Differential Effects of TGF-β1 on Lymphohemopoiesis in Long-Term Bone Marrow Cultures

By Shin-Ichi Hayashi, Jeffrey M. Gimble, Anna Henley, Larry R. Ellingsworth, and Paul W. Kincade

Latent transforming growth factors beta (TGF-β) are easily detectable in embryonic and adult hematopoietic tissues and in vitro studies show that they are potent antagonists of lymphopoiesis and myelopoiesis when converted to biologically active form. To learn more about possible roles in hematopoiesis, active TGF-β was added to cultures prepared to support myeloid cells (Dexter conditions) or B lineage lymphocytes (Whitlock-Witte conditions) and studied in detail. Hematopoiesis was permanently arrested in Dexter cultures treated with 40 pmol/L (1 ng/mL) of active TGF-β from initiation. In addition, adipogenesis was inhibited in a dose-dependent manner, and adherent layers from treated cultures were defective when rechallenged with fresh bone marrow cells. Ongoing neutrophil production was terminated in established cultures when addition of the factor was delayed for 8 weeks. In contrast, in experiments with Whitlock-Witte cultures, some of the flasks produced lymphocytes in the continuous presence of TGF-β1 (40 pmol/L). Lymphopoiesis was completely arrested by ten-fold higher concentrations, and this was most effective when added at the beginning of culture. Precursors of lymphocytes as well as the microenvironmental elements necessary for supporting their growth survived 2 weeks of cytokine treatment (400 pmol/L) in Dexter cultures. Normal outgrowth of lymphocytes occurred when the cultures were switched to Whitlock-Witte conditions. Surface marker expression on lymphocytes growing in TGF-β1 resistant or previously treated cultures was not unusual. These studies demonstrate that TGF-β1 is a negative regulator of hematopoiesis in long-term cultures and show that this includes effects on microenvironmental elements. At low concentrations, production of myeloid cells was preferentially affected.

MANY CYTOKINES have been discovered that stimulate the replication and differentiation of lymphoid and myeloid progenitors. Transforming growth factors beta (TGF-β) are among the few known antagonists for these responses. Members of this highly conserved and closely related family of molecules were first identified through stimulation of anchorage-independent fibroblast growth and collagen synthesis. Subsequent studies revealed that they are potent inhibitors of certain lymphocyte responses and myeloid colony formation. TGF-β are made in latent form and stored in platelets and bone. Acidification renders them biologically active form. To learn more about possible roles in hematopoiesis, active TGF-β was added to cultures prepared to support myeloid cells (Dexter conditions) or B lineage lymphocytes (Whitlock-Witte conditions) and studied in detail. Hematopoiesis was permanently arrested in Dexter cultures treated with 40 pmol/L (1 ng/mL) of active TGF-β from initiation. In addition, adipogenesis was inhibited in a dose-dependent manner, and adherent layers from treated cultures were defective when rechallenged with fresh bone marrow cells. Ongoing neutrophil production was terminated in established cultures when addition of the factor was delayed for 8 weeks. In contrast, in experiments with Whitlock-Witte cultures, some of the flasks produced lymphocytes in the continuous presence of TGF-β1 (40 pmol/L). Lymphopoiesis was completely arrested by ten-fold higher concentrations, and this was most effective when added at the beginning of culture. Precursors of lymphocytes as well as the microenvironmental elements necessary for supporting their growth survived 2 weeks of cytokine treatment (400 pmol/L) in Dexter cultures. Normal outgrowth of lymphocytes occurred when the cultures were switched to Whitlock-Witte conditions. Surface marker expression on lymphocytes growing in TGF-β1 resistant or previously treated cultures was not unusual. These studies demonstrate that TGF-β1 is a negative regulator of hematopoiesis in long-term cultures and show that this includes effects on microenvironmental elements. At low concentrations, production of myeloid cells was preferentially affected.

MATERIALS AND METHODS

Mice. BALB/cJ, C57BL/6J, CBA/H-T6T6, and CBA/N mice were originally purchased from Jackson Laboratory (Bar Harbor, ME). All animals were maintained in the OMRF Laboratory Animal Resources Facility.

Cell cultures. Long-term bone marrow cultures of B-lineage cells were prepared according to Whitlock et al (Whitlock-Witte cultures). Briefly, the pooled bone marrow cells from femora and tibiae were placed into 100 mm tissue culture dishes (10 to 16 x 10^6 cells/dish) (#25020, Corning, Corning, NY) or 25 cm^2 Flasks (5 to 6.5 x 10^6 cells/dish) (#25100, Corning). The culture medium consisted of RPMI-1640 (GIBCO, Grand Island, NY), 2 mmol/L L-glutamine (GIBCO), 100U/mL penicillin, 100 µg/mL streptomycin (GIBCO), 5 x 10^-3 mol/L 2-mercaptoethanol and 5% fetal calf serum (FCS; Hyclone, Lot #1110638, Logan, UT). The cultures were maintained in 5% CO₂ at 37°C and fed by replacing one half volume of fresh medium once a week. Lymphopoiesis was harvested by gentle pipetting or treatment with Hanks balanced salt solution without Ca²⁺ or Mg²⁺ (HBSS; GIBCO) containing 0.02% ethylene diamine tetracetic acid (EDTA; Sigma Chemical Company, St Louis) for 5 minutes at 37°C. Long-term cultures of myeloid cells were initiated and maintained using methods described by Dexter et al (Dexter cultures). Briefly, 10 to 13 million bone marrow cells were cultured in 25 cm² flasks. The culture medium consisted of alpha-MEM (GIBCO, without ribonucleosides and deoxynucleosides), penicillin-streptomycin, 10⁻¹ mol/L hydrocortisone sodium salt (Elkins-Sinn, Inc, Cherry Hill, NJ) and 20% horse serum (GIBCO). These flasks were incubated in 5% CO₂ at 37°C, and one half of the medium (4 mL) was replaced with fresh medium weekly.

After Dexter cultures were established (6 to 8 weeks) in some experiments conditions were switched to those of Whitlock-Witte culture (switch cultures). After gentle agitation all of the original medium was aspirated, the recovered cells spun down, and then washed twice in Whitlock-Witte medium. Adherent cells were also washed in 5 mL of new medium and one half of the nonadherent cell fraction was returned to the flasks. The cultures were then maintained at 37°C.

From the Oklahoma Medical Research Foundation, Oklahoma City, OK, and the Collagen Corporation, Oklahoma City, OK. Submitted February 14, 1989; accepted June 13, 1989. Supported by Grant Nos. AI-19884 and AI-20069 from the National Institutes of Health. Address reprint requests to Paul Kincade, MD, Oklahoma Medical Research Foundation, 825 NE 13th St, Oklahoma City, OK 73104. The current address for Dr Hayashi is the Department of Immunopathology, Kumamoto University Medical School, Kumamoto, Japan. The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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Antibodies

Monoclonal rat 14.8 antibody was produced in our laboratory.17 Hybridoma derived antibodies against MAC-1 (M1/70)24 and H-2D\(^{d}\) (34-5-85) were produced by lines acquired from the American Type Culture Collection (Rockville, MD). BP-I antibody'9 was the gift of Dr M.D. Cooper, University of Alabama (Birmingham, AL). Fluorescein labeled antibodies against \(\mu\) heavy chains (331.12)26 Thy 1.2 (30-H12),21 or unlabeled H-2K\(^{k}\)(11.4.1)21 and I-A\(^{b}\) (MK-D6)23 were purchased from Becton-Dickinson (Sunnyvale, CA). Goat anti-mouse \(\kappa\), \(\lambda\), and mouse anti-rat IgG antibodies conjugated with FITC were purchased from Southern Biotechnology Associates (Birmingham, AL). FITC-rat anti-mouse D6)22 were purchased from Becton-Dickinson (Sunnyvale, CA).

Fluorescence Analysis

Indirect immunofluorescence was detected with an EPICS V flow cytometer (Coulter, Hialia, FL) as described previously.14

Colony-Forming Assays

Myeloid progenitor cells capable of forming colonies in soft agar (CFU-c)23 were assayed by culturing 10\(^3\) cells in 35 mm plastic petri dishes (Miles Laboratories) containing 1 mL of 15% FCS (GIBCO), McCoy’s medium (GIBCO), and various colony stimulating factors. Dishes were cultured in 5% CO\(_2\) at 37\(^\circ\)C for seven days.

Factors

TGF-\(\beta\) was purified to homogeneity from bovine bone.24 Ten times concentrated supernatants from WEHI-3 (5%) or L cells (LCCM, 2%) were prepared as described previously25 as sources of colony stimulating factors.

Separation of Cells on Sephadex G-10 Columns

Cell suspensions from fresh bone marrow were passed through Sephadex G-10 (Pharmacia, Uppsala, Sweden) as in our previous studies to remove adherent cells.26 Approximately 500 million bone marrow cells were loaded onto a 20 mL G-10 column and nonadherent cells eluted with first 10 mL and then 15 mL of medium. The eluted cells were then passed through a second column under the same conditions. Incubations were for 45 minutes on each column.

Fig 1. Dose-dependent inhibition of myeloid long-term bone marrow cultures by addition of TGF-\(\beta\). Cultures were initiated and maintained in the indicated concentrations of cytokine as detailed in the Materials and Methods. Data are presented as means of triplicate cultures ± SE and are representative of three independent experiments involving one to three different strains of mice.

Fig 2. Dexter type long-term cultures are also sensitive to TGF-\(\beta\) (10 ng/mL) added 8 weeks after culture initiation. The results are from six cultures per group and were similar in two separate experiments done with two strains of mice and final cell recoveries ranged from 7% to 18% of the initial bone marrow cells.

RESULTS

Dose-Dependent Inhibition of Long-Term Myeloid Cultures

As little as 1 ng/mL (40 pmol/L) of TGF-\(\beta\) blocked establishment of myeloid cell growth when added at the initiation of Dexter type long-term cultures (Fig 1). Addition of TGF-\(\beta\) to well-established cultures also completely inhibited myelopoiesis (Fig 2). TGF-\(\beta\) had an early effect on myeloid progenitor cells (Table 1). After 1 week of treatment with 10 ng/mL of cytokine, total recoveries of nonadherent cells were not notably different (Fig 1). However, the incidence of clonable cells was already reduced by more than half (Table 1). Addition of TGF-\(\beta\) to semisolid agar cultures prepared from treated long-term cultures reduced them even more, but there were still small numbers of resistant colonies.

Table 1. Myeloid Progenitors Harvested From TGF-\(\beta\)-Treated Cultures

<table>
<thead>
<tr>
<th>Assay Conditions</th>
<th>Number of Colonies 10(^6) Cells Derived From 1-Week-Old Dexter Culture With</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of CSA</td>
<td>TGF-(\beta), (ng/mL)</td>
</tr>
<tr>
<td>WEHI-3</td>
<td>0.1</td>
</tr>
<tr>
<td>WEHI-3</td>
<td>1</td>
</tr>
<tr>
<td>WEHI-3</td>
<td>10</td>
</tr>
<tr>
<td>None</td>
<td>0.1</td>
</tr>
<tr>
<td>LCCM</td>
<td>0.1</td>
</tr>
<tr>
<td>WEHI-3</td>
<td>1</td>
</tr>
<tr>
<td>LCCM</td>
<td>10</td>
</tr>
</tbody>
</table>

\*Balb/c bone marrow cells were held for 1 week under Dexter type long-term culture conditions with or without TGF-\(\beta\) (10 ng/mL). Harvested nonadherent cells were then plated in semi-solid agar with WEHI-3 or L-cell conditioned medium as sources of CSF. The TGF-\(\beta\) resistance of myeloid progenitor cells was also evaluated by addition of the cytokine at that stage. Each value is the mean number of colony forming cells in triplicate cultures ± SE.
The appearance of adherent layers was dramatically influenced by TGF-$\beta_1$ addition (Fig 3). Adipocytes were notably absent in cultures containing 10 ng of factor, and very few were observed in cultures that received 1 ng. There was little three-dimensional organization in treated cultures. Cobblestone areas of myelopoiesis were absent and the adherent layers contained only highly spread stromal cells and macrophages. Similar results were obtained in many independent experiments with bone marrow from CBA as well as BALB/c background mice.

Persisting effects of TGF-$\beta_1$ on adherent layer cells were then investigated. CBA/H-T6T6 cultures were maintained for 8 weeks with factor, washed, and kept in medium alone for 1 additional week. At that time fresh marrow suspensions from BALB/c mice were passed through G-10 Sephadex columns and added to the pre-established adherent layers. Small numbers of nonadherent, H-2D$^d$ positive (BALB/c) myeloid cells appeared 4 weeks later and then declined (Fig 4). In control experiments column passaged marrow cells did not survive when placed in culture alone (Fig 4), but typical myeloid cultures resulted when they were placed on untreated adherent layers selected for low numbers of endogenous nonadherent cells. These results indicated that TGF-$\beta_1$
cultures were analyzed by flow cytometry. Percentages of cells with above background staining were determined and median fluorescence intensities (MFI) expressed as the channel numbers beyond which 50% of the positive cells were found (shown in parentheses). There is typically a substantial group of cases in which flasks were scored as positive for lymphoid growth if they contained typical adherent foci and viable lymphocytes.

has effects on microenvironmental cells required for granulopoiesis, which are not completely reversible.

Dose- and Time-Dependent Effects on Lymphocyte Cultures

Continuous treatment with 10 ng/mL of TGF-β1 totally prevented outgrowth of lymphocytes in cultures established under Whitlock-Witte conditions (Table 2, group D). However, in contrast to Dexter type cultures, a fraction of the flasks were resistant to the continuous presence of 1 ng/mL of factor (group C), and a series of experiments were done to determine which stage of culture development was being affected. As little as 1 week of treatment with 10 ng/mL totally prevented lymphocyte growth, provided this was done during the first week of culture (Table 2, group G). However, some cultures resisted this concentration of factor once the lymphocytes were established in the adherent layer is being established. Lymphoid precursors could be more resistant than their counterparts in myeloid lineages.

Addition of TGF-β to “Switch” Cultures

Preliminary experiments revealed that, in contrast to our findings with myeloid cultures, TGF-β1-treated stromal cells could support lymphocyte growth. As before, cultures were treated with 10 ng/mL of TGF-β1 for 7 weeks, washed, and held for an additional week. G-10 column passed C57BL/6 bone marrow was then added and this resulted in normal lymphocyte producing cultures (data not shown).

One of the innovations in long-term culture technology involves shifting Dexter type myeloid cultures to Whitlock-Witte conditions. Granulopoiesis declines in this circumstance and the subsequently emerging nonadherent cells are B-lineage lymphocytes. This provided an opportunity to compare progenitors of lymphocytes and myeloid cells with...

<table>
<thead>
<tr>
<th>Group</th>
<th>TGF-β, Treatment Protocols</th>
<th>Lymphocyte-Positive Flasks/Total</th>
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<tbody>
<tr>
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<td>0 1 2 3 4 5</td>
<td>2 3 4 5 6 7-19</td>
</tr>
<tr>
<td>A</td>
<td>0 0 0 0 0 0</td>
<td>0/16* 8/12 10/12 8/11 5/7 5/7</td>
</tr>
<tr>
<td>B</td>
<td>0.1 0.1 0.1 0.1 0.1 0.1</td>
<td>0/4 3/4 3/4 3/4 3/4 3/4</td>
</tr>
<tr>
<td>C</td>
<td>1 1 1 1 1 1</td>
<td>0/8 3/8 2/4 2/4 2/4 2/4</td>
</tr>
<tr>
<td>D</td>
<td>10 10 10 10 10 10</td>
<td>— — 0/4 0/4 0/4 0/4</td>
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<tr>
<td>E</td>
<td>1 1 1 1 1 1</td>
<td>0/8 3/8 1/4 1/4 1/4 1/4</td>
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<tr>
<td>F</td>
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<tr>
<td>J</td>
<td>0 0 0 0 0 0</td>
<td>0/16 8/12 10/12 8/11 2/4 1/4</td>
</tr>
</tbody>
</table>

0, 1, 2, 3, 4, 5, and 2, 3, 4, 5, 6, 7-19 are number of weeks.

*Whitlock-Witte cultures were prepared with BALB/c bone marrow with the indicated doses and durations of TGF-β1 treatment. The data are pooled from two experiments in which flasks were scored as positive for lymphoid growth if they contained typical adherent foci and viable lymphocytes. Reduction in total numbers of cultures over time resulted from loss due to contamination or use in other analyses.

Table 2. Effect of TGF-β1 on Lymphopoiesis in Long-Term Cultures

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Recovery x 10^-4</th>
<th>Mu</th>
<th>Kappe</th>
<th>14.8</th>
<th>60.1</th>
<th>BF-1</th>
<th>I-A*</th>
<th>Mac-1</th>
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<td>7 weeks</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.4*</td>
<td>5.9 (&lt;5)</td>
<td>ND</td>
<td>13.8 (43)</td>
<td>92.8 (81)</td>
<td>ND</td>
<td>2.0 (18)</td>
<td></td>
</tr>
<tr>
<td>Treated flask</td>
<td>3.4</td>
<td>10.1 (&lt;5)</td>
<td>ND</td>
<td>56.6 (61)</td>
<td>41.0 (62)</td>
<td>ND</td>
<td>13.9 (22)</td>
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<tr>
<td>12 weeks</td>
<td></td>
<td></td>
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<tr>
<td>Control pool</td>
<td>1.5</td>
<td>0.9 (&lt;5)</td>
<td>ND</td>
<td>4.5 (10)</td>
<td>94.1 (69)</td>
<td>ND</td>
<td>1.1 (12)</td>
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<tr>
<td>Treated pool</td>
<td>1.2</td>
<td>8.0 (31)</td>
<td>ND</td>
<td>66.1 (63)</td>
<td>28.7 (20)</td>
<td>ND</td>
<td>11.7 (7)</td>
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<tr>
<td>19 weeks</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control flask 1</td>
<td>1.7</td>
<td>28.1 (27)</td>
<td>0.5 (&lt;5)</td>
<td>1.3 (5)</td>
<td>95.2 (54)</td>
<td>1.2 (&lt;5)</td>
<td>0.8 (&lt;5)</td>
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<tr>
<td>Control flask 2</td>
<td>0.7</td>
<td>31.2 (31)</td>
<td>1.7 (&lt;5)</td>
<td>58.2 (39)</td>
<td>98.5 (102)</td>
<td>1.9 (15)</td>
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<tr>
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<td>ND</td>
<td>69.1 (37)</td>
<td>88.6 (56)</td>
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<tr>
<td>Treated flask</td>
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<td>1.0 (&lt;5)</td>
<td>0.6 (&lt;5)</td>
<td>41.1 (48)</td>
<td>68.9 (34)</td>
<td>1.7 (&lt;5)</td>
<td>1.5 (19)</td>
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</tr>
</tbody>
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Abbreviation: ND, not done.

*Long-term bone marrow cultures were initiated with BALB/c bone marrow and maintained under Whitlock-Witte conditions. After 5 weeks one group was treated continuously with 10 ng/mL of TGF-β1. Lymphocyte growth was conspicuous in only a small fraction of the treated flasks, and these cultures were analyzed by flow cytometry. Percentages of cells with above background staining were determined and median fluorescence intensities (MFI) expressed as the channel numbers beyond which 50% of the positive cells were found (shown in parentheses). There is typically a substantial degree of flask-to-flask variation in percentages of cells that express most leukocyte antigens.

Table 3. Normal Surface Marker Expression on Lymphocytes Growing in TGF-β1-Resistant Long-Term Cultures

<table>
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<tr>
<th>Group</th>
<th>Cell Recovery x 10^-4</th>
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Fig 5. Lymphocyte precursors survive in TGF-β1-treated
Dexter cultures and grow out when switched to Whitlock-Witte
conditions. After 8 weeks of culture under myeloid permissive
conditions, 10 ng/ml of cytokine was added for an additional 2
weeks. One half of the medium and nonadherent cells were
removed at that time, and conditions were shifted to permit
lymphocyte outgrowth in one half of the flasks (lower panel). Each
group included three replicate cultures, and this experiment is
representative of three done with two strains of mice.

respect to TGF-β1 sensitivity (Fig 5). A 2-week treatment of
established Dexter cultures with 10 ng/ml of factor resulted in
disappearance of all nonadherent cells. In contrast, when
the cultures were switched to Whitlock-Witte conditions
after treatment, lymphocyte outgrowth occurred normally.
The sizes of these cells and their expression of B lymphocyte
markers did not reveal any influence of factor exposure.
Forward angle light scatter (size) decreased after switching
(data not shown) and cells bearing the Mac-1 myeloid
antigen disappeared while lymphocytes bearing 14.8, BP-1
appeared (Fig 6). None of these events were influenced by
TGF-β1 treatment. Therefore, mechanisms associated with
lymphocyte and granulocyte maintenance appear to be dif-
ferentially affected by this cytokine.

DISCUSSION

A variety of treatment protocols were used in this study to
investigate the effects of exogenous TGF-β1 in long-term
bone marrow cultures. Exposure to the cytokine during the
first week caused a dose-dependent inhibition of subsequent
myeloid culture establishment. This occurred, at least in
part, due to an influence on the microenvironment. Dramatic
effects were also observed when cultures were established
under lymphocyte support conditions. However, a fraction of
those cultures were productive in the continuous presence of
moderate amounts of TGF-β1, and any influence on stromal
cells must have been reversible. These findings extend previ-
ous studies that demonstrate that TGF-β can directly inter-
act with and modulate the activities of lymphoid and myeloid
progenitors.6-10,12

TGF-β are true multifunctional mediators and their
effects on various tissues are not uniform.4,5 Our findings
revealed a differential sensitivity of lymphopoiesis and mye-
lopoiesis to these cytokines. Although both were inhibited by
400 pmol/L (10 ng/ml) concentrations, only lymphocyte
growth proceeded at a tenfold lower dose. This amount
causes incomplete inhibition of IL-7–dependent proliferation
of pre-B cells in culture,27 and it is possible that particular
lymphocyte subpopulations are affected. However, no dis-
tinctive characteristics were noted for lymphocytes growing
in TGF-β–resistant cultures (Table 3). Differential sensitiv-
ity was also obvious in the switch culture experiments and
may be relevant to diseases, such as cyclic neutropenia. In
that circumstance lymphocyte precursors expand recipro-
cally with declines in myeloid progenitors,28 and it is conceiv-
able that this results from periodic activation of TGF-β.

Whitlock and Witte’s modification of the Dexter culture
system made it possible to selectively propagate immature
lymphocytes and further innovations provided a means to
move between the two culture situations.13,16 It is possible
that multipotential stem cells that are maintained under
myeloid conditions give rise to lymphocytes when medium
constituents and temperature are changed. However, our
switch culture experiments might also be interpreted to mean
that the lymphocytes derived from committed precursors
that were uniquely TGF-β–resistant. If that were true, the
cytokine could be useful to experimentally resolve and
manipulate early hemopoietic progenitors. Alternatively, the
selectivity of the inhibition may have resulted from differen-
tial effects on stromal cell functions.

The composition of the adherent layer of long-term cul-
tures was markedly affected by TGF-β1, and this persisted
long after nonadherent cells disappeared. This suggested that
the microenvironment might be directly or indirectly
influenced by the cytokine. Co-cultures done with G-10
passaged bone marrow revealed that prior treatment of
adherent layers diminished support capacity for myeloid,
but not lymphoid cell growth. Lymphopoiesis was significant
in this case because expanding nonadherent cells were clearly
of donor type. The results also correspond to findings from
the “switch” culture experiments. A 2-week treatment proto-
col that terminated maintenance of myeloid cells permitted
subsequent lymphocyte outgrowth. Thus, any TGF-β1 effects
on stromal cell support of lymphocyte growth must be
reversible. A variety of effects on hematopoietic cells and
tissues have been recorded following in vivo administration of
active TGF-β, but these are completely reversible within 2
weeks of cessation of treatment. Future studies might
address the question of whether treated adherent layers ever
regain the ability to support myelopoiesis in vitro. Alterna-
tively, microenvironmental elements might be able to regen-
erate from TGF-β resistant precursors in vivo, which are not
present in vitro.
Adipocytes become increasingly conspicuous with time in Dexter type long-term cultures.\textsuperscript{28} We found that this progression was partially inhibited with as little as 40 pmol/L (1 ng/mL) concentrations of TGF-β, consistent with a prior study of Ignottz and Massague.\textsuperscript{30} They found adipocyte formation to be inhibited in the 3T3 L1 preadipocyte line with 100 pmol/L of TGF-β. The differentiation of microenviron-mental elements to adipocytes may be an important determinant of the volume of hematopoietically active “red” marrow in vivo.\textsuperscript{31,32} These culture studies indicate that TGF-β might be important antagonists of that response. Recent experiments with cloned stromal cell lines revealed that levels of TGF-β transcripts increased at least twofold by exposure of the cells to the purified cytokine and expression of a series of adipocyte genes was inhibited.\textsuperscript{34}

Temporal effects were also apparent with the different treatment protocols, and the first week of establishment of lymphocyte cultures was particularly susceptible to TGF-β. This cytokine can profoundly affect extracellular matrix formation,\textsuperscript{35,36} and further investigation might reveal how construction of an in vitro microenvironment is influenced.

These findings add to the growing list of responses that are experimentally modulated by TGF-β and invite speculation about their importance in hematopoiesis. In addition to influences on progenitor cells, effects on the microenvironment must be considered and stromal cells are probably critical components of a complex cytokine communication network. Localized events involving the TGF-β family of cytokines could lead to a coordinate change in several bone marrow functions.

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Differential effects of TGF-beta 1 on lymphohemopoiesis in long-term bone marrow cultures

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