T Gamma (Tγ) Cells Suppress Growth of Erythroid Colony-forming Units In Vitro in the Pure Red Cell Aplasia of B-Cell Chronic Lymphocytic Leukemia

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ABSTRACT In vitro studies were performed in two patients with B-cell chronic lymphocytic leukemia who developed pure red cell aplasia (CLL-PRCA). During the active phase of their red cell aplasia, there was a marked reduction in the numbers of erythroid colony-forming units (CFU-E). Unfractionated sera or separated IgG fractions from these patients did not impair CFU-E proliferation from either autologous or allogeneic marrows. Increased numbers of T lymphocytes were present in marrow aspirates of these patients. Analysis of these T cells indicated that 90 and 35%, respectively, bore Fc receptors for IgG (Tγ cells). Removal of T cells by E-rosetting techniques augmented CFU-E growth in CLL-PRCA 10-fold. Similar treatment of normal marrows did not cause similar enhanced growth of CFU-E. Co-cultures of marrow T cells or Tγ cells obtained during the active phase of CLL-PRCA suppressed CFU-E growth from autologous or allogeneic marrows. After achieving drug-induced remission of the PRCA, marrow T cells were no longer inhibitory. In contrast, BFU-E (erythroid burst-forming units) or granulocyte proliferation in diffusion chambers were not suppressed by CLL-PRCA T cells. These findings suggest that the development of PRCA in B-cell CLL may result from suppression of CFU-E proliferation by Tγ cells.

INTRODUCTION

The majority of patients with B-cell chronic lymphocytic leukemia (CLL) develop anemia sometime during the course of their disease. In 10 to 20% of these patients, the anemia results from an autoimmune hemolytic process (1). However, in most patients, the anemia of CLL is due to decreased red cell production (2, 3). An extreme manifestation of this production defect is the complete cessation of red cell production (pure red cell aplasia, PRCA), a syndrome noted in a small number of CLL patients (4). This latter group have provided an opportunity to examine the operative mechanism(s) for the anemia associated with this leukemia. Previous studies indicated that serum IgG inhibitors are not responsible for the PRCA in CLL (5, 6); by contrast, these can account for up to 50% of idiopathic PRCA (5–7).

Hoffman et al. (8) recently found that T cells from a patient with a T-cell variant of CLL suppressed proliferation of the mature erythroid colony-forming cells (CFU-E). Nagasawa et al. (9) subsequently showed that the malignant T cells bearing Fc receptors for IgG, termed T gamma (Tγ) cells, suppressed both erythroid colony formation and B-cell differentiation in

1 Abbreviations used in this paper: BFU-E, erythroid burst-forming units; CFU-E, erythroid colony-forming units; CLL, chronic lymphocytic leukemia; CVP, cyclophosphamide, vincristine, and prednisone; DC, diffusion chamber; E-rosetting, sheep erythrocyte rosetting; FH, Ficoll-Hypaque; Hb, hemoglobin; HCT, hematocrit; LDMNC, light density mononuclear cells; α-MEM, alpha minimal essential medium; PRCA, pure red cell aplasia; TD-LDMNC, T-cell-depleted LDMNC; WBC, leukocyte(s).
vitro. Moreover, we have shown that T cells from four patients with B-cell CLL-PRCA were defective stimulators of the primitive blood erythroid burst-forming units (BFU-E) (6). The defective burst-promoting function of these T cells was correlated with the presence of increased numbers of Ty cells (10).

We recently had the opportunity to study the serum and cellular interactions of marrow CFU-E in two additional patients with B-cell CLL who developed PRCA. This report provides evidence for T-cell suppression of marrow erythropoiesis in B-cell CLL with PRCA. Furthermore, the suppressor cells appear to be confined to the Ty cell subset.

METHODS

Case reports. Patient A, a 45-yr-old White female was seen in December of 1979 with complaints of profound fatigue and weakness. Physical examination revealed diffuse lymphadenopathy, splenomegaly, and absent cutaneous lesions. Laboratory examination revealed a hematocrit (HCT) of 15%, hemoglobin (Hb) 5.0 g/dl, platelets 350,000/μl, and reticulocyte 0.1%. The leukocyte (white blood cell, WBC) count was 49,000/μl with a differential count of 84% small round lymphocytes, 14% segmented neutrophils, and 2% monocytes. Direct Coombs test, serum bilirubin, haptoglobin, iron, and total iron binding capacity, vitamin B₁₂, folic acid, creatinine, serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase were normal or negative. Serum IgG and IgM levels were decreased to 7.6 m/ml (normal 8-18) and 0.4 m/ml (normal 0.6–2.8 m/ml), respectively. Bone marrow aspirate and biopsy revealed a hypercellular marrow infiltrated with >50% small round lymphocytes. Granulocytic and megakaryocytic maturation was normal, however, no erythroid precursors could be identified. A diagnosis of B-cell CLL with PRCA (CLL-PRCA) was made. Direct immunofluorescence studies on blood lymphocytes confirmed the presence of B-cell CLL, IgM, lambda type. Nuclear morphologic findings by light and electron microscopy, sheep erythrocyte rosetting (E-rosetting) studies and acid phosphatase reaction of blood lymphocytes further excluded a diagnosis of T-cell CLL. The patient was transfused and treated for 2 mo with chemotherapy (chlorambucil, prednisone, vincristine, methotrexate, and adriamycin) without improvement of anemia. 3 wk after chemotherapy was discontinued, a repeat bone marrow aspirate and biopsy showed again, CLL-PRCA. Marrow was taken for in vitro studies. Treatment with splenic irradiation, splenectomy, and total body irradiation induced a reticulocytosis of 5.4% and the HCT rose to 42%, 6 mo later, the HCT dropped to 26.9% with 0.1% reticulocytes. The WBC count was 65,000/μl with 72% small round lymphocytes. A repeat marrow was consistent with B-cell CLL-PRCA. Treatment with oral chlorambucil and prednisone induced a second reticulocytosis of 4.9% after 6 wk. The HCT rose to 42% and a repeat culture study was performed off therapy. The patient has remained transfusion free for the last year, on monthly cycles of cyclophosphamide, vincristine, and prednisone (CVP).

Patient B, a 54-yr-old White female presented in January of 1976 with diffuse peripheral lymphadenopathy and mild splenomegaly. Cutaneous lesions were absent. Laboratory examination revealed a WBC count of 21,200/μl with 71% small round lymphocytes. T cell nuclear morphologic features were absent. The HCT, platelet count, and reticulocyte count were normal. A diagnosis of B-cell CLL was made. WBC rose to >100,000/μl over the ensuing months and the patient was treated with daily chlorambucil for 36 mo followed by 12 mo of pulse chlorambucil and prednisone. The therapy was discontinued. 5 mo later the patient complained of profound fatigue. The HCT was 19.3%, the WBC count 59,900/μl with 96% small round lymphocytes and 4% segmented neutrophils. Platelets were 166,000/μl. Five daily reticulocyte counts ranged between 0 and 0.1%. Direct Coombs test, serum bilirubin, haptoglobin, vitamin B₁₂, folic acid, creatinine, iron and total iron binding capacity were all normal or negative. Serum immunoglobulin levels revealed a panhypogammaglobulinemia (IgG 1.95 m/ml, IgA 0.5 m/ml, and IgM 0.13 m/ml). Bone marrow aspirate and biopsy revealed a diffuse lymphocytic infiltrate with absent erythroid precursors. Maturation of granulocytic and megakaryocytic series were normal. Direct immunofluorescence studies of blood lymphocytes confirmed the presence of B-cell CLL IgM/D lambda type. Marrow samples were taken for in vitro studies. The patient was transfused and begun on daily oral cyclophosphamide and prednisone for 1 mo followed by six monthly cycles of CVP. After 7 mo, a reticulocytosis of 5% ensued and the control was discontinued and repeat in vitro culture studies were performed. The patient remains transfusion free, off therapy 10 mo later.

Preparation of marrow or blood target cells. Marrow aspirations or venipunctures were performed on patients or normal volunteers giving informed consent, as approved by the Institutional Human Subjects Committees. Marrow aspirations were limited to 2.0 ml to avoid dilution with peripheral blood.

Light density mononuclear cells (LDMCN) or T-cell-depleted LDMCN (TD-LDMCN) retrieved from bone marrow were cultured alone or co-cultured with marrow T cells or Ty cells in assays for marrow CFU-E. Assays for primitive BFU-E used blood null cells in co-cultures with marrow T cells or Ty cells. In brief, marrow was aspirated into heparinized syringes diluted 1:1 with alpha minimal essential medium (α-MEM, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), strained through small bone needles and layered at a Ficoll-Hypaque (FH) gradient (specific gravity 1.077 g/cm³). After centrifugation at 400 g for 30 min, whole mononuclear cells (WMNC) were retrieved from the interface and depleted of adherent monocytes by incubation on fetal calf serum-coated petri dishes (25 × 10⁵/78 cm²) for 1 h. The nonadherent cells referred to as LDMCN contained <5% monocytes as judged by α-naphthylesterase activity and morphology. Marrow LDMCN were washed and further depleted of T cells by E-rosetting, as described below, to retrieve TD-LDMCN. The recovery of TD-LDMCN was 75% in patient A and 92% in patient B.

For BFU-E assays, blood LDMCN were further depleted of surface immunoglobulin-bearing B cells by incubation for 1 h on plastic petri dishes coated with goat anti-human immunoglobulins [F(ab')₂] fragment, N. L. Cappel Laboratories Inc., Cochranville, PA), as described previously (11). The B cell-depleted LDMCN were then depleted of T cells by E-rosetting, as described below for marrow T cells, to retrieve BFU-E enriched null cell targets. These null cells contain <5% B and T cells as judged by surface immunoglobulin analysis and E-rosetting, and <5% monocytes as judged by α-naphthylesterase activity.

Isolation of T cells and Ty cells. T cells used in co-cultures were obtained by rosetting LDMCN from marrow (or
blood) with 2-aminoethylisothio uronium bromide (AET)-treated sheep erythrocytes followed by a second FH density gradient centrifugation (12). Greater than 95% of the predicted number of T cells were found in the pellets. Based on rosetting, these T cells were >96% pure. T cells were freed of sheep erythrocytes by lysis with Tris-buffered ammonium chloride, washed with α-MEM and used in co-cultures with marrow or blood target cells.

For isolation of Ty cells (10, 12), ox erythrocytes were sensitized with a subagglutinating titer of rabbit anti-ox erythrocyte IgG (Cappell Laboratories) to make EALgG complexes. 5.0 × 10^5 T cells were then mixed with an equal volume of a 1:2 solution of EALgG complexes for 30 min at 37°C, centrifuged at 200 g for 5 min and kept at 4°C for 1 h. The rosetted Ty cells were then retrieved by FH density gradient centrifugation followed by lysis with Tris-buffered ammonium chloride as described above. The purity of Ty cells were >90% when checked by rosetting with 1% EALgG complexes. Less than 1% of these cells were monocytes as judged by morphology and α-naphthylesterase activity.

**Co-culture studies and stem cell assays.** A methylcellulose erythroid colony system was used (13) for assays of marrow CFU-E and blood BFU-E. Co-cultures were performed as follows: 1 × 10^5 patient or control normal T cell or Ty cells were mixed with 1 × 10^5 patient or control marrow TD-LDMNC and scored for day 7 CFU-E. For BFU-E, 2 × 10^5 patient or control blood T cells were mixed with 2 × 10^5 patient or control blood null cells (6) and scored for day 14 BFU-E. Erythroid colonies were always absent in control cultures of T cells or Ty cells plated alone. Each co-culture was plated in triplicate. Human urinary erythropoietin kindly provided by the Erythropoietin Subcommittee, (National Heart, Lung, and Blood Institute, Bethesda, MD) was present in cultures at a final concentration of 1 IU for marrow CFU-E and 2 IU for blood BFU-E. Aggregates containing eight or more benzidine-positive cells on day 7 were defined as 1 CFU-E. Aggregates containing >50 benzidine-positive cells on day 14 were defined as 1 BFU-E.

**Diffusion chamber studies.** In some studies, the in vivo mouse diffusion chamber (DC) culture system was used (14) to study the cellular interaction of patient B's T cells on granulocyte production. In this system, mature and immature granulocytes retrieved for the chambers after 14 d are identified on Wright-Giemsa-stained smear. In these studies, 4 × 10^5 patient blood T cells were inoculated into quadruplicate DC with 4 × 10^5 control T cells and 1 × 10^5 control TD-LDMNC. DC inoculated with 4 × 10^5 normal T cells plus 10^5 TD-LDMNC served as controls. Granulocytes did not proliferate in DC inoculated with only patient or control T cells.

**Surface marker analyses.** Surface immunoglobulin typing was performed on blood WMNC obtained by FH density gradient centrifugation by direct immunofluorescence using fluorescein-conjugated goat F(ab')2 fragments (Meloy Laboratories, Inc., Springfield, VA) directed toward the human heavy or light chains (15). Blood or marrow E-rosetted cells or Ty cells were further subtyped into helper (OKT4+) or suppressor/cytotoxic (OKT8+) monoclonal antibody-defined subsets (Ortho Pharmaceutical, Raritan, NJ) using an indirect immunofluorescence method with fluorescein-conjugated goat anti-mouse IgG (12, 16).

**Serum inhibitor studies.** Serum samples obtained from patients during active phase of PRCA and two type AB control donors were stored at −20°C before testing. IgG fractions were isolated by ammonium sulfate precipitation followed by DEAE cellulose column chromatography (17). The IgG fractions gave identical single bands of reactivity with anti-human whole sera and anti-human IgG on immunodiffusion plates (Hyland Diagnostics Div., Costa Mesa, CA). Patient or control IgG fractions were dialyzed against α-MEM and added to the cultures at a final concentration equivalent to 10% of patient's serum IgG level. Whole sera or IgG fractions were tested in the presence of a 1:10 dilution of rabbit complement (Low Tox, Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). In some serum studies, exogenous erythropoietin was omitted in order to test for the presence of erythropoietin in the samples. Serum or IgG fractions were continuously present throughout the culture period. 2 × 10^5 normal or patient marrow LDMNC obtained after initial treatment (but before complete recovery) were used as target cells.

**Statistics.** Comparison of cohorts was made using Student's t test.

**RESULTS**

_Marrow CFU-E before and after resolution of PRCA._ CFU-E in LDMNC from CLL-PRCA patients were barely detectable (3±1 CFU-E/10^5 LDMNC) during the active phase of PRCA (i.e., at presentation) compared with 12 normal controls (155±41/10^5 LDMNC, Fig. 1). 4 and 6 wk after initial treatment but before development of a reticulocytosis, CFU-E numbers were 48±5 and 28±7 CFU-E/10^5 LDMNC in patient A and B. After remission of PRCA, CFU-E rose to 90±10 and 42±7/10^5 LDMNC in patients A and B, respectively. These numbers, however, were still reduced compared with normal (P < 0.05).

**Serum inhibitor studies.** The effects of patient serum or IgG fractions on autologous erythroid colony formation from initial posttreatment patient marrows are summarized in Table I. Compared with normal control sera or IgG fractions, CLL-PRCA whole sera or IgG fractions obtained during the active phase of PRCA did not suppress erythroid colony growth from autologous marrow target cells (P > 0.1). Similar re-

![Figure 1](image_url) The effects of removal of marrow T cells by E-rosetting on growth of CFU-E in T-cell-depleted fractions. Compared with nonadherent cells that contained T cells, CFU-E numbers were increased 10-fold in CLL-PRCA patients but not in 12 normal controls.
**TABLE I**

*Effects of Whole Sera or IgG Fractions from CLL-PRCA Patients on Erythroid Colony Growth from Autologous Marrows*

<table>
<thead>
<tr>
<th>Serum additions*</th>
<th>Patient A marrow</th>
<th>Patient B marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement only</td>
<td>48±3</td>
<td>28±7</td>
</tr>
<tr>
<td>Patient serum</td>
<td>67±11 (139)</td>
<td>52±11 (185)</td>
</tr>
<tr>
<td>Normal serum</td>
<td>61±12 (127)</td>
<td>48±10 (171)</td>
</tr>
<tr>
<td>Patient IgG</td>
<td>45±9 (94)</td>
<td>22±7 (78)</td>
</tr>
<tr>
<td>Normal IgG</td>
<td>41±7 (85)</td>
<td>29±8 (103)</td>
</tr>
<tr>
<td>Patient serum</td>
<td>65±12 (135)</td>
<td>45±5 (161)</td>
</tr>
</tbody>
</table>

* Cultures were incubated with 10% patient serum (active phase of PRCA) normal AB serum, or their respective IgG fractions. All samples were cultured with 0.1 ml of rabbit complement (C') and 1.0 IU/ml erythropoietin (EPO). Each patient serum was also added to cultures in the absence of exogenous EPO (no EPO) to measure serum EPO activity. Nonadherent patient marrow cells retrieved after initial treatment but before complete recovery of PRCA served as targets.

† Values are means±1 SD of triplicate cultures; values in parentheses are percentages of C' control. Colony growth with patient sera or IgG fractions did not differ significantly from normal controls.

Results were observed when normal marrows served as target cells (data not shown). The CLL-PRCA sera stimulated growth of autologous (Table I) or normal marrow CFU-E in the absence of exogenous erythropoietin suggesting that erythropoietin was present and antierythropoietin antibodies were absent.

*T cell and Tγ cell composition of marrow and blood.* A striking increase in E-rosetted (T cells) was observed in mononuclear marrow cells from both CLL-PRCA patients during the active phase of PRCA (Table I, columns 1 and 3). In contrast, in five untreated patients with Rai stage 0-III (3 stage III, 1 stage II and 1 stage 0 patients) B-cell CLL marrow T cells comprised only 7±2% of mononuclear cells. 90 and 35% of marrow T cells in the CLL-PRCA patients bore Fc receptors for IgG i.e., were Tγ cells. 53 and 55% of these Tγ cells reacted with the OKT8 suppressor antibody, whereas only 37 and 34% reacted with the OKT4 helper antibody. In the three patients with untreated B-cell CLL (Rai stage III) 51, 35, and 39% of marrow T cells were Tγ cells. Thus, although Tγ cells were increased proportionally in both CLL-PRCA and advanced stage III common B cell CLL marrows, the total numbers of Tγ cells were less in patients without PRCA. In patient A, after resolution of PRCA, the proportion of marrow T cells and Tγ cells decreased towards normal but were still increased (Table II, column 2). In patient B, the numbers of T cells and Tγ cells were normal after resolution of PRCA (Table II, column 4).

Before treatment, blood E-rosettes in the CLL-PRCA patients were decreased consistent with B-cell CLL (Table II, columns 1 and 3). However, the proportions of Tγ cells were markedly increased (Table II, columns 1 and 3). OKT4/OKT8 helper suppressor ratios were 0.78:1 and 0.86:1 in patients A and B, respectively, compared with controls (1.8:1). After resolution of PRCA posttreatment, the proportion of Tγ cells in blood decreased (Table II, columns 2 and 4). The proportion of T cells in blood had risen slightly to 21 and 11% (Table II, columns 2 and 4) but were still abnormally low.

**TABLE II**

*T-Cell Surface Markers in B-Cell CLL*

<table>
<thead>
<tr>
<th>Test</th>
<th>Percentage of cells rosetting*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRCA(+)</td>
</tr>
<tr>
<td>BM ER*</td>
<td>70</td>
</tr>
<tr>
<td>BM Tγ*</td>
<td>90</td>
</tr>
<tr>
<td>PB ER*</td>
<td>10</td>
</tr>
<tr>
<td>PB Tγ*</td>
<td>75</td>
</tr>
</tbody>
</table>

* Values represent the percentage of E-rosette-positive (ER*) T cells from whole mononuclear cells or percent T cells rosetting with IgG-coated ox erythrocytes (Tγ cells) from bone marrow (BM) or blood (PB) before (PRCA*) or after (PRCA-) resolution of PRCA.

† Indicates range for 12 controls.
**T-cell depletion studies.** Removal of T cells from CLL-PRCA marrows before erythroid colony-forming assays, increased CFU-E numbers ~10-fold compared with CFU-E numbers in LDMNC (Fig. 1). The percent recovery of TD-LDMNC in patient A and B was 75 and 92%, respectively. Thus, concentration of CFU-E due to loss of TD-LDMNC could not explain this increase. Furthermore, based on the percentage of T cells in marrows of this patient only a 3.3- and 1.3-fold increase in patients A and B, respectively, would be expected due to enrichment of CFU-E alone. Moreover, separate studies in 12 normal volunteers did not show a similar augmentation of CFU-E numbers after removal of T cells (P > 0.05, Fig. 1).

**Co-culture studies.** To confirm that increased colony formation was due to removal of T suppressor cells, 10^6 T cells from CLL-PRCA marrows were retrieved and mixed with autologous marrow TD-LDMNC. The ratio of T cells of TD-LDMNC was 1:1 (Table III). Readuction of autologous CLL-PRCA marrow T cells, obtained during the active phase of PRCA, suppressed CFU-E proliferation to 56 and 67% of control cultures without T cells (P < 0.05). By contrast, an equal number of normal T cells stimulated CFU-E three- to sixfold. When CLL-PRCA Tγ cells were mixed with autologous marrow target cells, CFU-E growth was suppressed to 23 and 28% of controls (P < 0.025). Suppression of CFU-E from CLL-PRCA marrow cells by normal Tγ cells was not observed.

When CLL-PRCA T cells or Tγ cells were co-cultured with normal marrow TD-LDMNC in 1:1 ratios, CFU-E growth was suppressed to 34 and 47% of controls (P < 0.025, Table IV). Addition of normal T cells or Tγ cells to normal autologous marrows resulted in no significant change in CFU-E numbers.

Co-culture studies were repeated in patient A and B after they had achieved drug-induced remissions of their PRCA and when the proportions of Tγ cells in blood and marrow T cells were normal or nearly normal (Table II). At this time, CFU-E numbers in marrow TD-LDMNC (Table V) were increased 1.7- to 2.3-fold compared with CFU-E numbers in TD-LDMNC during PRCA. However, they were still significantly reduced compared with normals (P < 0.05). During remission of their PRCA, the effects of patients’ T cells on CFU-E growth from autologous TD-LDMNC did not differ significantly from the effects of control T cells (P > 0.05, Table V). Similar results were observed when normal TD-LDMNC were used as target cells in co-cultures (data not shown).

Additional co-culture studies were performed in patient B to determine whether the T-suppressor effect was specific for CFU-E. As shown in Table VI, addition of blood T cells from patient B at the time of PRCA stimulated autologous or allogeneic BFU-E growth.

### Table III

**Effects of T Cells or Tγ Cells from CLL-PRCA Patient Marrows on Erythroid Colony Growth from Autologous Marrows**

<table>
<thead>
<tr>
<th>Cell additions</th>
<th>Patient A bone marrow</th>
<th>Patient B bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>39±5</td>
<td>25±3</td>
</tr>
<tr>
<td>Patient T cells</td>
<td>26±5 (67)</td>
<td>14±2 (56)</td>
</tr>
<tr>
<td>Normal T cells</td>
<td>127±19 (325)</td>
<td>137±32 (548)</td>
</tr>
<tr>
<td>Patient Tγ cells</td>
<td>9±1 (23)</td>
<td>7±1 (28)</td>
</tr>
<tr>
<td>Normal Tγ cells</td>
<td>26±9 (67)</td>
<td>30±7 (120)</td>
</tr>
</tbody>
</table>

*10^6 T cells or Tγ cells from patients or normal marrows were co-cultured with 10^6 T-cell-depleted marrow cells from patients A and B. Studies were performed with patients during the active phase of PRCA. Controls represent the numbers of CFU-E in T-cell-depleted marrows alone. CFU-E did not proliferate in T cells or Tγ cells cultured alone.

### Table IV

**Effects of T Cells or Tγ Cells from CLL-PRCA Patients on Erythroid Colony Growth from Normal Marrows**

<table>
<thead>
<tr>
<th>Cell additions</th>
<th>Normal bone marrow I</th>
<th>Normal bone marrow II</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>152±31</td>
<td>208±20</td>
</tr>
<tr>
<td>Patient T cells</td>
<td>51±11 (34)</td>
<td>97±15 (47)</td>
</tr>
<tr>
<td>Normal T cells</td>
<td>139±22 (91)</td>
<td>209±35 (100)</td>
</tr>
<tr>
<td>Patient Tγ cells</td>
<td>24±5 (16)</td>
<td>45±15 (22)</td>
</tr>
<tr>
<td>Normal Tγ cells</td>
<td>155±21 (102)</td>
<td>221±20 (106)</td>
</tr>
</tbody>
</table>

*10^6 T cells or Tγ cells from patients or normal marrows were co-cultured with 10^6 T-cell-depleted marrow cells from normal donor I or II. Studies were performed with patients during the active phase of PRCA. Controls represent the numbers of CFU-E in normal T-cell-depleted marrows alone. CFU-E did not proliferate in T cells or Tγ cells cultured alone.

Values are mean±1 SD of triplicate cultures; values in parentheses indicate the percentage of control CFU-E. Addition of active phase patient T cells or Tγ cells decreased CFU-E numbers from patient marrows as compared with addition of normal T cells or Tγ cells (P < 0.025).
from blood null cells, although the stimulatory effect was less than that observed in the normal T cells (P < 0.05) under identical conditions. Furthermore, when 4 X 10^5 T cells from patient B were mixed with equal numbers of normal T cells before inoculation into DC and scored for total granulocytes (mature and immature), no significant differences in granulocyte precursor cells (myeloblasts-polymorphonuclear leukocytes) were observed from 10^5 TD-LDMNC of two normal donors compared with DC containing only 4 X 10^5 normal T cells (Table VII).

**TABLE VI**

<table>
<thead>
<tr>
<th>Additions to null cells</th>
<th>Controls</th>
<th>Patient B</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>60±24</td>
<td>10±2</td>
</tr>
<tr>
<td>Patient T cells</td>
<td>110±21</td>
<td>70±24</td>
</tr>
<tr>
<td>Control T cells</td>
<td>278±52</td>
<td>185±25</td>
</tr>
</tbody>
</table>

* 2 X 10^5 normal control T cells or patient T cells retrieved during active phase of PRCA were co-cultured with 2 X 10^5 normal or patient blood null cells and scored for day 14 BFU-E. BFU-E were absent in T cells cultured alone.  
* Values indicate mean±1 SD. BFU-E/2 X 10^5 null cells in triplicate plates. Patient T cells stimulated normal or control T cells but the burst-promoting effect was significantly less than observed with normal T cells under identical conditions (P < 0.05).
exogenous erythropoietin. These studies indicate that erythropoietin was present and antierthropoietin antibodies were unlikely (20, 22).

Previous investigators have emphasized the need for cautious interpretation of in vitro coculture studies due to the possible effects of allosensitization and histoincompatibility (23, 24). Although the allosensitization of T suppressor cells by transfusion therapy cannot be excluded in patient A, studies in patient B were performed before transfusions. Furthermore, the in vitro inhibitory effects of T cells were found using both autologous and allogeneic conditions; thus, it is unlikely that suppression can be attributed to histocompatibility differences alone.

The B cell origin of the lymphocytic leukemia in our patients was confirmed by the findings of a monoclonal immunoglobulin (IgM lambda) on the surface of a majority of blood lymphocytes, and the absence of typical clinical and morphologic features that may be seen in T-cell CLL. Thus, these cases differ from those previously described by Hoffman et al. (8) and Nagasawa et al. (9), who found CFU-E suppression to be mediated by T cells from two patients with T-cell CLL. In the former case report, B cells from patients with B-cell CLL did not suppress CFU-E proliferation in vitro (8). We have also been unable to demonstrate suppression of erythropoiesis in vitro by B cells from normals (11) or patients with B-cell CLL (unpublished observations).

Our E-rosette T-cell depletion studies performed on normal controls (Fig. 1) are consistent with the concept that the mature erythroid stem cells (CFU-E) can proliferate in the absence of T cells (25). CFU-E numbers were, in fact, increased after T cell depletion in normals, which may reflect some enrichment of CFU-E by the separation procedure. However, the 10-fold increase in CFU-E numbers of CLL PRCA patients after T cell depletion could not be attributed to either enrichment by loss of T cells or TD-LDMNC in the separation procedure. Moreover, readdition of normal T cells to TD-LDMNC marrow cells consistently increased CFU-E numbers from normal or patient marrow cells (Tables III and IV). Therefore, suppression of CFU-E growth under these conditions is quite unexpected. The T-cell enhancing effect of CFU-E is not due to addition of CFU-E, since control T cells cultured separately were devoid of erythroid colonies. It is possible that T cells may enhance the responsiveness of CFU-E to erythropoietin or recruit resting pre-CFU-E.

The localization of the CFU-E T-suppressor activity in CLL-PRCA to the Tγ cell subset is consistent with the observed effects of these cells in other cell cooper-
served in DC studies under allogeneic conditions; and (c) primitive BFU-E proliferation was suboptimal but not suppressed in the presence of CLL PRCA T cells. Differences in target antigens on erythroid and granulocyte stem cells may account for these findings (35).

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