T Lymphocytes Involved in Inhibition of Granulopoiesis in Two Neutropenic Patients Are of the Cytotoxic/Suppressor (T3+T8+) Subset

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ABSTRACT T lymphocyte-mediated bone marrow failure is a recently recognized clinical disorder, but the T lymphocyte subsets responsible for mediating the inhibitory effect have not been identified. We obtained T lymphocytes from the bone marrow of two patients with T lymphocyte-mediated granulopoietic failure, exposed them to monoclonal antibodies (OKT3, OKT4, and OKT8) in cytotoxicity assays, then recombined the treated T cells with autologous T-depleted marrow cells in clonal assays for granulocyte/macrophage progenitors (CFU-GM). Treatment of T cells with OKT3 and OKT8 abrogated their granulopoietic inhibitory effect but treatment with OKT4 did not. Therefore, in these two patients, the lymphocytes that played a role in the inhibition of granulopoiesis were of the cytotoxic/suppressor subset.

INTRODUCTION

Thymus-dependent lymphocytes and their subsets function as critical components of normal mammalian hematopoiesis. For example, T lymphocytes promote the growth of pluripotent (1–3) and committed (4–8) hematopoietic progenitor cells and interact with mononuclear phagocytes to regulate granulopoiesis in vitro (8). In vitro studies in a number of laboratories, including our own (9–12), have also provided evidence that a major pathophysiologic abnormality in some patients with bone marrow failure derives from T lymphocytes that inhibit hematopoiesis (9–14). While the term "suppressor" has been sometimes used to describe the hemopoietic activity of these T lymphocytes, it is not yet known whether these cells are the same as those more widely recognized (and more thoroughly studied) suppressor T lymphocyte subpopulations that inhibit certain phases of the immune response. To address this concern we have used monoclonal antibodies against the suppressor/cytotoxic and helper/inducer subsets (15, 16) in cytotoxicity assays against T lymphocytes obtained from the bone marrow of two patients with T lymphocyte-mediated granulopoietic failure.

METHODS

Patients. We studied cells from two patients in whom we had previously documented T lymphocyte-mediated hematopoietic failure. The first patient is a 52-yr-old woman with aplastic anemia (10) who had been in complete remission for 2 yr while taking 50 mg azathioprine daily. Progressive neutropenia and thrombocytopenia developed 6 mo after discontinuation of azathioprine. Cells were obtained at that time. A second study was performed 5 mo later during remission.

The second patient is a man with prednisone-responsive T lymphocyte-mediated granulopoietic failure and Felty's syndrome (9). Prednisone had been discontinued for 11 mo before neutropenia (0.4 × 10^6 neutrophils/ml) recurred at which time cells were obtained. A second study was performed 7 mo later during a second relapse.

Volunteers. Bone marrow cells were obtained from five paid consenting normal adult volunteers and were processed as described below.

Bone marrow cells. Single-cell suspensions of low density marrow cells were prepared as described previously using Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N. J.) (8–10, 12). Phagocyte-depleted low density marrow cells were prepared using an iron magnet technique (9) and T lymphocytes were removed by depleting those phagocyte-depleted low density cells that formed sheep erythrocyte rosettes on a second Ficoll-Paque gradient (8–12). The T lymphocytes removed were isolated by gentle agitation and osmotic lysis (8, 9, 17). 0.1 ml of 5 × 10^6 T lymphocytes/ml.

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TABLE I
Granulocyte Colony Growth (Mean Colonies ± 10x LDBMC ± SD)
of Marrow Cells from Two Patients (P1 and P2) and Four Normal Volunteers (V1–V5)

<table>
<thead>
<tr>
<th>Subject</th>
<th>LDBMC</th>
<th>TD</th>
<th>TD+T</th>
<th>TD+OKT3-T</th>
<th>TD+OKT4-T</th>
<th>TD+OKT8-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>64±15</td>
<td>163±20</td>
<td>41±11</td>
<td>107±20</td>
<td>20±4</td>
<td>114±20</td>
</tr>
<tr>
<td>P2</td>
<td>62±10</td>
<td>110±11</td>
<td>64±10</td>
<td>85±16</td>
<td>2±2</td>
<td>245±39</td>
</tr>
<tr>
<td>V1</td>
<td>162±10</td>
<td>163±33*</td>
<td>176±17</td>
<td>192±26</td>
<td>54±21</td>
<td>184±30</td>
</tr>
<tr>
<td>V2</td>
<td>—</td>
<td>80±18*</td>
<td>91±25</td>
<td>77±16</td>
<td>44±7</td>
<td>45±8</td>
</tr>
<tr>
<td>V3</td>
<td>202±31</td>
<td>212±32*</td>
<td>200±11</td>
<td>176±17</td>
<td>74±14</td>
<td>166±17</td>
</tr>
<tr>
<td>V4</td>
<td>85±10</td>
<td>—*</td>
<td>61±10</td>
<td>64±6</td>
<td>112±13</td>
<td>75±4</td>
</tr>
<tr>
<td>V5</td>
<td>182±39</td>
<td>160±8</td>
<td>—</td>
<td>—</td>
<td>181±10</td>
<td>162±16</td>
</tr>
</tbody>
</table>

TD, T lymphocyte depleted (E rosette depletion) LDBMC.
TD+T, Complement treated T lymphocytes added back to TD.
TD+OKT3-T, OKT3 antibody and complement treated T-cells added back to TD.
TD+OKT4-T, OKT4 treated T-cells added back to TD.
TD+OKT8-T, OKT8 treated T-cells added back to TD.

* Depletion of T lymphocytes from LDBMC from 27 normal volunteers has never augmented colony stimulated activity-stimulated granulocyte colony growth.

were treated with 0.1 ml of 1:25 dilution of one of the monoclonal antibodies, OKT3, OKT4, or OKT8 (Ortho Pharmaceutical Corp., Raritan, N. J.) for 20 min at 2°C. 0.5 ml (0.1 ml in the second studies on the two patients) rabbit complement was then added and the suspension was incubated at 37°C for 60 min as previously described (8). Cells so treated were thrice washed and added back to autologous T-depleted, phagocyte-depleted low density bone marrow cells (LDBMC)\(^1\) in clonal assays for granulocyte/macrophage progenitors (CFU-GM) (8). In two volunteers and both patients cytotoxic treatment of T\(^+\), T4\(^+\), or T8\(^+\) cells resulted in 85–98% lysis of the appropriate subset.

On cytofluorographic analysis (8) of cells from patients one and two, T4\(^+\) cells were 7 and 15% of LDBMC, respectively, and T8\(^+\) cells were 14 and 21%, respectively. In the volunteers T4\(^+\) cells ranged from 16 to 29% and T8\(^+\) cells ranged 9 to 18% of LDBMC. In each case, the number of T cells added back to (T-depleted LDBMC) were adjusted to match the original fraction of T cells in each patient. Aliquots of T cells in this adjustment were prepared prior to their exposure to antibodies and/or complement so that potentially misleading enrichment of antigen-negative T cells would not occur.

Clonal assays (CFU-GM) were performed using methylcellulose (0.9%) in alpha medium with 15% fetal calf serum (8, 11, 12), and 10% human placental conditioned medium (18) as the source of colony stimulating activity. Colonies (>40 cells per aggregate) and clusters (<40, >8 cells per aggregate) were counted after 7 d of culture (7.5% CO\(_2\) in air, 37°C). Each variable was tested in four replicate plates. Inhibition or enhancement of granulopoiesis was considered to be statistically significant if mean clonal growth was different than control clonal growth with \(P < 0.05\).

RESULTS

Colony growth of normal (V1–V5) and abnormal (P1 and P2) bone marrow cell fractions and recombinations thereof are shown in Table I. In patient 1, results of a second study (during another relapse) were similar. In patient 2, a second study (during remission) showed markedly improved colony growth (216±21 colonies per plate) no T-mediated inhibition and no change when T8\(^-\) (229±28) or T4\(^-\) (208±30) cells were added. Colony-stimulated activity—stimulated colony growth of cells from both patients increased significantly (\(P < 0.001\)) when T lymphocytes were removed by E rosette depletion. When untreated (not shown) or complement-treated T lymphocytes (Table I) were added back, suppression was again noted but when OKT3 and OKT8 treated T-cells were added there was no inhibition of colony growth. In fact, in P2 the combination of marrow cells with T8\(^-\) T lymphocytes yielded colony growth that exceeded that of cells from which T cells had been removed by rosette depletion or by OKT3 antibody (Table I). Treatment with OKT4 antibody did not abrogate T suppression.

In cell cultures from normal volunteers T removal by OKT3 cytotoxicity or E rosette depletion failed to enhance colony growth. Selective removal of T8\(^+\) cells by cytotoxicity failed to enhance colony growth but removal of T4\(^+\) cells inhibited colony growth (Table I).

DISCUSSION

There are interesting parallels between suppressor T-cells of immunity (19, 20) and T cells that inhibit granulopoiesis (9–12). For example, in patients with prednisone-responsive hypogammaglobulinemia cortisol-sensitive T lymphocytes account for suppression of B cell function (19, 20). Similarly, cortisol sensitive T lymphocytes that mediate prednisone-responsive

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\(^1\) Abbreviations used in this paper: CFU-GM, clonal assays for granulocyte/macrophage progenitors; LDBMC, low density bone marrow cells.
hemopoietic failure have also been described (9–11). Furthermore, both types of “suppressor” T cells produce soluble factors that inhibit target cell function in vitro (9, 10, 19, 20).

The studies described herein were designed to test the hypothesis that the T lymphocyte subset that inhibits granulopoiesis in patients with T lymphocyte-mediated hemopoietic failure possesses some surface antigens in common with T lymphocyte suppressors of immunity. In vitro granulopoiesis in both patients was significantly enhanced by removal of T lymphocytes from the marrow cell suspension, and was inhibited when autologous T lymphocytes were added back (Table I). T lymphocytes treated with a monoclonal antibody directed against either a common T cell antigen (OKT3) or against an antigen found on the cytotoxic/suppressor subset (OKT8) failed to inhibit the granulopoietic activity of autologous T-depleted marrow cells (Table I). Indeed, in one patient colony growth was maximal when T lymphocytes were selectively T8−, which suggests that some T8− cells may have functioned as granulopoietic helpers. None of the above phenomena were noted in studies on cells from normal volunteers or in a study on cells from patient one obtained during azathioprine-induced remission.

A major limitation of cytotoxicity assays is that critical cell-cell interactions may be missed. For this reason although our study indicates that T8+ cells are obviously involved in suppression of granulopoiesis, we cannot state with certainty that they are the effectors of suppression. They could, for example, recruit other lymphocytes to effect suppression. Positive controls using living sorted cells will be required to answer this question. However, because the T4− cells were fully suppressive and the T8− cells were not, we can state that if T8+ cells recruit a non-T8 effector population they should be T4+. Similarly if T cell help is required for T8+ cells to suppress colony growth the help should be T4−.

Of additional interest is the observation that in both patients T lymphocytes treated with antibody directed against an antigen found on the “helper/inducer” T lymphocyte subset (OKT4) potently inhibited granulopoiesis. This initially suggested to us that T4+ cells may have functioned as enhancers of colony growth which, when present with T8− cells, partially competed with the inhibitory activity of T8+ cells. However, an inhibitory effect of T4+ cells was also seen in three of five studies on normal cells (Table I). It is possible that two or more subsets of T cells are capable of antipodal function. A recent report (21) that normal T cell subsets exhibit opposing functions in a clonogenic erythroid system is compatible with this point of view. It is also possible that a purely technical artifact might account for the observations in our volunteers. For example, treatment of T8+ cells with OKT4 may induce them to function as granulopoietic suppressors. Until more detailed studies are performed using isolated T cell subsets the explanation of this OKT4 related augmentation of suppression will remain enigmatic.

Despite these new concerns, our results document that enhancement of marrow cell colony growth in patients with T lymphocyte-mediated marrow failure occurs after exposure of those inhibitory T cells to OKT8 and OKT3 but not to OKT4 antibody and that similar experiments in five volunteers and one patient in remission did not result in enhancement of colony growth. Thus, in these two patients, the T lymphocytes involved in the pathophysiology of granulopoietic failure are of the “suppressor/cytotoxic” subset.

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REFERENCES