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

By Olay 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 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.

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MATERIALS AND METHODS

Patients. Fifteen patients (13 males and two females) were studied (Table I). 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 siderobластs 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 31 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, Bionetics Laboratory Products, Litton Bionetics, Kensington, MD). Unfractionated mononuclear cells were depleted of macrophages by adherence to plastic. Nonadherent, mononuclear cells were separated into E rosette-positive (E+) 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% SRBCs. E+ T cell population was <1% surface immunoglobulin (Slg+) positive, and <3% a-naphthylacetate-esterase (ANAE) positive, 97% Slg+ and 95% ± 2% reacted with anti-Leu 5 monoclonal antibody (MoAb) (Becton Dickinson, Immunocytochemistry 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 Slg+, <1% were E+ and <3% were ANAE positive. Viability of both E+ and E- populations was determined 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) or allogeneic (allo) MLR.

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THE PRIMARY myelodysplastic syndrome (MDS) consists of a heterogeneous group of bone marrow disorders characterized by ineffective and dysplastic hemopoiesis in one or more cell lines.1 The basic abnormality in this syndrome is a clonal disorder of the hemopoietic stem cells.2,4 In 20% to 75% of cases, MDS evolves to acute nonlymphocytic leukemia.5,6 Recent evidence suggests that lymphocytes of MDS patients are derived from the same clone as the abnormal erythroid and myeloid cells.7 There is often a peripheral blood lymphopenia with8,9 without10,11 T cell subset distribution abnormalities. T cells may show increased radiosensitivity, reduced proliferative response to mitogens, and poor colony formation in vitro.10,12 B cells may be deficient in Epstein-Barr virus (EBV) receptors.13 Natural killer (NK) cell functions may be abnormal, and their numbers may be reduced.14,15 Although phenotypic and functional abnormalities of T cells and NK cells are reported, immunoregulatory mechanisms in MDS have not been studied.

Alterations in immunoregulatory T cell functions and in lymphokine production, especially interleukin 2 (IL 2) are observed in patients with acute lymphoblastic leukemia and aplastic anemia.16,18 Because T cells contribute to the regulation of hemopoiesis, abnormalities in their numbers and functions are believed to play a pathophysiologic role in these hematologic dyscrasias.19,20 To determine the immunoregulatory lymphocyte functions in MDS, 15 patients were studied.
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-CD 19 (Becton Dickinson), B cells; anti-IL 2 receptor (IL 2R) (CD25) (Becton Dickinson), 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 (GAM IgG). Results were analyzed by a Consort 30 Data Management System (Hewlett Packard, Co).

Flow cytometry analysis. Flow cytometry analysis of cells stained by either indirect (single-color) or direct (two-color) immunofluorescence was performed using a FACScan analyser (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 ng/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 microtissue culture plates (No. 3040 Falcon Labware, Oxnard, CA) for 6 days at 37°C in a humidified air atmosphere of 5% CO2.

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 (x 10^3/µL)</th>
<th>Platelets (µL)</th>
<th>Karyotype</th>
<th>Time From Diagnosis (mo)</th>
<th>RBC Transfusions (U PRBCa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.P.</td>
<td>74</td>
<td>RA</td>
<td>7.3</td>
<td>7.0</td>
<td>478,000</td>
<td>46XY, 5q-</td>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>O.S.</td>
<td>75</td>
<td>RA</td>
<td>9.9</td>
<td>3.0</td>
<td>198,000</td>
<td>47XY, +8</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>L.K.</td>
<td>75</td>
<td>RA</td>
<td>13.1</td>
<td>7.4</td>
<td>121,000</td>
<td>46XY</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>W.H.</td>
<td>64</td>
<td>RA</td>
<td>8.2</td>
<td>4.1</td>
<td>166,000</td>
<td>46XY</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>S.H.</td>
<td>76</td>
<td>RAEB</td>
<td>8.7</td>
<td>12.7</td>
<td>760,000</td>
<td>46XY</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>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>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>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>J.H.</td>
<td>56</td>
<td>RA</td>
<td>7.3</td>
<td>5.0</td>
<td>28,000</td>
<td>46XY</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>E.S.</td>
<td>65</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>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>
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<tr>
<td>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>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>J.B.</td>
<td>73</td>
<td>RAEB</td>
<td>8.1</td>
<td>4.3</td>
<td>39,000</td>
<td>53XY, -20, +5</td>
<td>6</td>
<td>+8, +10, +14, +22, +MI</td>
</tr>
<tr>
<td>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; PRBCs, packed RBCs; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; ND, not done.
Statistical analysis. All statistical analysis was performed using Bell Laboratories’ "S" statistical software program and a VAX-30 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.31,32 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.33 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 with its high-affinity receptor on the activated T cells, resulting in their proliferation. The auto MLR has specificity and memory,34 and generates helper, suppressor, and cytotoxic T cells.35,37 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>MDS (15)</th>
<th>C (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of PBls (μL)</td>
<td>1,457 ± 166*</td>
<td>2,011 ± 149</td>
</tr>
<tr>
<td>CD2</td>
<td>929 ± 140*</td>
<td>1,584 ± 170</td>
</tr>
<tr>
<td>CD4</td>
<td>545 ± 89*</td>
<td>1,015 ± 65</td>
</tr>
<tr>
<td>CD8</td>
<td>305 ± 57*</td>
<td>532 ± 60</td>
</tr>
<tr>
<td>CD19</td>
<td>120 ± 40*</td>
<td>231 ± 20</td>
</tr>
<tr>
<td>Leu 7</td>
<td>150 ± 48*</td>
<td>440 ± 97</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>611 ± 21</td>
<td>457 ± 72</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 (RA) or refractory anemia with excess blasts (RAEB) and those with refractory anemia with excess blasts in transformation (RA-EB) in the auto or allo MLR cultures.

To verify these findings, we added recombinant human IL 2 to MLR cultures. The addition of various concentrations of recombinant IL 2 increased the T cell proliferation in both patient and control MLR cultures. In the auto MLR of MDS patients, although the T cell proliferation increased with the addition of IL 2, this proliferative rate was still significantly lower than that of the control cultures (Fig 1). In the allo MLR with MDS T cells as responders and normal B cells as stimulators, proliferation in experimental and control cultures was similar (Fig 2). These results confirmed the presence of impaired T and B cell interactions in MDS; i.e., in the auto MLR, T cell IL 2R generation is impaired. Therefore, in these cultures, the low T cell proliferation cannot 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 IL 2.

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 in lymphocyte subset distribution in the peripheral blood occur in the recipients of blood products, such that the degree of inversion in the ratio of CD4 to CD8 T cell ratio correlated with the number of RBC transfusions received by MDS patients. We did not find such a correlation in our study between transfusion and an inverted ratio of CD4 to CD8 T cells. Because many of our transfused patients had long-standing (>2 years) MDS, during this period myelodysplastic lymphopoiesis may have resulted in lymphopenia of both helper and suppressor T cell subsets, masking an initially present T cell subset abnormality.

Alterations in T and B interactions in MDS patients were studied with the use of the auto MLR. Abnormalities of this reaction have been reported in various immunologic and hematologic diseases, suggesting its possible role in vivo as a mechanism of immunoregulation. In patients with MDS, T cell responsiveness in auto MLR was significantly diminished as compared with that of age-matched controls. The low T cell proliferation in the auto MLR could not be due to a lack of CD4 T cells, which are thought to proliferate in this reaction, since their ratio to CD8 cells was unchanged. In addition to a low proliferative response, IL 2R generation by T cells in the auto MLR was also impaired.
Number of persons studied given in parentheses.

**Abbreviations:** PHA, phytohemagglutinin; PMA, phorbol myristate acetate.

**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.
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. 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, 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, 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


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