiesis. Int J Cell Cloning 6:125, 1988
51. Hines DL: Lipid accumulation and production of colony-stimulating activity by the 266 AD cell line derived from mouse bone marrow. Blood 61:397, 1983
55. Temeles DS, McGrath HE, Shadduck RK, Quesenberry PJ: Induction of growth factor production in populations of murine stromal cells. (in preparation)
Further studies on growth factor production by the TC-1 stromal cell line: pre-B stimulating activity

TA Woodward, IK McNiece, PL Witte, P Bender, R Crittenden, DS Temeles, BE Robinson, GB Baber, DH Deacon and PC Isakson