Lymphocyte Interleukin 2 Production and Responsiveness Are Altered in Patients With Primary Myelodysplastic Syndrome

By Olcay Ayanlar-Batuman, Jerry Shevitz, Ursula C. Traub, Scott Murphy, and Daniel Sajewski

Immunoregulatory T and B cell functions in 15 patients with primary myelodysplastic syndrome (MDS) were studied by measuring the proliferative and the stimulatory capacity of T and B cells, respectively, in autologous (auto) and allogeneic (allo) mixed lymphocyte reaction (MLR). T cell proliferation in the auto MLR was 25% of the control (P < .02), whereas proliferation in the allo MLR was normal. When control T cells were stimulated by MDS B cells, their proliferative response was only 57% of the control (P < .01). The mechanism responsible for these abnormalities was studied by determining the capacity of MDS and normal T cells to produce interleukin 2 (IL 2) and to generate IL 2 receptors (IL 2R) following stimulation with control and MDS B cells. In the auto MLR of MDS patients, only 3% ± 2% of T cells developed IL 2R positivity, whereas in control cultures 12% ± 2% of T cells were positive, as determined by immunofluorescence, using a monoclonal antibody (MoAb) directed against the IL 2R, and FACS analysis. When MDS T cells were stimulated by control B cells, IL 2R generation and the production of IL 2 were within normal limits. In contrast, when control T cells were stimulated by MDS B cells or control B cells, the MDS B cells induced production of only 26% of IL 2 as compared with control B cells. In parallel experiments, IL 2R generation in control T cells stimulated by either MDS or control B cells was similar. We conclude that in the primary MDS, T and B cell interactions are impaired. Although MDS T cells develop normal quantities of IL 2R and produce normal amounts of IL 2 when stimulated by control B cells, they are markedly impaired when stimulated by self B cells. Similarly, MDS B cells can induce IL 2R generation in control T cells but not in MDS T cells. Myelodysplastic B cells are also defective in inducing IL 2 production by normal T cells in an allo MLR. These in vitro abnormalities strongly suggest that generation of lymphocytes with immunoregulatory functions is impaired in patients with MDS.

MATERIALS AND METHODS

**Patients.** Fifteen patients (13 males and two females) were studied (Table 1). Diagnosis of primary MDS was confirmed by morphological study of bone marrow aspirates and peripheral smears stained with Wright's Giemsa. The percentage of ring sideroblasts was determined by prussian blue reaction for iron. The French-American-British (FAB) group criteria were used for diagnosis and classification of each patient. Patients with refractory anemia or refractory anemia with ring sideroblasts had <5% blasts in the bone marrow. Patients with refractory anemia with excess blasts had <5% blasts in the peripheral blood and 5% to 20% blasts, which did not contain Auer rods, in the bone marrow. None of the patients had received cytotoxic agents or corticosteroids within the 6 months prior to the study of their lymphocytes. Five patients had not received transfusion with packed RBC (PRBC) transfusions. Ten patients were transfused; of these, 8 received 10 to 312 U and 2 had <10 U of PRBC. Controls were healthy, age-matched volunteers.

**Isolation of lymphocytes and lymphocyte subpopulations.** Peripheral blood lymphocytes were separated from heparinized whole blood by Ficoll-Hypaque centrifugation (Lymphocyte separation medium, Becton Dickinson, Mountain View, CA) that reacts with the receptor for SRBCs. Unfractionated mononuclear cells were depleted of macrophages by adherence to plastic. Nonadherent, mononuclear cells were separated into E rosette-positive (E+) T cells, E rosette-negative (E−) B cells, and E rosette-negative (E−) populations with neuraminidase (Sigma Chemical, St Louis) treated 5% sheep RBCs (SRBCs) (GIBCO Laboratories, Madison, WI). The E+ T cell population was <1% surface immunoglobulin (S Ig) positive, and <3% α-naphthylacetate-esterase (ANAE) positive, 97% formed rosettes with SRBCs and 95% ± 2% reacted with anti-Leu 5 monoclonal antibody (MoAb) (Becton Dickinson, Immunocytometry Systems, Mountain View, CA) that reacts with the receptor for SRBCs. Unrosetted cells at the Ficoll-medium interface are referred to as B cells. Of these, 96% were S Ig+ , <1% were E+ and <3% were ANAE positive. Viability of both E+ and E− populations was determined within 99% for the proliferative and stimulatory functions of T and B cells, respectively, as well as production of IL 2 and generation of IL 2 receptors (IL 2R) in an autologous (auto) and allogeneic (allo) MLR.
immunofluorescent staining with MoAb. The following MoAbs were used to identify the lymphocyte populations and subsets: anti-Leu 5 (CD2), mature T cells; anti-Leu 2 (CD8), cytotoxic/suppressor T cells (Becton Dickinson); anti-Leu 3 (CD4), inducer/helper T cells; anti B4 (CD19) (Coulter Immunology, Hialeah, FL), B cells; anti-IL 2 receptor (IL 2R) (CD25) (Becton Dickinson), the class II major histocompatibility (MHC) antigen complex and HLA-DR (nonpolymorphic) (Becton Dickinson) is directed against monococytes/macrophages, and activated T cells; anti-IL 2 receptor (IL 2R) (CD25) (Becton Dickinson), activated T cells; anti-Leu 7 (Becton Dickinson), and NK cells. Cell surface markers were determined by indirect immunofluorescence, applying first the relevant monoclonal antibody and then fluorescein conjugated (FITC) goat anti-mouse IgG (GAMIgG). Cell surface fluorescence was determined by flow cytometry. The coexistence of a pair of cell surface antigens by dual staining, the percentage positive for each surface marker was determined by indirect immunofluorescent method, applying first a FITC-labeled MoAb followed by a phycoerythrin (PE)-labeled MoAb to the cells. In all studies with two-color staining, the percentage positive for each surface marker was confirmed by single-color analysis. Control cells were incubated with isotype-matched FITC-mouse IgG and PE-mouse IgG.

Flow cytometry analysis. Flow cytometry analysis of cells stained by either indirect (single-color) or direct (two-color) immunofluorescence was performed using a FACS analyzer (Becton Dickinson). The percentage positive refers to the percentage of 25,000 lymphocyte-sized cells with more intense fluorescence than 99% of the background determined by incubation of lymphocytes with isotype-matched mouse IgG followed by staining with FITC-GAM IgG and/or PE-GAM IgG. Cell surface fluorescence was determined by flow cytometry. The coexistence of a pair of cell surface antigens by dual staining was performed by indirect immunofluorescent method, applying first a FITC-labeled MoAb followed by a phycoerythrin (PE)-labeled MoAb to the cells. In all studies with two-color staining, the percentage positive for each surface marker was confirmed by single-color analysis. Control cells were incubated with isotype-matched FITC-mouse IgG and PE-mouse IgG.

Table 1. Clinical Data of Patients With Primary MDS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Hemoglobin (g/dL)</th>
<th>WBC (× 10^9/L)</th>
<th>Platelets (μL)</th>
<th>Karyotype</th>
<th>Time From Diagnosis (mo)</th>
<th>RBC Transfusions Total (U PRBCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A.P.</td>
<td>74</td>
<td>RA</td>
<td>7.3</td>
<td>7.0</td>
<td>478,000</td>
<td>46X, 5q--</td>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>2. O.S.</td>
<td>75</td>
<td>RA</td>
<td>9.9</td>
<td>3.0</td>
<td>198,000</td>
<td>47X, +8</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>3. L.K.</td>
<td>75</td>
<td>RA</td>
<td>13.1</td>
<td>7.4</td>
<td>121,000</td>
<td>46X</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>4. W.H.</td>
<td>64</td>
<td>RA</td>
<td>8.2</td>
<td>4.1</td>
<td>166,000</td>
<td>46X</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>5. S.H.</td>
<td>76</td>
<td>RA</td>
<td>8.7</td>
<td>12.7</td>
<td>760,000</td>
<td>46X</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>6. P.S.</td>
<td>58</td>
<td>RA</td>
<td>11.6</td>
<td>3.6</td>
<td>53,000</td>
<td>ND</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>7. F.S.</td>
<td>59</td>
<td>RA</td>
<td>12.3</td>
<td>4.9</td>
<td>238,000</td>
<td>ND</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>8. W.N.</td>
<td>70</td>
<td>RARS</td>
<td>9.0</td>
<td>2.3</td>
<td>65,000</td>
<td>ND</td>
<td>48</td>
<td>312</td>
</tr>
<tr>
<td>9. J.H.</td>
<td>56</td>
<td>RARS</td>
<td>7.3</td>
<td>5.0</td>
<td>28,000</td>
<td>46X</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>10. E.S.</td>
<td>85</td>
<td>RAEB</td>
<td>8.4</td>
<td>2.2</td>
<td>32,000</td>
<td>ND</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>11. S.S.</td>
<td>74</td>
<td>RAEB</td>
<td>12.5</td>
<td>4.1</td>
<td>97,000</td>
<td>ND</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>12. M.M.</td>
<td>80</td>
<td>RA</td>
<td>7.0</td>
<td>3.0</td>
<td>78,000</td>
<td>ND</td>
<td>108</td>
<td>36</td>
</tr>
<tr>
<td>13. J.R.</td>
<td>63</td>
<td>RAEB</td>
<td>8.6</td>
<td>1.9</td>
<td>24,000</td>
<td>ND</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>14. J.B.</td>
<td>73</td>
<td>RAEB</td>
<td>8.1</td>
<td>4.3</td>
<td>39,000</td>
<td>53X, -20, +5</td>
<td>6</td>
<td>8, +10, +14, +22, +MI</td>
</tr>
<tr>
<td>15. J.F.</td>
<td>80</td>
<td>RAEB</td>
<td>9.0</td>
<td>2.6</td>
<td>73,000</td>
<td>ND</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: MDS, myelodysplastic syndrome; PR BCs, packed RBCs; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; ND, not done.

by exclusion of 0.05% (vol/vol) trypan blue in phosphate-buffered saline (PBS) and was >95%.

Cell surface phenotype analysis using immunofluorescent staining with MoAb. The following MoAbs were used to identify the lymphocyte populations and subsets: anti-Leu 5 (CD2), mature T cells; anti-Leu 2 (CD8), cytotoxic/suppressor T cells (Becton Dickinson); anti-Leu 3 (CD4), inducer/helper T cells; anti B4 (CD19) (Coulter Immunology, Hialeah, FL), B cells; anti-IL 2 receptor (IL 2R) (CD25) (Becton Dickinson), activated T cells; anti-Leu 7 (Becton Dickinson), and NK cells. Cell surface markers were determined by indirect immunofluorescence, applying first the relevant monoclonal antibody and then fluorescein conjugated (FITC) goat anti-mouse IgG (GAMIgG). Control cells were incubated with isotype-matched mouse IgG and FITC-GAM IgG. Cell surface fluorescence was determined by flow cytometry. The coexistence of a pair of cell surface antigens by dual staining was determined by direct immunofluorescent method, applying first a FITC-labeled MoAb followed by a phycoerythrin (PE)-labeled MoAb to the cells. In all studies with two-color staining, the percentage positive for each surface marker was confirmed by single-color analysis. Control cells were incubated with isotype-matched FITC-mouse IgG and PE-mouse IgG.

Flow cytometry analysis. Flow cytometry analysis of cells stained by either indirect (single-color) or direct (two-color) immunofluorescence was performed using a FACS analyzer (Becton Dickinson). The percentage positive refers to the percentage of 25,000 lymphocyte-sized cells with more intense fluorescence than 99% of the background determined by incubation of lymphocytes with isotype-matched mouse IgG followed by staining with FITC-GAM IgG and/or PE-GAM IgG. Results were analyzed by a Consort 30 Data Management System (Hewlett Packard, Co).

The Auto and Allo MLR. For the auto MLR, responder T cells and stimulator B cells were obtained steriley from a single individual. For the allo MLR, T cells and B cells were obtained from unrelated individuals. B cells to be used as stimulator cells were treated with mitomycin C (66 μg/mL) (Sigma). Responder and stimulator cells were cultured in RPMI, supplemented with 10% heat-inactivated pooled AB serum, 10 mmol/L of Hepes (GIBCO, Grand Island, NY), 2 mmol/L of L-glutamine (GIBCO), and antibiotics (complete medium) in flat-bottomed microtiter tissue culture plates (No. 3040 Falcon Labware, Oxnard, CA) for 6 days at 37°C in a humidified air atmosphere of 5% CO2. Eighteen hours before harvesting 1 μCi of tritiated thymidine (3HTrd) (2 Ci/mmol, Schwarz/Mann, Spring Valley, NY) was added to each well. Cultures were performed in triplicate with controls consisting of T cells and of stimulator cells by themselves. Each patient was tested for MLR twice, and the mean cpm was used in the statistical analysis of the results.

To determine the generation of IL 2 receptors on T cells activated with mitogen or in an auto or an allo MLR, T cells were cultured and prepared for immunofluorescent staining and flow cytometric analysis as described above. Cell viability in these cultures was >95%.

IL 2 production and assay. T cells (1 x 10^6/mL) were incubated with 2 μg/mL of phytohemagglutinin (PHA-P, Burroughs Wellcome, Greene, NC) in a humidified air atmosphere of 5% CO2 for 48 hours in complete medium supplemented with 2% human AB serum (heat inactivated). Cell viability after culture was >95%. The supernatant was collected and filtered through a 0.45-μm filter (Nalgene Labware, Nalge Co., Rochester, NY) before storage at −70°C. The IL 2 standard, a 1:16 dilution of rat IL 2 that was the supernatant obtained from spleen lymphocytes (10 x 10^6/mL) incubated with 5 μg/mL of concanavalin A (Con A) for 48 hours, was denoted as 1 U/mL.

Response to IL 2. Both auto and allo MLRs were cultured in medium alone or medium supplemented with recombinant human IL 2 (Amgen Biologicals, Thousand Oaks, CA). Fifty microliters of recombinant IL 2 (0.25 U/mL) was added to the cultures.
Statistical analysis. All statistical analysis was performed using Bell Laboratories' "S" statistical software program and a VAX-50 computer. Student's t test was used to determine the significance of the difference between experimental results obtained from patients and controls.

RESULTS

Surface characteristics and quantitation of lymphocyte subsets. Table 2 summarizes the surface reactivity of lymphocytes of MDS patients with MoAbs and compares them with those from age-matched healthy controls. Absolute numbers of total lymphocytes, T cells, and B cells were significantly diminished in all patients. Both CD4+ and CD8+ T cells were decreased to a similar extent (53.6% and 57.3% of control, respectively), keeping the helper/suppressor T cell ratio in the peripheral blood within normal limits, ie, 1.75. B cells, as assessed by the presence of the CD19 antigen, were comparable in percentage but significantly diminished in numbers, ie, 51.9% of control. In addition, the total numbers and percentage of NK cells were significantly diminished in all patients. Percentage of distribution and the absolute number of monocytes/macrophages determined by surface HLA-DR expression were normal. Both B cells and macrophages express HLA-DR antigens; therefore, the peripheral blood lymphocytes were simultaneously stained with FITC-labeled anti–HLA-DR and PE-labeled anti-CD19 antibodies and only the HLA-DR+ cells that did not express CD19 antigen were counted as macrophages.

Lymphocyte distribution in the peripheral blood varies with time. To determine the consistency of our findings, we performed the peripheral blood lymphocyte analysis on three occasions in four patients at 3-week intervals. We did not observe a significant variation in lymphocyte numbers or subset distribution. Changes in lymphocyte subset distribution also occur after multiple transfusions with blood products. Although 8 of 15 patients required frequent transfusions with RBCs (Table 1), no correlation was found between lymphopenia (total lymphocyte, T and B cells numbers) and the number of RBC transfusions in these patients.

Mixed lymphocyte reaction. To determine T cell functions in patients with MDS, we first investigated the T cell proliferative response to stimulation with autologous B cells in the auto MLR. T cells proliferate when cocultured with autologous non-T cells; this phenomenon is termed auto MLR. Class II MHC antigens expressed by the non-T cells play an essential role in activation of responding T cells in this reaction. Thus, HLA-DR antigens from stimulator cells render resting T cells sensitive to IL 2 and induce production of IL 2, which engages its high-affinity receptor on the activated T cells, resulting in their proliferation. The auto MLR has specificity and memory, and generates helper, suppressor, and cytotoxic T cells. In the patient population, the mean T cell proliferation in the auto MLR was significantly lower than control values (P < .0002) (Fig 1). To determine if MDS B cells were defective as stimulatory cells in the auto MLR, normal B cells were also used to stimulate MDS T cells. MDS T cells proliferated less than did control T cells in the allo MLR, but this difference was not statistically significant (Fig 2). To determine if an impairment in the stimulatory B cell function played a role in the low auto MLR, an allo MLR was performed using control T cells as responders and MDS B cells as stimulators. As shown in Fig 2, the MDS B cells induced a T cell proliferation that was 57% of that induced by control allogeneic B cells (P < .01). These findings showed that MDS B cells were impaired in stimulating autologous or allogeneic T cells to proliferate in a MLR and suggested the presence of defective T and B cell interactions in patients with MDS.

To determine the mechanism of low auto and allo MLR proliferation, we measured the IL 2 production and receptor generation in MLR-stimulated T cells of MDS patients. The amount of IL 2 generated in control and patient auto MLR was below the sensitivity of the CTLL assay. Therefore, we determined the capacity of T cells to produce IL 2 on activation with phytohemagglutinin (PHA) or with allogeneic B cells. IL 2 production by MDS T cells was normal when these cells were stimulated by either control B cells in an allo MLR or by lectin (Table 3). In contrast, when patients’ B cells were used as stimulators, control T cells produced only 26% of the IL 2 produced after their stimulation with control allogeneic B cells. These results show that T cells from MDS patients can produce IL 2 normally when stimulated with mitogen or by control B cells; however, MDS B cells are defective stimulators of IL 2 production of both MDS and control T cells. One possibility that could be responsible for the defective B cell stimulatory activities in MDS is a decreased expression of cell surface DR antigen. The fluorescence intensity of DR antigen on the B cell surface was not different in patients and controls (Fig 3).

Table 2. Immunofluorescence Analysis of Lymphocyte Populations in MDS

<table>
<thead>
<tr>
<th>Surface Antigen + (Absolute Numbers/μL)</th>
<th>CD2</th>
<th>CD4</th>
<th>CD8</th>
<th>CD19</th>
<th>Leu 7</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS (15)</td>
<td>1,457 ± 166*</td>
<td>929 ± 140*</td>
<td>545 ± 89*</td>
<td>305 ± 57*</td>
<td>120 ± 40*</td>
<td>150 ± 48*</td>
</tr>
<tr>
<td>C (15)</td>
<td>2,011 ± 149</td>
<td>1,584 ± 170</td>
<td>1,015 ± 65</td>
<td>532 ± 60</td>
<td>231 ± 20</td>
<td>440 ± 97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surface Antigen + (% positive)</th>
<th>CD2</th>
<th>CD4</th>
<th>CD8</th>
<th>CD19</th>
<th>Leu 7</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS (15)</td>
<td>67 ± 4</td>
<td>42 ± 4</td>
<td>24 ± 3</td>
<td>7 ± 1</td>
<td>9.6 ± 3*</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>C (15)</td>
<td>70 ± 2.3</td>
<td>49 ± 1.3</td>
<td>25 ± 1.7</td>
<td>10 ± 0.8</td>
<td>16 ± 2.1</td>
<td>20 ± 2.2</td>
</tr>
</tbody>
</table>

Results are mean ± SEM; number of persons studied is given in parentheses.

Abbreviations: C, controls, PBL, peripheral blood lymphocytes.

*Significant difference (P < .02) on the basis of 95% of confidence limits between patients and normal control groups.
The presence of IL 2R on activated T cells was determined using a MoAb, anti-CD25, with specificity for the IL 2R. Table 4 shows the percentage of IL 2R expressing nonactivated, auto MLR-activated, or allo MLR-activated T cells from patients and compares them with identically activated control T cells. In these experiments, MDS or control T cells were stimulated with autologous or allogeneic B cells, and expression of IL 2R on activated T cells was determined by dual immunofluorescence. Results of these experiments are summarized in Table 4 and demonstrate that MDS T cells are unable to generate IL 2R when stimulated by autologous B cells, whereas they are able to develop normal IL 2R positivity when stimulated by control B cells. This was not due to the defective stimulatory activity of MDS B cells alone, since these B cells could activate normal T cells to develop IL 2R. Therefore, although MDS B cells are defective stimulators of IL 2 production by normal T cells, they are able to stimulate normal generation of IL 2R by these cells. In addition, we did not find a statistically significant difference in the proliferative rate of T cells and the stimulatory capacity of B cells between patients with refractory anemia with ringed sideroblasts (RA) or increased.8'9 Variations in phenotype may reflect the immunologic differences in each category of MDS.

Altering lymphocyte subset distribution in the peripheral blood occur in the recipients of blood products, suggesting its possible role in vivo masking an initially present T cell subset abnormality. In addition to a low proliferative response, IL 2R generation by T cells in the auto MLR could not be completely reversed by exogenous IL 2. In contrast, when stimulated by control B cells, MDS T cells develop normal IL 2R positivity and proliferate in response to IL2.

The addition of IL 2 to allow MLR cultures containing normal T cells and MDS B cells corrected the low proliferative response to control levels (Fig 2), confirming the presence of adequate IL 2R on the T cells stimulated by MDS B cells.

**DISCUSSION**

This study demonstrates the presence of two major lymphocyte abnormalities in patients with MDS. The first abnormality is lymphopenia, involving B cells, T cells of both helper/inducer (CD4) and cytotoxic/suppressor (CD8) subsets, and NK cells. The second abnormality is an ineffective interaction between T and B cells, an interaction that is necessary for optimal immune responses.

Our results are in agreement with previous reports of lymphopenia. The pathogenesis of the lymphopenia is unknown. In our patients, both CD4 and CD8 T cells were decreased in numbers to a similar extent, resulting in a normal ratio of CD4 to CD8 T cells. This is in contrast to studies in which T cells with a helper phenotype were diminished while those with a suppressor phenotype were normal or increased. Variations in phenotype may reflect the immunologic differences in each category of MDS.

Alterations of lymphocyte subset distribution in the peripheral blood occur in the recipients of blood products, suggesting its possible role in vivo masking an initially present T cell subset abnormality. In addition to a low proliferative response, IL 2R generation by T cells in the auto MLR could not be completely reversed by exogenous IL 2. In contrast, when stimulated by control B cells, MDS T cells develop normal IL 2R positivity and proliferate in response to IL2.

The addition of IL 2 to allow MLR cultures containing normal T cells and MDS B cells corrected the low proliferative response to control levels (Fig 2), confirming the presence of adequate IL 2R on the T cells stimulated by MDS B cells.
resulting in their failure to respond to exogenous IL 2 addition. Inability of MDS T cells to become activated in an auto MLR suggests that regulatory T cell generation in these patients is impaired. T cell responsiveness to self class II MHC antigens expressed by B cells and macrophages is particularly important because antigen is recognized by T cells in the context of their antigen receptors and the cell membrane DR determinants on the antigen-presenting cell. The inability to respond to self class II MHC antigens could lead to impaired T cell responsiveness to foreign antigen. The result could be an inability to cope with viral, fungal, and other infective agents, as occurs in MDS patients.

In contrast to their poor responsiveness in the auto MLR, MDS T cells generated IL 2R, produced IL 2, and proliferated normally when stimulated by control B cells. These experiments showed that MDS T cells could be activated by foreign cellular antigens but not by autologous DR antigens and suggested that B cells from MDS patients were defective in their ability to activate MDS T cells.

To pursue a possible B cell defect, we examined the capacity of B cells from MDS patients to elicit a proliferative response by allogeneic T cells in addition to autologous T cells. B cells were significantly impaired in both capacities. The diminished proliferation was most likely due to the inability of MDS B cells to induce the generation of normal quantities of IL 2 from control T cells, since the expression of IL 2R by these cells was not defective. Thus, the addition of IL 2 normalized the T cell proliferation in these cultures.

### Table 3. IL 2 Production by T Cells in MDS

<table>
<thead>
<tr>
<th>Source of T Cells</th>
<th>PHA/PMA (U/mL)</th>
<th>B Cells (U/mL)</th>
<th>MDS B Cells (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (5)</td>
<td>2.44 ± 0.4</td>
<td>1.87 ± 0.3</td>
<td>0.50 ± 0.1</td>
</tr>
<tr>
<td>MDS (5)</td>
<td>1.92 ± 0.7</td>
<td>1.42 ± 0.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

Number of persons studied given in parentheses.

Abbreviations: PHA, phytohemagglutinin; PMA, phorbol myristate acetate.
Although all patients expressed normal concentrations of DR antigens on their B cells and macrophages, structural abnormalities not detectable by immunofluorescence in the DR determinants may exist or these MDS cells may lack a DR-linked determinant, such as DQ or DP, which could be required for stimulation of IL 2 production by T cells. Abnormalities of B cells, such as lack of surface EBV receptors in MDS patients, suggest that these cells are relatively immature. We previously showed that B cells in chronic lymphocytic leukemia (CLL) patients are also poor stimulators of IL 2 production but not IL 2R generation in normal T cells.41 CLL B cells are not fully mature and, as MDS B cells, also express DR antigens. The capacity of stimulation of IL 2 production by T cells may be acquired only by the fully mature B cells and therefore is lacking in MDS B cells and CLL cells, whereas stimulation of IL 2 expression is acquired earlier and is present in MDS and CLL B cells.

These results raise two important questions: (a) Why are lymphocytes deficient in number and impaired in function in MDS? (b) Do immunoregulatory abnormalities, ie, defective T and B cell interactions, affect the course of MDS? Lymphocytes in MDS patients may be quantitatively and functionally abnormal because they descend from an abnormal stem cell. Lack of self-recognition, as demonstrated by a low auto MLR and impaired regulatory T cell generation, exists in malignancies such as CLL, Hodgkin's disease, and colon carcinoma.42 The absence of regulatory T cells in these disorders may allow cells that carry phenotypic or karyotypic mutations to escape surveillance and other regulatory differentiation effects the T cells induce. In primary MDS, abnormal clones with karyotype aberrations occur in 50% of patients,34,43 and their presence is associated with leukemic change and a more rapid progression of the disease. Although the relationship between prognosis and cytogenetic abnormalities is established,3,4 the correlation of cytogenetic abnormalities with T cell regulatory functions and their relationship to the prognosis has not been studied. Surveillance by regulatory T cells may be necessary to prevent the unstable hemopoietic stem cells with karyotype abnormalities from gaining a growth advantage and developing leukemic transformation.

MDS offers a unique opportunity to study the changes in immunoregulatory T and B cell interactions in a preleukemic state. Investigating the mechanisms of effector and regulatory lymphocyte defects in these patients will contribute to our understanding of the contribution of these cells to the regulation of normal and leukemic hemopoiesis.

### REFERENCES


Lymphocyte interleukin 2 production and responsiveness are altered in patients with primary myelodysplastic syndrome

O Ayanlar-Batuman, J Shevitz, UC Traub, S Murphy and D Sajewski