# Factors That Affect Human Hemopoiesis Are Produced by T-Cell Growth Factor Dependent and Independent Cultured T-Cell Leukemia-Lymphoma Cells

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**Some laboratory results and clinical situations suggest that human T cells may be important in the regulation of growth of hematopoietic cells. Since the discovery of T-cell growth factor (TCGF), systems are now available for the long-term specific in vitro propagation of mature normal or neoplastic human T cells, providing an opportunity to study the influence of T cells on hematopoiesis. Recently, 24 cell lines from patients with cutaneous T-cell lymphoma (CTCL) and T-cell acute lymphoblastic leukemia (T-ALL) were grown with TCGF and then assessed for release of humoral factors that affect hematopoiesis. Conditioned media (CM) from these cell lines were tested for erythroid burst-promoting activity (BPA) and granulocyte colony-stimulating activity (CSA). BPA was detected in CM from 3/6 cultures of T-ALL patients and 4/6 CTCL cultures. CSA was found in the CM from 6/8 cultures of T-ALL patients, 7/12 CTCL cultures, and 3/4 CTCL cell lines that become independent of exogenous TCGF for growth. The CSA from several of the neoplastic T-cell cultures stimulated high levels of eosinophil colonies, a possible source of the eosinophilia seen in these patients. The ability of continuously proliferating human T lymphocytes, which retain functional specificity and responsiveness to normal humoral regulation, to produce factors that directly or indirectly stimulate myeloid and erythroid colony formation lends further credence to the role of T lymphocytes in regulating hematopoiesis.**

THE INTERACTIONS underlying the regulation of normal hematopoiesis[1] have been partially opened to study by the development of in vitro cloning techniques. For instance, in these systems, monocyte-macrophages have been shown to have an important role in the production of CSA,[2,3] the regulators of in vitro growth of granulocyte-macrophage precursors, and more recently to have a function in erythroid colony formation.[4,5] There is some evidence suggesting that T lymphocytes are involved in the regulation of myeloid and erythroid colony formation in both mouse[6,7] and man.[8,10] Indeed, the major role of T cells seems to be regulatory. With the exception of cytotoxic T cells, it appears that all classes of T cells function at least in part by secretion of factors that modulate the response of other cells.[10-13] However, the heterogeneity of cells in peripheral blood leukocytes (PBL) and spleen cell cultures used in these studies as a source of these factors makes it difficult to be certain of their cellular origin(s).

One approach to determine the cellular origin of these activities is to examine pure populations of cultured cells. Cultured cell lines may also provide a practical source of these factors. In fact, results have already been reported indicating that pure populations of continuously proliferating neoplastic T cells and macrophages do constitutively release these factors in certain instances.[8,15]

An opportunity to study the role of human T cells in influencing hematopoiesis was opened with the discovery of T-cell growth factor (TCGF).[16] When TCGF is appropriately used, pure populations of normal[16,17] and neoplastic[21] human T cells can be routinely grown in long-term suspension cultures. Moreover, the TCGF used can be purified free of myeloid and erythroid stimulating factors.[18] Therefore, constitutive and inducible release of hematopoietic growth-affecting factors can be measured with pure populations of cultured T cells from normal donors and patients with T-cell neoplasias by using this system. We report results from such studies. Our findings support the notion that T cells affect growth of other hematopoietic cells, and they further suggest that in T-cell lymphomas and leukemias, normal hematopoiesis might be influenced by release of such factors from neoplastic T cells.

## MATERIALS AND METHODS

### *Specimen Collection*

Peripheral blood samples from people with T-cell neoplasias were obtained by venipuncture and used as a source of T cells as previously described.[16,17] Human bone marrow as was obtained by posterior iliac crest puncture under local anesthesia from normal volunteers and used for target cells for assays as described below. Specimens were collected in polystyrene tubes containing 0.3 ml of preservative-free heparin (100 U/ml).

### *Establishment of T-Cell Lines*

Peripheral blood or bone marrow samples from normal donors or patients with T-cell malignancies were separated using Ficoll-Hypaque density centrifugation. The mononuclear cells were washed twice and resuspended at $10^6$ cells/ml under the following conditions: (1) RPMI-1640 media containing 20% fetal calf serum

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and (2) RPMI-1640 media containing 20% serum and 10% partially purified TCGF (ppTCGF). The cells were counted twice weekly and either were subcultured or refed with fresh media. The response of normal T cells to TCGF is dependent on initial activation with lectin or antigen,[18,19] whereas some human neoplastic T cells can be grown directly with TCGF without prior activation as previously described.[20] Cell lines were derived from patients with Sézary's syndrome, mycosis fungoides (designated CTCL for cutaneous T-cell lymphomas), and T-cell lymphoid leukemia (ALL). Three cell lines (HUT102, HUT78, and CTCL-2), originally dependent on addition of TCGF or mitogen for in vitro growth, sequentially become independent of exogenous TCGF.[20,21]

### *Preparation of ppTCGF*

Crude TCGF was prepared by incubating human mononuclear cells from multiple healthy donors at a density of $10^6$ cells/ml in RPMI-1640 media without serum but containing 0.25% bovine serum albumin and 10 μg/ml PHA-P (Difco, Detroit, Mich.) at 37°C for 72 hr.[22] The conditioned media was then concentrated by ammonium sulfate precipitation using 50%–80% saturated solutions. The precipitate was dissolved in a minimal amount of 0.01 *M* Tris-HCl (pH 8.0) and dialyzed extensively against that buffer. This material was then placed on a DEAE-Sepharose column equilibrated with the same buffer, and the absorbed proteins were eluted with a linear 0–0.2 *M* NaCl gradient in the same buffer. The active fractions, which elute at approximately 0.07 *M* NaCl, were pooled and diluted 1:1 with RPMI-1640 containing 0.01% polyethylene glycol. This product, ppTCGF, which is concentrated twofold over the starting material, usually lacks CSA, BPA, interferon, and direct mitogenic activity for fresh PBL, but each lot must be pretested. Further purification of TCGF is described elsewhere.[18]

### *Preparation of T-Cell Conditioned Media*

Cultured T cells were in cultures for at least 30 days. At various times, these cells were washed and resuspended at $10^6$ cells/ml in the following conditions: RPMI-1640–10% FCS 25; RPMI-1640–10% FCS + 10% ppTCGF. Cell-free conditioned media were harvested daily for 72 hr.

### *Light-Density Nonadherent Marrow Cells*

Light-density, nonadherent marrow cells were obtained as described elsewhere.[23] Briefly, light-density cells (1.070 g/ml) obtained by Ficoll-Hypaque gradient centrifugation were placed in 75 sq cm plastic flasks (Corning Glass Works, Corning, N.Y.) at 2–5 × $10^6$ cells/ml. After 2 hr incubation at 37°C, the cells in suspension were placed into a new flask for another 2-hr incubation. These cells, which were less than 1% monocyte/macrophages as judged by nonspecific esterase staining,[24] were used as target cells for CSA and BPA assays.

### *BFU-E and CFU-E Culture*

Cells were cultured for BFU-E assays by the method of Iscove;[25] 3 × $10^5$ in 1 ml final volume were plated in tissue culture dishes (35 × 10 mm, Lux Sci. Corp., Thousand Oaks, Calif.). Colony-forming unit erythroid (CFU-E), defined as cells forming small (4–64 cells) rapidly developing colonies, were counted on day 7 and BFU-E, defined as cells forming large multicentric aggregates, were counted on day 16. The standard medium used was α-medium (Flow Labs, Rockville, Md.), which contained 0.8% methylcellulose (Dow Chemical Co., Midland, Mich.), 30% FCS, $10^{-4}$*M* α-thioglycerol, 1% penicillin-streptomycin solution, and 2 U/ml sheep erythropoietin (step 3; Connaught Labs). BFU-E were scored after 10–11 days of incubation in a fully humidified atmosphere of 95% air and 5% $CO_2$ at 37°C. Colonies of more than 500 cells were defined as BFU-E colonies. Presence of hemoglobin in colonies was determined using 3.3'-dimethoxybenzidine.[9] A number of colonies were picked at random and stained with Wright's stain for morphological identification.

### *CFU-C Culture*

All agar cultures were performed as 1-ml cultures in 35-mm Petri dishes using an equal volume mixture of double strength modified α-medium and 0.6% agar. Modified α-medium contained *L*-asparagine (20 μg/ml), DEAE-dextran (75 μg/ml), penicillin-streptomycin (1%), and 10% FCS. Bone marrow cells at 2 × $10^5$ cells/plate were added to the liquid agar medium and 1 ml was pipetted into each dish containing 0.1 ml of material to be tested for CSA activity.[26] Colony-stimulating factor was also assayed in the soft gel system by using methylcellulose as described.[27] Briefly, each milliliter of culture contained 0.8% methylcellulose, 15% preselected fetal calf serum, 2 × $10^5$ human bone marrow cells, and 10% test sample. At day 7 and day 14, all separate clusters of 50 or more cells were scored as colonies using an inverted microscope. Discrete colonies were individually picked using a Pasteur pipette air-dried and stained with either Wright-Giemsa or Luxol. The method for staining colonies for Luxol was essentially the method of Johnson and Metcalf.[28]

## RESULTS

### *Characteristics of Cultured Neoplastic T Cells*

Cell lines established from peripheral blood of patients with various T-lymphocytic neoplasias[20] were used in this study. The population of growing cells appear to be purely T lymphocytes in that they are E-rosette positive (50%–95%) and negative for B cells and myeloid markers. In addition, these cell lines have no adherent cells and they are nonspecific esterase negative, with the exception of CTCL cell lines, indicating the absence of monocyte-macrophages. Growth characteristics, karyotypic abnormalities, and cytochemical analysis suggest that these cell lines represent a transformed neoplastic cell population.[20] These data are summarized in Table 1 and in Fig. 1.

### *CSA Production by Cultured T Cells Derived From Patients With T-Cell Neoplasias*

The majority of these T-cell lines derived from patients with T-cell malignancies, whether TCGF-dependent or not, constitutively released material that stimulated myeloid colony formation (Table 2). Proliferating T cells derived from patients with Sézary's syndrome, mycosis fungoides, and T-cell lymphoma leukemia constitutively released this material. Since T-cell-released factors can stimulate macrophages to release many humoral factors, including CSA,[15–17] the effect of CM from these T-cell lines was tested on both unseparated marrow and light-density nonadherent

**Table 1. Characteristics of the Human Neoplasia T-Cell Lines Studied**

| Sample* Number | Diagnosis† | TCGF‡ Dependency | Karyotype Alterations | Nonspecific Esterase | Acid Phosphatase§ I | II | III |
|---|---|---|---|---|---|---|---|
| CTCL-2 | SS | – | – | + | + | – | – |
| CTCL-3 | MF | + | + | + | + | – | – |
| CTCL-11 | MF | + | – | + | + | – | – |
| CTCL-12 | SS | + | – | + | + | – | – |
| CTCL-16 | MF | + | – | + | + | – | – |
| CTCL-20 | SS | + | + | + | + | – | – |
| ALL-3 | T-ALL | + | – | – | – | + | – |
| ALL-4 | T-ALL | + | – | – | – | + | – |
| ALL-8 | T-ALL | + | – | – | – | + | – |
| ALL-9 | T-ALL | + | – | – | – | + | – |
| ALL-10 | T-ALL | + | – | – | – | + | – |
| ALL-12 | T-ALL | + | – | – | – | + | – |
| HUT-102 | MF | – | + | + | + | – | – |
| HUT-78 | MF | – | + | + | + | – | – |
| CCRF-CEM | T-ALL | – | + | – | – | + | + |
| MOLT-4 | T-ALL | – | + | – | – | – | + |
| PBL | Normal | + | – | – | – | – | + |

*All samples were derived from peripheral blood except HUT-102, which was derived from lymph node tissue.

†Diagnosis: SS, Sézary's syndrome; MF, mycosis fungoides; T-ALL, acute T-lymphoid leukemia; PBL, normal donor-stimulated PHA for 72 hr.

‡(+) Cells require TCGF for growth, while (–) means they do not require it.

§Acid phosphatase type I, heavy diffuse grandular pattern; type II, concentration of stain in the Golgi region; type III, light diffuse stain.

marrow (Table 3). These CM stimulated myeloid colony formation on the nonadherent marrow as well as on the unseparated marrow. Neither the concentrated pooled ppTCGF nor 10- and 50-fold dilutions of the ppTCGF stimulated any colony formation by itself. The CM from HUT102, CTCL-2, and CTCL-3

**Table 2. Granulocyte-Macrophage Colony Formation of Human Marrow Cells Stimulated by CM From T-Cell Lines Derived From T-Cell Neoplasias**

| CM Source* | TCGF† Dependency | Colony Formation Range | Colony Formation Mean SE‡ | Colony Size§ |
|---|---|---|---|---|
| CTCL-2 | – | 24-65 | 38 ± 19.7 | 200 |
| CTCL-3 | + | 35-153 | 61 ± 32.3 | 200 |
| CTCL-11 | + | 0-11 | 7 ± 6.0 | 70 |
| CTCL-20 | + | 22-48 | 29 ± 7.7 | 150 |
| CTCL-16 | + | 26-33 | 30 ± 4.0 | 100 |
| HUT-78 | – | 19-26 | 23 ± 3.3 | 100 |
| HUT-102 | – | 19-46 | 33 ± 13.7 | 150 |
| ALL-3 | + | 21-54 | 26 ± 14.0 | 150 |
| ALL-8 | + | 0-47 | 19 ± 24.0 | 150 |
| ALL-9 | + | 47-89 | 61 ± 23.7 | 150 |
| ALL-10 | + | 0-31 | 14 ± 15.3 | 70 |
| CCRF-CEM | – | 0 | — | — |
| PBL | NA | 25-45 | 34 ± 11.0 | 100 |
| ppTCGF | NA | 0 | — | — |

*All cell lines were in culture at least 30 days before CM was collected for assay except PBL, which were freshly collected. All CM were collected 72 hr after the cell lines were subcultured.

†(+) Means that the cells required TCGF for growth, while (–) means they did not.

‡CM prepared as described above were tested for their ability to stimulate colony formation of normal bone marrows at 2 × $10^5$/ml in agar as described in Methods. Results indicate CM harvested from three successive passages of each T-cell line (mean ± SE) done in triplicate.

§This refers to the average size of the colonies.

**Table 3. Effect of Removal of Bone Marrow Adherent Cells on Colony Formation Stimulated by CM From Cultured T-Cell Lines**

| Cm Source* | Colony Formation/ 2 × $10^5$ Unseparated Bone Marrow Cells† | Colony Formation/ 2 × $10^5$ Nonadherent Bone Marrow Cells† |
|---|---|---|
| CTCL-2 | 26 ± 7.3 | 28 ± 4.7 |
| CTCL-3 | 31 ± 9.0 | 31 ± 8.3 |
| ALL-3 | 44 ± 11.3 | 45 ± 9.3 |
| PBL | 23 ± 6.0 | 48 ± 10.3 |

*All cell lines were in culture at least 30 days before CM was collected for assay, except PBL which were freshly collected. All CM were collected 72 hr after the cell lines were subcultured.

†CM prepared as described above were tested for their ability to stimulate colony formation of normal bone marrows at 2 × $10^5$/ml in agar as described in Methods. Results indicate CM harvested from three successive passages of each T-cell line (mean ± SE) done in triplicate.

showed a sigmoid dose–response curve similar to that observed with other CSAs[1] (data not shown). Analysis of the morphology of 200 colonies, individually picked from the methylcellulose and stained with Wright-Giemsa, was made for colonies stimulated by several CM derived from T-cell lines. These results are summarized in Table 4.

A high percentage of eosinophil colonies was detected on microscopic examination of individual colonies stained with Wright-Giemsa. Colonies stimulated by CM from CTCL-2, CTCL-3, and ALL-3 had between 70% and 90% eosinophil colonies. CM from HUT102 and from PHA-stimulated peripheral blood leukocytes (PBL) stimulated only 20%–30% eosinophil colonies. The results were also confirmed by staining individual colonies with Luxol blue, a stain specific for eosinophils[28] (Fig. 2). The noneosinophil colonies were generally pure macrophage or a macrophage-granulocyte mixture with only a rare granulocyte colony.

**Table 4. Morphological Characterization of Bone Marrow Colonies Stimulated by CM From Cultured T-Cell Lines**

| CM Source* | Eosinophil Colonies† (%) | Granulocyte/Macrophage Colonies† (%) | Luxol Stain‡ (%) |
|---|---|---|---|
| CTCL-2 | 83 | 17 | 77 |
| CTCL-3 | 79 | 21 | 78 |
| ALL-3 | 89 | 11 | 87 |
| HUT-102 | 19 | 81 | 18 |
| PBL | 32 | 68 | 33 |

*All cell lines were in culture at least 30 days before CM was collected for assay except PBL, which were freshly collected. All CM were collected 72 hr after the cell lines were subcultured.

†CM prepared as described above were tested for their ability to stimulate colony formation of normal bone marrows at 2 × $10^5$/ml in agar as described in Methods. Results are with CM harvested from three successive passages of each T-cell Line. Two-hundred colonies for each CM were picked from the agar by using a Pasteur pipette and stained with Wright-Giemsa. Morphology of each colony was determined by microscopic examination.

‡The Luxol stain, as described in Methods, was performed on 200 individual colonies picked from the agar as described above.

Fig. 1. Acid phosphatase cytochemical appearance of cultured T lymphocytes. Acid phosphatase staining was performed as previously described.[21] Cells were grown in growth media supplemented with 10% ppTCGF. (Top) Cultured T cells from a normal donor after 35 days in culture; (middle) cultured T cells from a patient with T-cell ALL after 23 days in culture; (bottom) cultured T cells from a patient with CTCL after 75 days in culture.

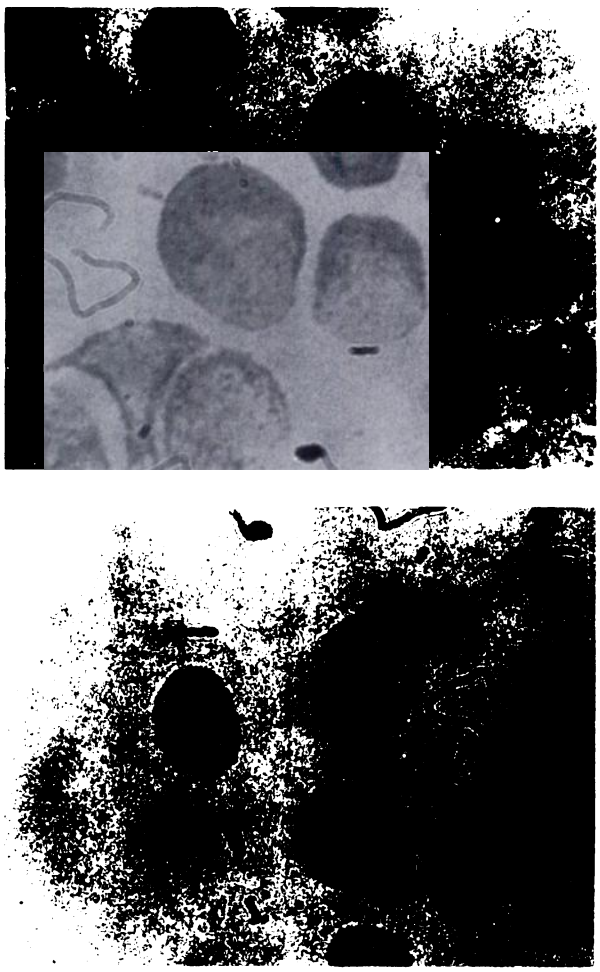

Fig. 2. Luxol fast blue cytochemical appearance of bone marrow colonies. Luxol fast blue staining was performed as described in Materials and Methods. Bone marrow colonies were stimulated by CM from CTCL-2 (Top) Colony that is positive for luxol; (bottom) colony that is negative for luxol.

### *Effect of CM From Cultured T Cells on Erythroid Colony Formation*

The effect of CM derived from T-cell lines on erythroid colony formation was also tested (Table 5). It is evident that several CM caused a significant increase in BFU-E-derived colonies ( $p < 0.05$) relative to the control not treated with CM. Addition of the CM not only stimulated an increase in the number of colonies, but also in the size of the colonies. These CM, with the exception of one CM from ALL-3, had little effect on the number of CFU-E present in the culture system. No increase in the ability of these CM to stimulate CFU-E was observed, even when the cell concentration was decreased to $10^5$ cells/plate. CM from several cell lines were also able to stimulate colony formation on light-density adherent cell-depleted bone marrow cells (Table 6). When nonadherent bone marrow cells were cultured in the absence

**Table 5. Erythroid Colony Formation of Human Bone Marrow Stimulated by CM From Cultured T-Cell Lines**

| CM Source* | TCGF Dependency† | CFU-E‡ ($3 \times 10^5$ Cells) | Colony Enhancement | BFU-E‡ ($3 \times 10^5$ Cells) | Colony Enhancement§ |
|---|---|---|---|---|---|
| None | NA | 155 ± 44.0 | — | 52 ± 3.3 | — |
| CTCL-3 | + | 96 ± 24.7 | 0.58 | 87 ± 5.0 | 1.60 |
| ALL-3 | + | 381 ± 63.0 | 2.30 | 105 ± 31.0 | 2.00 |
| ALL-4 | + | 114 ± 81.0 | 0.70 | 111 ± 29.0 | 2.10 |
| ALL-9 | + | 149 ± 18.3 | 0.90 | 120 ± 36.0 | 2.20 |
| ALL-10 | + | 185 ± 38.0 | 1.20 | 83 ± 18.0 | 1.55 |
| PBL | NA | 76 ± 11.7 | 0.45 | 125 ± 33.0 | 2.25 |
| CCRF-CEM | − | 190 ± 48.0 | 1.25 | 110 ± 25.0 | 2.10 |
| ppTCGF | NA | 73 ± 15.0 | 0.40 | 60 ± 31.0 | 1.15 |

*All cell lines were in culture at least 30 days before CM was collected for assay, except PBL that were freshly collected. All CM were collected 72 hr after the cell lines were subcultured.

†(+) Means TCGF is required for cell growth. NA means nonapplicable.

‡Bone marrow cells were cultured at $3 \times 10^5$ cells/ml in methylcellulose as described in Methods in triplicate. CM from three successive passages of the cells were assayed. Results represent the mean ± SE.

§Colony enhancement is determined by taking the ratio of erythroid colonies formed in the presence of CM divided by the colonies formed in the absence of CM.

of CM, the proliferation of BFU-E was severely suppressed (20% of unseparated control). The addition of CM from these T-cell lines stimulated a 4–5-fold increase in the number of BFU-E-derived colonies. All the colonies contained hemoglobin. Several CM from T-cell lines had a profound inhibitory effect on erythroid colony formation. Tenfold dilutions of these inhibiting CM did not alter their inhibitory effect. Some of these CM contained CSA activity and others did not. ppTCGF by itself did not enhance or inhibit BFU-E formation.

## DISCUSSION

The ability of T lymphocytes to induce proliferation of myeloid and erythroid committed progenitors has been suggested in a variety of studies both in mice[6,7,12] and in humans.[8-10] However, the heterogeneous nature of the culture systems used to prepare the CM makes it difficult to establish the cellular origin of these factors and how their production is regulated. A few continuously growing neoplastic cell lines of T-cell and macrophage origin have been shown to produce hematopoietic factors.[8,14,15]

**Table 6. Effect of Removal of Bone Marrow Adherent Cells on Erythroid Colony Formation Stimulated by CM From Cultured T-Cell Lines**

| CM Source* | Bone Marrow Cells Source† | CFU-E‡ /$3 \times 10^5$ Cells | BFU-E‡ /$3 \times 10^5$ Cells |
|---|---|---|---|
| None | Unseparated | 190 ± 67.0 | 48 ± 17.0 |
| None | Nonadherent | 410 ± 92.0 | 11 ± 4.3 |
| ALL-3 | Nonadherent | 520 ± 110.0 | 68 ± 28.0 |
| ALL-9 | Nonadherent | 260 ± 78.0 | 45 ± 16.0 |
| PBL | Nonadherent | 375 ± 103.0 | 52 ± 19.3 |

*All cell lines were in culture at least 30 days before CM was collected for assay, except PBL that were freshly collected. All CM were collected 72 hr after the cell lines were subcultured.

†Bone marrow cells were separated by adherence to plastic as described in Methods.

‡Bone marrow cells were cultured at $3 \times 10^5$ cells/ml in methylcellulose as described in Methods in triplicate. CM from three successive passages of the cells were assayed. Results represent the mean ± SE.

In this report, several cell lines derived from patients with Sézary's syndrome, mycosis fungoides, and some other T-cell lymphoid leukemias were able to constitutively release CSA while continuously proliferating in the presence of either added or constitutively produced TCGF. These results suggest that activated T cells, still controlled by apparently normal regulatory factors, can produce other normal hemotopoietic factors without any further signals. Morphological analysis of the myeloid colony formation stimulated by these CM showed that three cell lines derived from patients with T-cell malignancies released factors that selectively stimulated eosinophil colonies (80%–90% of colonies were eosinophils). CM from fresh mitogen-stimulated normal cells were much less stimulatory of eosinophil colonies (20%). These results are in agreement with published data suggesting that T cells can produce eosinophil-stimulating factors.[29] It is possible that the in vivo eosinophilia observed in several T-cell malignancies[30] is due to the abnormal production of this factor by neoplastic T-cells.

The CM from neoplastic T-cell lines were also tested for capacity to induce erthyroid colony formation. Several of these CM caused a doubling of the maximal erythropoietin-induced BFU-E response. This is in agreement with other workers who have found that CM from antigen/mitogen-treated mononuclear cells stimulated an increase in BFU-E.[9]

Since T cells are known to elaborate factors that stimulate proliferation of macrophages and their production of some hematopoietic factor(s),[10-13,31] light-density nonadherent bone marrow cells were isolated and used as target cells in the CFU-C and BFU-E assays. The CSA and BPA activity of these CM from

cultured T-cell lines was not altered by using nonadherent bone marrow cells as target cells. This suggests that the factor(s) present in these CM act directly on the progenitor cells rather than by stimulating factor production from the macrophages. However, we cannot eliminate the possibility that the CM are stimulating a nonadherent accessory cell still present in the light-density bone marrow fraction.[32] Nevertheless, it is clear that the neoplastic T cells, which are still under the apparent normal cellular growth control by TCGF, can influence other hematopoietic cells by secretion of these factors.

In contrast to the constitutive release of CSA and BPA from T cells of neoplastic origin, results have been reported[33] showing that CSA was released from a cloned TCGF-dependent mouse helper T-cell line only after stimulation with macrophage and antigen. However, there is a report that conflicts with this. Normal cloned mouse helper T-cell lines were reported to release CSA constitutively.[34] Whether this discrepancy is due to a difference in function of a T-cell subset or methods of activation is not known. However, the use of purified TCGF to grow T cells of defined characteristics and precise function should help to further clarify the role of T cells in regulating hemotopoiesis.

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