In Vitro Evidence for Disappearance of Erythroid Progenitor T Suppressor Cells Following Allogeneic Bone Marrow Transplantation for Severe Aplastic Anemia

By Kenneth F. Mangan, Mark T. Mullaney, Craig S. Rosenfeld, and Richard K. Shadduck

In vitro coculture studies were performed in five patients with severe aplastic anemia (SAA) and their normal HLA-matched donors before and after allogeneic bone marrow transplantation (BMT) to determine whether the erythropoietic function of T cells is abnormal in this disorder. These coculture studies used fresh or cryopreserved marrow T lymphocytes with fresh or cryopreserved marrow T cell-depleted target cells. Four of five aplastic patients had little or no transfusion exposure before studies. The composite results showed that, in comparison to the erythropoietic effects of normal HLA-identical marrow T lymphocytes or engrafted T lymphocytes, T lymphocytes collected from the aplastic patients before BMT consistently suppressed or failed to support CFUe and BFUe growth optimally from autologous marrow, HLA-identical marrow, or engrafted aplastic T cell-depleted marrows. This T cell abnormality was not observed in four multiply transfused leukemias and three patients with myelodysplastic syndrome. Marker analyses of SAA marrow T lymphocytes performed before and after BMT suggested that the erythropoietic functional abnormality was due to abnormal marrow T cell composition reflecting an excess of activated Tac+, T3+, T11+ lymphocytes. Collectively, these in vitro studies provide firmer in vitro evidence implicating T cells in the pathogenesis of SAA. The erythropoietic T cells abnormalities in SAA are fully corrected by allogeneic BMT.

A PROXIMATELY 50% of patients with severe aplastic anemia (SAA) respond to immunosuppressive agents such as antithymocyte globulin (ATG), corticosteroids, or cyclosporin A.1,4 Intensive immunosuppressive conditioning regimens may also induce autologous recovery in SAA patients who fail to engraft with donor marrow following allogeneic bone marrow transplantation (BMT).3 About one-half of identical twin pairs with SAA will require intensive immunosuppressive conditioning to allow acceptance of their syngeneic graft.6 These clinical observations implicate immune lymphocytes, especially T lymphocytes, in the pathogenesis of SAA.

Several laboratories have performed coculture studies in patients with SAA to provide in vitro evidence for a pathologic role of T lymphocytes in this disorder.7-17 Interpretation of these in vitro coculture studies, however, has been hampered by several methodologic pitfalls: (a) Unseparated blood lymphocytes were often used rather than marrow lymphocytes or target cells;8,12,13,14; (b) HLA-mismatched, allogeneic target cells were used rather than autologous or HLA-matched target cells;10,12,13,14; (c) patients were often studied after multiple transfusions leading to allosensitization effects;7,12; and (d) studies were not always performed before and after clinical manipulations, making correlations between in vitro and in vivo findings difficult.

To minimize these problems, we performed in vitro coculture studies in five aplastic anemia patients and their HLA-matched donors before and after they received allogeneic BMT. Aplastic anemia and normal HLA-identical marrow T lymphocytes and their T cell-depleted narrow target cells were cryopreserved before BMT to assess their erythropoietic function in vitro after BMT. The results showed that in comparison to normal T lymphocytes, aplastic anemia T lymphocytes collected from marrow before BMT suppressed or failed to stimulate erythroid colony growth from autologous marrow or HLA-matched normal donor marrow. Three weeks following allogeneic BMT, the in vitro erythropoietic T cell function returned to normal.

MATERIALS AND METHODS

Isolation of marrow T lymphocytes and T cell-depleted target cells. Informed consent was obtained before marrow aspirations were performed on all patients and their HLA-identical normal donors. These studies were approved by the Human Investigations Committee at Montefiore Hospital. Multiple 2-cc marrow samples were aspirated from the posterior iliac crest into sterile preheparanized syringes immediately before and 21 days following an allogeneic bone marrow transplant. Light-density whole mononuclear cells (WMNCs) were prepared by a standard Ficoll-Hypaque (D = 1.077 g/dl) density-gradient centrifugation technique.18 Aliquots of WMNCs were analyzed for T cell and natural killer cell (NK) subsets (described below) or further depleted of T lymphocytes by rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes and subjected to a second FH centrifugation.19 Rosetted T cells were freed of sheep erythrocytes by lysis with Tris-buffered ammonium chloride, washed three times in a-minimum essential medium, (a-MEM, GIBCO, Grand Island, NY) and used directly (fresh T cells) in cocultures. Some T cells were cryopreserved for future studies. T cell-depleted (TD) marrow cells that remained at the interface of the FH gradient were used as progenitor-cell-enriched target cells in erythroid colony assays or cryopreserved. Isolated T cells were >95% pure as judged by immunofluorescent analysis with the OKT11 E rosette receptor monoclonal antibody. TD target cells contained <5% residual T cells.

Cryopreservation techniques. T cells or TD target cells from SAA patients or their matched donors were suspended in RPMI

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media containing 20% fetal calf serum (FCS, HyClone, Logan, UT) at a concentration of 10 x 10^6 cells/mL. Cold RPMI media containing 20% FCS and 20% dimethylsulfoxide (DMSO) were mixed on ice with equal volumes of cell suspensions to give a final concentration of 5 x 10^6 cells/mL in 10% DMSO and 20% FCS–RPMI. Cells were placed in 2-mL vials (Nunc; Gibco, Grand Island, NY) at −20°C for 60 minutes and then transferred to a −70°C freezer. Alternatively, cells in vials were placed in a controlled rate freezer (Cryomed, Mt Clemens, MO) and frozen in liquid nitrogen at a rate of −1°C/min to −40°C and then −5°C/min to −90°C, and were then stored under liquid nitrogen (−186°C) until further use. Before repeat cocultures, cells were thawed rapidly in a 37°C water bath and washed in cold RPMI with 20% FCS to remove dead cells. Cell fractions used in coculture were >90% viable as judged by the Trypan blue dye exclusion technique.

**Erythroid colony assay.** A methylcellulose erythroid culture system containing 10⁻¹ mercaptoethanol, 10% deionized bovine serum albumin (BSA), and 30% horse serum (Hyclone, Logan, UT) was used as described previously. For day 14 BFU₆, Mo cell-conditioned medium (10%) was added as a source of burst-promoting activity. In coculture studies, 2 x 10⁶ aplastic or normal donor TD target cells were mixed directly with aplastic or normal donor T cells at a ratio of 1:1. When adequate T cells were available, ratios of 1:0.5 or 1:0.25 were also tested. TD cells and T cells were also plated alone as separate controls. Each variable was tested in triplicate. Erythropoietin 0.5 IU or 1.0 IU (Alpha Therapeutics, Los Angeles—specific activity 500 IU/mg protein) was added to each dish for CFU₆ and BFU₆ cultures, respectively. Cultures were placed in a 37°C, 5% CO₂ humidified atmosphere for 7 or 14 days. Day 14 benzidine-positive colonies containing >50 cells/aggregate were scored as BFU₆. Day 7 benzidine-positive colonies containing >8 cells/aggregate were scored as CFU₆ using an inverted microscope.

**Cell marker analysis.** Aplastic or normal donor WMNCs were labeled with a battery of T cell (OKT1, E rosette receptor; OKT3, pan T cell, OKT4, helper/inducer T cell, OKT8 cytotoxic/suppressor (Ortho Pharmaceutical, Raritan, NJ); natural killer cell (Leu 11, Leu 7, Becton Dickinson, Mountainview, CA) or activation antigens [HLA-DR, interleukin 2 receptor (Tac), Becton Dickinson] fluorescein-conjugated monoclonal antibodies and analyzed on an Ortho Spectrum II flow cytometer as described previously.

**Statistics.** Comparison of cohorts was made with Student’s t tests.

**RESULTS**

**Clinical characteristics of the aplastic patients.** The pertinent clinical data of the aplastic patients is summarized in Table 1. All the patients met published criteria for SAA. All were conditioned with high-dose cyclophosphamide 50 mg/kg/day for 4 days and received either cyclosporin A alone or cyclosporin A/methotrexate for graft-vs-host disease (GVHD) prophylaxis beginning on day 1. All patients received >3 x 10⁶ cells/kg unmanipulated marrow from an HLA-identical MLC nonreactive donor. None of the donors were allosensitized. Four of the five SAA patients had been exposed to more than five blood donor exposures before the initial coculture study. In case 1, studies were performed before any transfusions were administered. All patients engrafted promptly by day 21. Engraftment was proven by informative RBC phenotyping and sex chromosome studies. Three patients are alive and well >6 months following BMT without any evidence of GVHD. Cases 4 and 5 died after 2 and 4 months, respectively, following onset of Grade III through IV acute GVHD.

**Effect of aplastic marrow T cells on CFU-E growth from autologous or HLA-identical T-depleted target cells before BMT.** The individual and composite results of coculture studies performed before BMT with aplastic T cells and autologous TD target cells are shown in Fig 1. Before BMT, 11.8 ± 10.2, (mean ± 1 SD, n = 5) CFU-E were detected in 2 x 10⁶ aplastic marrow WMNC (stippled bars, composite). Removal of marrow T cells by E rosetting significantly increased the number of CFU-E in the aplastic group to 65 ± 43 CFU-E/2 x 10⁵ TD cells (mean ± 1 SD, n = 5, P < .05) (clear bars). In the presence of aplastic T cells, CFU-E growth was suppressed to 46% of that in the TD fraction alone (striped bars, P > .05, n = 5). In the individual cases (cases 1, 2, 3, and 5), aplastic T cells significantly suppressed CFU-E growth from TD target cells (P < .05). In two cases, (cases 3 and 4), equal numbers of T cells from the HLA-identical donor marrow (solid bars) were cocultured with aplastic TD target cells. As shown in Fig 1, these cells failed to suppress (case 3) or stimulated CFU-E growth, (case 4), from aplastic TD cells. CFU-E were not detectable in aplastic T cells cultured alone and less than 20 CFU-E were detected in fresh 2 x 10⁶ normal T cells cultured separately (data not shown).

The individual and composite results of coculture studies performed before BMT with fresh HLA-identical TD target cells are shown in Fig 2. In four cases (cases 1, 2, 3, and 5), aplastic T cells decreased CFU-E growth on an average of 63% (49% to 79%) as compared with growth in TD target cells alone (P < .05). Moreover, CFU-E numbers in cultures containing aplastic T cells were significantly lower than CFU-E numbers in cultures with normal donor T cells (P < .01, n = 5).

**Effects of aplastic or engrafted T cells on CFU-E growth from cryopreserved HLA-identical T-depleted target or autologous engrafted target cells following BMT.** The individual and composite results of coculture studies performed after BMT with engrafted TD target cells are shown in Fig 3. Twenty-one days after BMT, CFU-E numbers

**Table 1. Clinical Characteristics of SAA Patients**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)/Sex</th>
<th>Conditioning Regimen</th>
<th>Donor Exposures</th>
<th>GVHD Prophylaxis</th>
<th>Engraftment Proof</th>
<th>GVHD</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17/M</td>
<td>CTX 50 mg/kg × 4</td>
<td>0</td>
<td>Cyclosporin A</td>
<td>Chromosomes, RBCs</td>
<td>0–I</td>
<td>A + W, 12 mo</td>
</tr>
<tr>
<td>2</td>
<td>15/F</td>
<td>CTX 50 mg/kg × 4</td>
<td>3</td>
<td>Cyclosporin A</td>
<td>Chromosomes, RBCs</td>
<td>0–I</td>
<td>A + W, 6 mo</td>
</tr>
<tr>
<td>3</td>
<td>16/F</td>
<td>CTX 50 mg/kg × 4</td>
<td>1</td>
<td>Cyclosporin A</td>
<td>RBCs</td>
<td>III–IV</td>
<td>Died, 2 mo</td>
</tr>
<tr>
<td>4</td>
<td>21/M</td>
<td>CTX 50 mg/kg × 4</td>
<td>5</td>
<td>Cyclosporin A/MTX</td>
<td>Chromosomes, RBCs</td>
<td>0–I</td>
<td>A + W, 6 mo</td>
</tr>
<tr>
<td>5</td>
<td>31/M</td>
<td>CTX 50 mg/kg × 4</td>
<td>&gt;20</td>
<td>Cyclosporin A</td>
<td>Chromosomes, RBCs</td>
<td>III–IV</td>
<td>Died, 4 mo</td>
</tr>
</tbody>
</table>
returned to normal (clear bars). In every case, engrafted T cells (hatched bars) obtained following BMT no longer suppressed CFU-E growth ($P > .05$, $n = 5$). There was no difference between the CFU-E erythropoietic effects of engrafted T cells and cryopreserved normal donor T cells in cocultures with fresh TD engrafted target cells. In contrast, in two cases (cases 1 and 4) in which aplastic T cells had been collected and cryopreserved before BMT, CFU-E growth was suppressed from the TD-depleted engrafted target cells ($P < .05$). Moreover, the T cell erythropoietic effects of aplastic T cells collected before BMT were significantly diminished when compared with the effects of the engrafted T cells or normal T cells ($P < .05$, $n = 3$) (cases 1, 2, and 4). Less than 10 CFU-E were detected in $2 \times 10^5$ engrafted or cryopreserved normal T cells cultured separately (data not shown).

The individual and composite results of coculture studies performed after BMT with cryopreserved HLA-identical normal donor marrow T-depleted cells are shown in Fig 4. As was noted with engrafted TD-depleted target cells, engrafted or normal donor T cells were equivalent in their ability to support CFU-E growth from normal cryopreserved marrow ($P > .05$, $n = 5$). In contrast, aplastic T cells in three cases tested (cases 1, 3, and 4) were significantly less supportive of CFU-E growth when compared with engrafted or normal T cells ($P < .05$) and frankly suppressed CFU-E growth from T-depleted targets in cases 3 and 4 ($P < .05$).

**Effects of aplastic, HLA-identical donor or engrafted T cells on BFU-E growth from aplastic or normal donor T-depleted target cells before and after BMT.** The composite
results of coculture studies performed in the marrow BFU-E cultures with aplastic, fresh normal donor, cryopreserved normal donor, and engrafted T-depleted target cells are summarized in Fig 5. The results mirror the findings in the CFU-E cultures. Namely, aplastic T cells (striped bars) collected before BMT frankly suppressed growth of BFU-E from fresh donor target cells ($P < .05$, $n = 5$) and engrafted target cells ($P < .05$, $n = 5$). In coculture studies using autologous or cryopreserved donor target cells, suppression was not as readily demonstrable ($P > .05$). As shown in Fig 5, the differences were more readily apparent (ie, statistically significant) when BFU-E growth was optimal, ie, when fresh donor or engrafted marrow target cells were used. Following BMT, BFU-E numbers returned to normal (engrafted aplastic clear bars). At that time, engrafted T cells (hatched bars) were equivalent to donor T cells (solid bars) in their ability to support BFU-E growth. In contrast, before BMT, there were significant differences in the ability of donor T cells v aplastic T cells to support BFU-E growth from either aplastic ($P < .05$, $n = 3$) or normal T-depleted target cells ($P < .05$, $n = 5$). Fewer than 3 BFU-E were detected in $2 \times 10^3$ donor or aplastic T cells cultured separately (data not shown).

**Effects of allosensitized v unsensitized T lymphocytes on growth of CFU-E from HLA-identical TD marrow cells.** To determine whether the CFU-E suppressing effects of T lymphocytes in SAA were unique to this disease or were a function of allosensitization from transfusion, additional control coculture studies were performed using normal marrow T lymphocytes retrieved from multiply transfused leukemic patients in first complete remission, myelodysplastic patients, and their HLA-identical normal donors. Patients had all been exposed to more than ten donors. Ablative therapy had been completed 2 to 3 months before the studies. Myelodysplastic patients had never been exposed to ablative therapy before the studies. Their HLA identical normal donors were not sensitized. These T cells were cocultured with patient or normal donor T cell-depleted marrow target cells before BMT at a ratio of 1:1. As shown in Table 2, in seven separate cases, patient allosensitized T cells were approximately equivalent to HLA-identical normal unsensitized T cells in their ability to support CFU-E growth ($P > .05$). Addition of T lymphocytes from leukemic or myelodysplastic marrow resulted in frank stimulation of CFU-E from either autologous or HLA-matched T-depleted marrow in six of seven cases ($P < .05$, no T v normal T or patient T, Table 2).

**Cell surface marker analysis of aplastic marrow cells before and after BMT.** To determine whether the functional differences in the T cell erythropoietic effects in culture reflected phenotypic differences in T cell composition, we performed flow cytometric analysis of aplastic marrow mononuclear cells before and 3 weeks after allogeneic BMT. Marker studies were also performed in blood mononuclear cells from aplastic patients before BMT. A battery of T cell and NK cell reactive fluorescein-conjugated monoclonal antibodies were used for the analyses. Similar marker studies were performed on their normal HLA-identical donors. Aplastic blood lymphocytes were significantly less reactive with HLA-DR than were marrow lymphocytes ($P < .05$); otherwise, the cell populations were statistically identical. As shown in Table 3, before BMT, the numbers of T3-positive, T11-positive, and Tac-positive antigen-bearing lymphocytes were significantly greater than normal ($P < .05$). The Leu 11 NK cell antigen was significantly less than normal ($P < .05$). After BMT, there were no significant differences between any of the marker values in the engrafted cells and normal values. Aplastic patients had a significant decrease ($P < .05$) in the expression of T3 and Leu 7 antigens and a significant increase ($P < .05$) in Leu 11 and HLA-DR antigens as compared with marker studies performed before BMT. There were decreases in T11+, T8+, and Tac-bearing cells following BMT in the aplastic patients ($P > .05$). The bone marrow T4/T8 ratio rose from $0.6 \pm 0.3$ to $0.8 \pm 0.6$ 3 weeks after allogeneic BMT, reflecting a decrease in T8 cells. There was no change in the proportion of T4-bearing cells.

**DISCUSSION**

These in vitro coculture studies in patients with SAA were designed to avoid several methodologic pitfalls that hampered interpretation of previous coculture studies. First, we used marrow-enriched T cells with marrow T cell-depleted target cells rather than blood lymphocytes to gain better understanding of marrow progenitor cell growth in SAA. The marrow also contained a significantly greater number of HLA-DR+ cells as compared with blood cells. Second, to minimize allogeneic effects in vitro, target cells were derived from autologous or HLA-matched, MLC nonreactive donors. Third, to determine the physiologic relevance of our in vitro findings, we repeated the coculture studies following BMT when patients were fully engrafted. By cryopreserving aplastic marrow T cells at presentation, we could compare
their erythropoietic effects in vitro with normal or engrafted T lymphocytes retrieved from patients after BMT. The results showed that, in comparison to the erythropoietic effects of normal HLA-identical T lymphocytes or engrafted cells, aplastic T cells collected before BMT consistently suppressed or failed to support optimally CFU-E and BFU-E growth from autologous marrow, HLA-identical normal marrow, or engrafted T cell-depleted target marrows. Twenty-one days after conditioning with high-dose cyclophosphamide, when blood counts were normal, engrafted T cells failed to suppress erythropoiesis in vitro and progenitor cell numbers had risen to the normal range.

Previous studies had suggested that in vitro inhibition of hematopoietic progenitor cell growth by SAA lymphocytes was largely due to transfusion-induced sensitization of patient lymphocytes to minor histocompatibility antigens. In the present studies, we used HLA-matched or autologous marrow target cells, which minimizes this artifact. We also studied one patient before any transfusions were administered and three other patients, who had been exposed to fewer than five donors. As other researchers have noted, T suppressor cell effects on progenitor cells growth in vitro may nonetheless be observed in untransfused patients. Moreover, in contrast to aplastic marrow T cells, marrow T cells from multiply transfused leukemia patients or myelodysplastic patients retrieved before BMT did not suppress CFU-E growth from autologous or HLA-identical normal target marrows. Indeed, they stimulated growth modestly, as we have observed in normal subjects. Collectively, these observations suggested that allosensitization effects alone cannot explain all the abnormal in vitro findings in SAA. Our findings confirm that when directly compared with normal T lymphocytes, aplastic anemia T lymphocytes in many if not all patients are fundamentally and functionally abnormal with respect to their ability to support erythroid colony growth in vitro. The CFU-E assay system may be more sensitive than the BFU-E assay in detecting this SAA T cell abnormality.

Although several laboratories have examined peripheral blood markers in SAA before and after treatment, there is very little information on marrow lymphocyte composition in aplastic anemia. The samples we analyzed were all taken directly from marrow aspirates, using multiple needle punctures in small volumes to minimize blood contamination. Aplastic marrow mononuclear cells expressed HLA-DR antigens at an increased frequency in comparison to blood. Otherwise, the markers were similar. This suggested that the marrow lymphocytes used for these studies were similar but not identical to blood lymphocyte populations. The marrow marker studies before and after BMT also suggested that the functional abnormalities of aplastic anemia T lymphocytes observed in the erythroid colony assays before BMT may be due to changes in the composition of marrow T cell subsets. These marrow marker studies appear to support the results of previous studies performed on SAA peripheral blood which implicated activated Tac-positive suppressor T cells in the pathogenesis of SAA. Thus, in our studies, the Tac, T11, and T3 antigens were the only antigens that were significantly increased in marrow whole mononuclear marrow cells (WMNCs) as compared with their expression in HLA-

Table 2. Effects of Normal or Allosensitized Patient T Cells on Growth of CFU-E From Normal or Patient TD Marrow Cells in HLA-Identical Pairs

<table>
<thead>
<tr>
<th>Case No</th>
<th>Diagnosis</th>
<th>HLA-Matched Donor TD Target Cells*</th>
<th>Patient TD Target Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No T (%)</td>
<td>Normal T (%)</td>
</tr>
<tr>
<td>1</td>
<td>ANLL</td>
<td>223 ± 21</td>
<td>275 ± 8†</td>
</tr>
<tr>
<td>2</td>
<td>ANLL</td>
<td>135 ± 13</td>
<td>173 ± 8†</td>
</tr>
<tr>
<td>3</td>
<td>ALL</td>
<td>315 ± 28</td>
<td>360 ± 14</td>
</tr>
<tr>
<td>4</td>
<td>ALL</td>
<td>253 ± 19</td>
<td>306 ± 45</td>
</tr>
<tr>
<td>5</td>
<td>ALL</td>
<td>184 ± 11</td>
<td>204 ± 18</td>
</tr>
<tr>
<td>6</td>
<td>MDS</td>
<td>135 ± 20</td>
<td>161 ± 18</td>
</tr>
<tr>
<td>7</td>
<td>MDS</td>
<td>166 ± 27</td>
<td>238 ± 26†</td>
</tr>
</tbody>
</table>

*Marrow T cells (2 x 10⁸) from multiply transfused (more than ten donors) leukemic patients (ANLL or ALL) in complete remission or myelodysplastic (MDS) patients or their HLA-identical donors were cocultured with 2 x 10⁷ T cell-depleted (TD) marrow target cells from patients or their HLA-matched normal donors. Values indicate mean ± 1 SD CFU-E for triplicate cultures. Values in parentheses indicate percentage of CFU-E as compared with cultures containing no T cells. There were no differences in the CFU-E erythropoietic effects of allosensitized patient T cells vs nonsensitized HLA-matched normal T cells (P > .05).

1 Significant (P < .05) increase in colony numbers vs "no T" control.

Table 3. Marrow Lymphocyte Markers in SAA Before and After Allogeneic BMT

<table>
<thead>
<tr>
<th>Subject</th>
<th>Marker (% of WMNCs)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T11</td>
</tr>
<tr>
<td>Aplastic patients before BMT</td>
<td>74 ± 6†</td>
</tr>
<tr>
<td>(70 ± 15.6)</td>
<td>(73 ± 9)</td>
</tr>
<tr>
<td>After BMT</td>
<td>50 ± 31</td>
</tr>
<tr>
<td>Normal donors</td>
<td>57 ± 16</td>
</tr>
</tbody>
</table>

*Percentage of WMNCs positive with fluorescein-conjugated monoclonal antibodies. Values are mean ± 1 SD for five aplastic patients and their HLA-identical donors. Markers in parentheses indicate blood lymphocyte markers tested simultaneously.

† P < .05 before BMT vs normal subjects.

‡ P < .05 blood vs marrow.

§ P < .05 before BMT vs 3 weeks after BMT.

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matched normal donor marrow cells. NK cells (as assessed by the Leu 11 monoclonal antibody) were decreased as shown by use of peripheral blood. This observation makes it less likely that our E rosetted T cells reflected effects of NK cells, which can suppress erythroid colony growth in vitro. Alternatively, reduction in NK cell frequency may contribute to reduced ability of marrow E rosette population to support colony growth. These abnormalities are unlikely to reflect transfusion sensitization. After BMT, there was a significant decrease in T cell expression of OKT8-positive antigens, which resulted in a modest rise in T4/T8 ratio. The T4/T8 ratio is normally <1 in bone marrow. Thus, the return to normal did not result in inversion of T4/T8 ratio. As previously noted, however, a persistently low level of T4-positive cells was observed before and after BMT.

In summary, the present studies provide firmer in vitro evidence to support the hypothesis that abnormalities in T cell composition and function contribute to the initiation and/or perpetuation of marrow failure in SAA. The current studies do not address the precise mechanism by which these cells act. Pure populations of progenitor cells and T cell subsets will be required to determine whether the T cells interact directly with the progenitor cells in an "autoreactive" fashion or whether they act through other accessory cells such as monocytes. Following less intensive immunosuppressive therapy with ATG or corticosteroid treatment, clinical improvements in blood counts may occur, but in vitro evidence suggests persistence of abnormal T cells with reduced progenitor colony growth. Indeed, up to 20% of these patients may eventually relapse. The present studies show that with the more intensive immunosuppressive conditioning treatment and an allogeneic BMT, both the in vivo and in vitro defects are fully corrected. Recent studies in our laboratory suggest that reemergence of suppressive T cells may occur with graft rejection. In vitro studies using appropriate controls may prove useful in predicting clinical events after BMT.

ACKNOWLEDGMENT

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In vitro evidence for disappearance of erythroid progenitor T suppressor cells following allogeneic bone marrow transplantation for severe aplastic anemia

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