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Bone Marrow Clonogenic Capability, Cytokine Production, and Thymic Output in Patients with Common Variable Immunodeficiency

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In patients with primary Ab deficiencies, hematological and immunological abnormalities are frequently observed. A regenerative failure of hemopoietic stem/progenitor cells has been hypothesized. We evaluated in the bone marrow (BM) of 11 patients with common variable immunodeficiency, the phenotype of BM progenitors and their in vitro growth by colony-forming cell (CFC) and long-term culture (LTC) assays. A significant decrease in erythroid and mixed CFC and, to a greater extent, in primitive LTC-CFC progenitors was observed in patients compared with healthy controls. The frequency of BM pre-B and pro-B cells correlated directly with the absolute number of CD19+ lymphocytes. BM cells cultured in vitro produced spontaneously lower amounts of IL-2 and elevated levels of TNF-α compared with controls, indicating a skewing toward a proapoptotic cytokine pattern. In addition, stromal cells generated after BM LTC secreted less IL-7 and displayed by immunohistochemistry an altered phenotype. These findings were associated with a significant decrease in naive Th cells coexpressing CD31 in the peripheral blood. These results indicate an impaired growth and differentiation capacity of progenitor cells in patients with common variable immunodeficiency. *The Journal of Immunology, 2005, 174: 5074–5081.*

In approximately one-third of patients with CVID, T cells are also decreased as absolute number, with an inversion of the CD4:CD8 ratio due to reduced CD4+ T cells, especially those expressing the CD4+CD45RA− (naive) phenotype, whereas the number of B cells is variable (normal to very low) (12–15), often with a deficiency in memory B cells along with somatic hypermutation (16). To explain these findings, several hypotheses have been proposed, including deficient IL-2 production (17) and/or increased T cell apoptosis due to the persistent Ag activation after infections (10). However, the production of T cell precursors in CVID patients was never investigated in detail. Thymic activity may be ineffective in replenishing the pool of peripheral T lymphocytes. Recently, TCR excision circles (TREC) frequencies in peripheral blood of CVID patients have been investigated and a significant diminished content of TREC levels both in CD4+ and in CD8+ T compartments, compared with those of age-matched healthy controls has been observed (18). These results suggest a more rapid reduction of thymic output in CVID individuals, but it remains to be clarified whether the damage of the T cell compartment is related to an altered generation of new T cells from hemopoietic stem/progenitor cells or to a defect in thymic and/or bone marrow (BM) epithelial cell compartment.

In vivo development of lymphoid progenitors requires a strict interaction of these cells with the BM stromal microenvironment that provides a rich milieu of cytokines, extracellular matrix proteins, and adhesion molecules (19, 20). Analysis of BM and stromal cytokine production may be important in understanding the causes of the hematological abnormalities occurring in CVID patients. Impaired stromal function, alteration of hemopoietic growth factor network, and abnormal apoptosis may be all involved in impaired hemolymphopoiesis of these patients.

At present, no data are available on BM functions, because the literature reports are limited to sporadic cases with identified hematological abnormalities.
To investigate the BM function in patients with CVID, we analyzed the BM progenitor cell growth and differentiation with colony-forming cell (CFC) and long-term culture (LTC)-CFC assays, the spontaneous cytokine production (IL-2 and TNF-α) at BM level, the BM stromal cell morphology, and the stromal IL-7 production. In addition, we analyzed, as thymic naive Th cells (21), the coexpression of CD31+ in peripheral CD45RA+ CD4+ T cells to extend the previous data about thymic output in CVID patients (18).

Materials and Methods

Patient population

The study group consisted of 11 patients with CVID, followed as outpatients at the Division of Allergy and Clinical Immunology, University of Rome “La Sapienza” (Rome, Italy) and recently at Interregional Center for Primary Immunodeficiencies (Rome, Italy). They were selected on a voluntary basis for BM aspirates and enrolled in this study. Two of them presented with leukopenia and thrombocytopenia. All subjects gave their written informed consent for the breastbone aspiration, according to the Ethical Committee procedures at our institution. The main clinical and immunological characteristics of the patients are reported in Table I. The mean age ranged from 22 to 59 years (42 ± 7). The diagnosis was made according to the criteria of the WHO experts’ group for primary immunodeficiency diseases (1). All men underwent the B tyrosine kinase test with negative results (data not shown). Five CVID patients received regular medications at the time of the study.

As controls, 10 healthy donors matched by sex and age with the patients were included. Eight of ten subjects also underwent BM aspirates (all had normal BM). None of the controls had acute infection and/or received medications at the time of the study.

Serum Ig analyses

Serum concentrations of IgG, IgM, and IgA were measured by immunephelometry (Behring 100), using N antisera to human Igs (IgG, IgM, and IgA) (Dade Behring Marburg). Normal laboratory ranges for serum IgGs were 690–1400 mg/dl for IgG, 70–370 mg/dl for IgA, and 40–240 mg/dl for IgM.

Flow cytometric analysis of BMNCs

Whole blood phenotype analysis consisted of lysing 500 μl of blood with 10 ml of Ortho-mune Lysing Reagent (Ortho Diagnostic Systems) at room temperature, washing, and labeling with a mixture of four mAbs for 30 min at 4°C. Anti-CD3-FITC, anti-CD4-allophycocyanin, anti-CD8-PerCP, anti-CD45RA-FITC, anti-CD62L-PE, and anti-CD19-PE were purchased from BD Biosciences. CD34+ cells were defined as positive events in the low side scatter region, CD34+CD45− cells: progenitor cell subpopulations were calculated within the CD34+CD45+ gate, expressed as relative percentages of the CD34+ cells.

CFC assay and long-term BM cultures (LTBMC)

BMMCs (1 × 10^6) were plated in duplicate cultures in 1 ml of methylcellulose assay medium containing recombinant human (rhu) erthropoietin (3 U/ml), rhu stem cell factor (10 ng/ml), rhu-SCF (10 ng/ml), rhu GM-CSF (10 ng/ml), and rhu IL-3 (10 ng/ml) (BIOSPA; Stem Cell Technologies). In addition, in three CVID patients, we analyzed the effects of anti-TNF-α and of cytokines IL-7, IL-2, and IL-2 plus IL-7 on BM colony formation by CFC assay. Briefly, 1 × 10^5 BMMCs were plated in duplicate cultures in 1 ml of methylcellulose assay medium with or without anti-TNF-α (1 μg/ml), IL-7 (20 ng/ml), IL-2 (10 ng/ml), or IL-2 plus IL-7 (10 and 20 ng/ml, respectively) (all from R&D Systems). According to standardized morphological criteria, the growth of the multipotent hemopoietic progenitor was evaluated as CFU-granulocyte-erythrocyte-monocyte-megakaryocyte (CFU-GEMM), burst-forming unit-erythroid (BFU-E), CFU-erythroid (CFU-E), and CFU-granulocyte-macrophage (CFU-GM) after 2 wk of incubation at 37°C in an atmosphere of 5% CO₂.

To analyze the most immature progenitors and the stem cell compartment, we used LTBMC. Stromal cell cultures and determination of LTC-CFC numbers were performed according to a modification of the described methods (22). The murine BM stromal cell line M210B4 was cultured until cell confluence and then trypsinized, irradiated (800 rad), washed, and placed in six-well plates. Total BMNCs (1 × 10^6 cells, in duplicate cultures) were applied on the pre-established, irradiated stromal feeder layers and cultured at 37°C for 5 wk. In three CVID patients, BMNCs were seeded on the stromal feeder layers and cultured with the weekly addition of 5M/5F 40

Preparation of BM mononuclear cells (BMNCs)

BM aspirates were initially collected into a tube containing EDTA as anticoagulant. The BM samples were diluted 1/3 with PBS plus 5 ml EDTA and then separated after centrifugation by Ficoll (Lymphoprep; Nycomed Pharma) and resuspended in RPMI 1640 supplemented with 50% FCS, 1-glutamine (2 mmol/L), and penicillin (250 U/ml) (all from Invitrogen Life Technologies).

Flow cytometry of BMNCs

Flow cytometric analysis was performed with freshly collected BM samples from patients or normal BM donors. Briefly, 1 × 10^6 BMNCs were washed twice with PBS and then labeled with the following fluorochrome-conjugated mAbs: anti-CD34-PerCP; anti-CD45-FITC; anti-CD38-PE; anti-CD19-PE; anti-CD10-FITC; and anti-HLA-DR-FITC (BD Biosciences). CD34+ cells were defined as positive events in the low side scatter region, CD34+CD45− windows: progenitor cell subpopulations were calculated within the CD34+CD45− gate, expressed as relative percentages of the CD34+ cells.

Table I. Clinical, serological, and hematological characteristics of CVID patients at enrolment for BM aspiration

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Score</th>
<th>IgG (μg/ml)</th>
<th>IgA (μg/ml)</th>
<th>IgM (μg/ml)</th>
<th>Hb (g/dl)</th>
<th>PTL (μl/μl)</th>
<th>WBC (μl/μl)</th>
<th>Lymph (μl/μl)</th>
<th>CD4+ (cells/μl)</th>
<th>CD8+ (cells/μl)</th>
<th>CD4+CD8+ (cells/μl)</th>
<th>CD19+ (cells/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. D.D.</td>
<td>M</td>
<td>38</td>
<td>1</td>
<td>183</td>
<td>&lt;4.3</td>
<td>&lt;5.8</td>
<td>14.6</td>
<td>120</td>
<td>4.5</td>
<td>630</td>
<td>296</td>
<td>195</td>
<td>1.5</td>
<td>16</td>
</tr>
<tr>
<td>2. G.C.</td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>100</td>
<td>&lt;4.3</td>
<td>&lt;5.8</td>
<td>12</td>
<td>66</td>
<td>2.3</td>
<td>650</td>
<td>234</td>
<td>247</td>
<td>0.9</td>
<td>6.5</td>
</tr>
<tr>
<td>3. S.M.</td>
<td>F</td>
<td>38</td>
<td>1</td>
<td>209</td>
<td>21</td>
<td>20</td>
<td>11.2</td>
<td>212</td>
<td>6.1</td>
<td>1016</td>
<td>417</td>
<td>224</td>
<td>1.9</td>
<td>264</td>
</tr>
<tr>
<td>4. G.Z.</td>
<td>M</td>
<td>55</td>
<td>0</td>
<td>150</td>
<td>&lt;4.3</td>
<td>&lt;5.8</td>
<td>9.9</td>
<td>57</td>
<td>0.6</td>
<td>451</td>
<td>135</td>
<td>573</td>
<td>0.6</td>
<td>14</td>
</tr>
<tr>
<td>5. P.E.</td>
<td>M</td>
<td>22</td>
<td>0</td>
<td>460</td>
<td>20</td>
<td>17</td>
<td>14.6</td>
<td>189</td>
<td>4.5</td>
<td>994</td>
<td>278</td>
<td>298</td>
<td>0.9</td>
<td>99</td>
</tr>
<tr>
<td>6. M.T.</td>
<td>F</td>
<td>53</td>
<td>1</td>
<td>310</td>
<td>&lt;4.3</td>
<td>26</td>
<td>15.4</td>
<td>385</td>
<td>13.6</td>
<td>5900</td>
<td>1652</td>
<td>3363</td>
<td>0.5</td>
<td>354</td>
</tr>
<tr>
<td>7. G.L.</td>
<td>F</td>
<td>59</td>
<td>1</td>
<td>300</td>
<td>&lt;4.3</td>
<td>28</td>
<td>11.4</td>
<td>133</td>
<td>4.9</td>
<td>955</td>
<td>257</td>
<td>420</td>
<td>0.6</td>
<td>134</td>
</tr>
<tr>
<td>8. R.C.</td>
<td>M</td>
<td>51</td>
<td>1</td>
<td>188</td>
<td>&lt;4.3</td>
<td>626</td>
<td>13.3</td>
<td>156</td>
<td>8.0</td>
<td>1902</td>
<td>361</td>
<td>1065</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>9. M.G.</td>
<td>M</td>
<td>44</td>
<td>1</td>
<td>230</td>
<td>&lt;4.3</td>
<td>5.8</td>
<td>15.3</td>
<td>182</td>
<td>6.9</td>
<td>1519</td>
<td>289</td>
<td>714</td>
<td>0.4</td>
<td>182</td>
</tr>
<tr>
<td>10. G.H.</td>
<td>M</td>
<td>48</td>
<td>1</td>
<td>290</td>
<td>&lt;4.3</td>
<td>5.8</td>
<td>12</td>
<td>73</td>
<td>6.9</td>
<td>774</td>
<td>248</td>
<td>348</td>
<td>0.7</td>
<td>89</td>
</tr>
<tr>
<td>11. S.W.</td>
<td>F</td>
<td>64</td>
<td>1</td>
<td>129</td>
<td>&lt;4.3</td>
<td>18</td>
<td>12.9</td>
<td>168</td>
<td>6.1</td>
<td>1723</td>
<td>982</td>
<td>362</td>
<td>2.7</td>
<td>68</td>
</tr>
</tbody>
</table>

*To assess patient’s clinical conditions a scoring system has been elaborated, and the following parameters were collected retrospectively during a 3-year follow-up: presence (=1) or absence (=0) of three or more severe bacterial infections and/or recurrent less severe infectious episodes. IgG, IgA, and IgM values (milligrams per deciliter) before starting IVIG treatment; IgGb values (milligrams per deciliter) after IVIG treatment (at time of breastbone aspiration).

*Naive for IVIG treatment.
of anti-TNF-α (1 μg/ml), IL-7 (20 ng/ml), or IL-2 (10 ng/ml) to evaluate their effect on immature progenitor cells.

The half-medium liquid was changed weekly. After 5 wk, the nonadherent and adherent cells were harvested by treatment with trypsin (Invitrogen Life Technologies), washed, and replaced in duplicate in methylcellulose to evaluate the number of cells able to determine secondary colonies. The number of CFC generated after 5 wk of cultures on stromal cells gives an indirect but consistent measurement of the content of LTC-initiating cells (LTC-IC) (22).

**Spontaneous cytokine production from BMMMC cultures**

To evaluate the cytokine production at BM level, BMMCC short-term cultures were performed with freshly collected BM samples from CVID patients, as well as from normal BM donors. Briefly, isolated BMMCCs were cultured in RPMI 1640 supplemented with 10% FCS, l-glutamine (2 mmol/L), and penicillin (250 U/ml) in a 5% CO2 atmosphere at 37°C, in the absence of stimuli to verify the spontaneous production of cytokines IL-2 and TNF-α. After 24 h of culture, supernatants were collected, and cytokines were measured by ELISA, according to the manufacturer’s instructions (R&D Systems). For each cytokine determination, a standard curve was set up. In addition BMMCCs (1×10^6/well), obtained from three CVID patients and two controls, were cultured, and the supernatants were collected after 24, 48, 72, and 96 h for IL-2 or TNF-α time kinetic study by ELISA.

**BM stromal cells characterization by immunohistochemistry**

BMMCCs were cultured in tissue culture chamber slides (Falcon) in IMDM (Invitrogen Life Technologies) supplemented with 20% FCS, 100 IU/ml penicillin-streptomycin, and 100 IU/ml glucoseamine at 37°C in humidified air at 5% CO2. At weekly intervals, cultures were fed by demipopulation of the nonadherent cells and replacement of 500 μl of fresh, supplemented IMDM. The cultures were maintained until stromal confluence (3–4 wk); then the cells were collected by trypsinization and cultured at a concentration of 1×10^6 cells/ml in a total volume of 1 ml/well. The plates were incubated at 37°C in humidified air at 5% CO2.

At weekly intervals, cultures were fed by demipopulation of the nonadherent cells and replacement of 500 μl of fresh, supplemented IMDM. The cultures were maintained until stromal confluence (3–4 wk); then the cells were collected by trypsinization and cultured at a concentration of 1×10^6 cells/ml in a total volume of 1 ml/well. Supernatants were then collected after 24 h of culture and cytokine IL-7 was measured by ELISA, according to the manufacturer’s instructions with an ultrasensitive kit (R&D Systems).

**Statistical analysis**

Nonparametric statistics were used (Mann-Whitney, Wilcoxon test) for unpaired and paired comparisons between the parameters analyzed in patients and healthy individuals. A simple regression test was used to correlate the group characteristics. A value of p < 0.05 and R > 0.5 or < −0.5 were considered significant. Statistical analyses were performed with Stat View 5.0 software (SAS Institute).

**Results**

**Patients’ features**

Patients showed pretherapy a mean value of serum IgG of 224 ± 105 mg/dl, generally associated with low or undetectable IgA and IgM.

**Table II. Analysis of CD4+ and CD8+ T cells and the coexpression of CD31 in peripheral naive CD45RA+ Th cells**

<table>
<thead>
<tr>
<th>Patients</th>
<th>CD4+ (%</th>
<th>CD4+ (cells/μl)</th>
<th>CD4+ naive (%)</th>
<th>CD4+ naive (cells/μl)</th>
<th>CD4+ CD45RA+ CD31+ (%)</th>
<th>CD4+ CD45RA+ CD31+ (cells/μl)</th>
<th>CD8+ (%)</th>
<th>CD8+ (cells/μl)</th>
<th>CD8+ naïve (%)</th>
<th>CD8+ naïve (cells/μl)</th>
<th>CD8+ naïve (cells/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVID</td>
<td>47 ± 12</td>
<td>296 ± 234</td>
<td>4 ± 6</td>
<td>12 ± 16</td>
<td>2 ± 5</td>
<td>6 ± 12</td>
<td>1 ± 10</td>
<td>31 ± 10</td>
<td>19 ± 12</td>
<td>10 ± 10</td>
<td>6 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>47 ± 6</td>
<td>296 ± 234</td>
<td>4 ± 6</td>
<td>12 ± 16</td>
<td>2 ± 5</td>
<td>6 ± 12</td>
<td>1 ± 10</td>
<td>31 ± 10</td>
<td>19 ± 12</td>
<td>10 ± 10</td>
<td>6 ± 10</td>
</tr>
</tbody>
</table>

**Table III. Evaluation of percentage of BMMCCs by flow cytometry in CVID patients vs controls**

<table>
<thead>
<tr>
<th>Patients</th>
<th>CD34+ CD45+ (%)</th>
<th>CD34+/CD38+ DR− (%)</th>
<th>CD34−/CD38+ DR− (%)</th>
<th>CD34+ CD19+ CD10+ (%)</th>
<th>CD34+ CD10+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ± 1.5</td>
<td>93 ± 8.9</td>
<td>1.7 ± 0.9</td>
<td>0.5 ± 0.5</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>CVID</td>
<td>5.6 ± 1.5</td>
<td>93 ± 8.9</td>
<td>1.7 ± 0.9</td>
<td>0.5 ± 0.5</td>
<td>4.8 ± 0.8</td>
</tr>
</tbody>
</table>

*p < 0.01; **p < 0.001.
*Within the lymphocyte/blast gate.
**Within the CD34+ CD45+ gate.

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IgM serum levels (Table I). According with the scoring system, some subjects had three or more infectious episodes per year despite IVIG treatment (see Table I). No one showed altered results of liver and kidney functions or a positivity for hepatitis B virus surface Ag and/or presented hepatitis C virus infection. The Dixon test was negative in all patients with thrombocytopenia.

Immunological and hematological data in PBMCs

Hematological and immunological data of the patients are reported in detail in Tables I and II. Patients had significantly lower CD4+ T cells than did the controls (351 ± 11006 233 cells/l vs 1024 ± 11006 162 cells/l, respectively; p = 0.0007), and this reduced number was observed mainly in CD45RA+CD62L+ (naive phenotype) subset (10 ± 7% in patients vs 52 ± 12% in controls; p = 0.0004). In addition, in nine CVID patients, we observed a significant decrease of peripheral CD45RA+CD31+ Th cells (thymic naive Th cells) (on average 8 ± 6% in patients vs 37 ± 10% in controls; p = 0.0004). CD8+ T cell numbers did not differ statistically between patients and controls (414 ± 272 cells/µl vs 404 ± 178 cells/µl; p NS), but the percentage of CD8+ naive T cells was significantly lower in patients (22 ± 11% vs 58 ± 15%; p = 0.0007). The CD4:CD8 T cell ratio was reduced (<0.75) in 6 of 11 patients (patients 4, 6, 7, 8, 9, and 10). Seven patients (patients 1, 2, 4, 5, 8, 10, and 11) displayed reduced numbers of B cells vs the control subjects, whereas three patients (n. 1, 2 and 8) had only 0–1% of CD19+ cells.

Hematological abnormalities were observed: decreased hemoglobin levels (<12 g/dl) in three patients (patients 3, 4, and 7), low white blood cell counts (<4.0 × 10^3 cells/l) in three patients (patients 2, 4, and 10), and thrombocytopenia (<150 × 10^3 cells/µl) in five patients (patients 1, 2, 4, 7, and 10).

Flow cytometric BMMCs analysis

Table III shows the results of the flow cytometric BM assessment. The mean proportion of CD34+ cells in BMMCs was in the range of normal controls, although in three patients (patients 4, 6, and 8) CD34+ cells were ≤1%. The percentage of phenotypically primitive CD34+CD38−DR− cells was 1.6 ± 1 (vs 5.6 ± 1.2% in normal subjects). The proportion of CD34+ cells expressing CD19 and CD10 (pro-B cells) was lower than that of the controls (8.7 ± 7.8% vs 15.9 ± 7.9%) and five patients (patients 2, 4, 8, 9, and 10) displayed very low levels of this progenitor cell subset (<5%). CD34+CD10+ cells (pre-B lymphocytes) did not differ from the normal range (4.6 ± 4% vs 5.1 ± 0.8%), despite the fact that eight
Cytokine production from BMMCs in patients with CVID. A. BMMCs (1 x 10^6 cells) were cultured in 1 ml of RPMI 1640 for 24 h. Cell supernatants were obtained from CVID patients and eight healthy controls (Ctr). Supernatants were assayed for the presence of cytokines by ELISA (see Materials and Methods). B. BMMCs (1 x 10^6/well), obtained from three CVID patients and two controls, were cultured, and the supernatants were collected after 24, 48, 72, and 96 h for IL-2 or TNF-α kinetic study by ELISA.

Measurements of spontaneous cytokine production in BMMCs

In vitro production of IL-2 and TNF-α by short-term culture of BMMCs was determined in CVID patients (Fig. 4A) and compared with healthy subjects. The amount of IL-2 produced was lower in patients vs healthy controls (16.9 ± 17 pg/ml vs 50.5 ± 7.4 pg/ml; p = 0.0009). On the contrary, the spontaneous production of TNF-α was significantly greater in patients (78.2 ± 87.4 pg/ml vs 10.4 ± 6.4 pg/ml; p = 0.0007). Fig. 4B shows the kinetic study of IL-2 and TNF-α production at 24, 48, 72, and 96 h in CVID patients and controls. In CVID patients, IL-2 production remained low with respect to the controls, whereas TNF-α production progressively increased.

Immunohistochemistry of BM stromal cells and IL-7 production

Next, we generated stromal cell layers from BMMCs of patients and controls. The stromal layers cultured on chamber slides were positive for CD68, vimentin, and CD14 but negative for S100 and CD34 molecules, indicating that these were preferentially of the macrophage/monocyte lineage origin. Upon light microscopy examination, the majority (75%) of these cells appeared as moderately large cells, frequently rounded, with abundant cytoplasm. In control subjects, ~90% of the stromal cells exhibited a different morphology characterized by irregular or spindle shape and branching cytoplasmic processes (fibroblast-like) as shown in Fig. 5.

Compared with healthy subjects, stromal cells from patients spontaneously produced lower levels of IL-7 (0.8 ± 0.1 pg/ml vs 0.3 ± 0.1 pg/ml, respectively; p = 0.02; Fig. 6).

Discussion

Regulation of hemopoiesis and maintenance of homeostasis in BM require a well balanced interaction between the hemopoietic cells and the immune system. It has been shown that immune deregulation in autoimmune and chronic inflammatory disorders may
modulate the function of BM hematopoietic progenitor cells and/or their microenvironment, either by inflammatory cytokine production or by cell to cell interactions (23). Occurrence of cytopenias may be observed in primary immunodeficiencies, and it is particularly frequent in CVID patients (5). Defects in T cell regulation and cytokine production, abnormal apoptosis, or abnormal secretion of IgA with autoimmune features are all potential mechanisms of cytopenias in association with congenital immunodeficiency (6–11). In addition, altered hemopoiesis may be associated with a variety of functional and immunophenotypic abnormalities at BM level, due to augmented local production of inflammatory cytokines, increased T cell activation, or intrinsic hemopoietic and stromal cell abnormalities.

In view of the current interest in exploring the role of hematopoietic stem cell transplantation, we evaluated BM progenitor cell reserve and function and stromal cells in 11 CVID individuals. In addition, we analyzed the cytokine production of BMMCs and the morphology of stromal cells established with long term cultures of BMMCs. The cytokines were selected on the basis of available data, showing the role of such molecules in impaired hemopoiesis in humans as well as for their regulatory effects on human hematopoietic progenitor cells (24, 25).

A significant quantitative and functional defect in hemopoietic progenitor cells was observed in our patients, as indicated by an altered clonogenic potential of BM-committed progenitor cells and a substantial reduction in primitive progenitors (LTC-CFC). These abnormalities may represent either an intrinsic progenitor cell defect or a secondary progenitor cell damage in response to an underlying inflammatory process within the BM microenvironment. In this regard, the reduced erythroid compartment and the tendency to an expansion of CFU-GM may be related to the frequent recurrences of infectious episodes observed in CVID patients. Compared with healthy controls, patients displayed an altered cytokine production by BMMCs, characterized by decreased levels of IL-2 and increased levels of TNF-α.

Although the proportion of total CD34+ cells was within the range of adult controls, phenotypically primitive CD34+CD38−DR− cells, which include lymphoid precursor cells, were decreased.

With regard to B cell development, we established a significant correlation between the frequency of CD34+CD19−CD10−CD38− (pro-B cells), the CD34+CD19−CD10+ (pre-B cells) in the BM, and the number of peripheral blood CD19+ cells. This finding, together with a decrease in the proportion of BM pre-B cells, indicates the involvement of BM in T and B cell differentiation, at least in selected CVID patients. These findings correlated with a significant decrease in total lymphocyte counts and depletion of CD4+ T cells expressing predominantly the CD45RA+CD62L+ phenotype. Also, the CD4+CD45RA−CD31+ T cell subset was significantly reduced in our cohort and correlated with the diminished content of TRECs previously observed (18), suggesting thymus involvement in these patients. Furthermore, we cannot rule out a defective egress of naive T lymphocytes from the thymus, which is regulated by sphingosine 1-phosphate receptor 1 (26). Indeed, it is possible that the T cell defect in CVID may occur at multiple levels, including egress from thymus.

IL-2 is a cytokine that increases proliferation and function of CD4+ and CD8+ T lymphocytes, B lymphocytes, and NK cells in vitro. Several studies have also shown positive effects of IL-2 on apoptosis; the addition of IL-2 to the lymphocyte cultures reduces apoptosis of PBMCs obtained from patients with secondary immunodeficiency (27). Recently, IL-2 was administered for a long period to CVID patients with associated numerous T cell defects, demonstrating a partial correction of cell-mediated immunity as well as of Ab production after immunization (28). In contrast, the suppressive effect of TNF-α on marrow progenitor cells has long been demonstrated and is mediated by affecting cell viability or by modulating the expression of numerous cytokine receptors on the cell surface (29). TNF-α is a well-known negative regulator of
hemopoiesis that induces functional FAS on CD34+ cells (30, 31) and promotes their differentiation (32). The elevated TNF-α levels and the decreased IL-2 production observed in our patients might be involved in mediating the apoptotic depletion of progenitor cells as demonstrated by the altered growth of in vitro colonies observed, also in the more primitive compartment. In fact, the addition of anti-TNF-α Ab and of the cytokines IL-7 and IL-2, alone or in combination, to CFC and LTBM C assays determined an increased growth of in vitro colonies, especially with IL-2 plus IL-7, suggesting an important role of these factors for committed and uncommitted progenitor cells.

In vivo and in vitro, hemopoiesis occurs in association with the complex network of cell types found in the stroma including non-hemopoietic (fibroblasts, adipocytes, and endothelial cells) and hemopoietic cells (macrophages and T cells) (19). Progenitor cell growth and differentiation depends on their interaction with stromal cells. Low levels of cytokine production can be more effective when local concentration is increased by cell-cell contacts and by the binding of cytokines to the extracellular matrix (20, 33). The prevalence of macrophage-like cells in long term bone marrow culture, rather than typical “fibroblast-like” cells, suggests an altered composition of the BM stroma, possibly linked to an underlying inflammatory process within the BM microenvironment.

A central function of stromal cells is the IL-7 production (34). Recent evidence shows that IL-7 acts as a master regulator of T cell homeostasis, expanding both the naive and memory T cell populations (35, 36). IL-7 primarily acts as a growth and antiapoptotic factor for B and T cell precursors, and its production is a critical step for the beginning of B and T lymphopoiesis starting from stem cells. Moreover, IL-7 induced and required the antiapoptotic protein MCL-1 to mediate lymphocyte survival. Thus, MCL-1, which selectively inhibits the proapoptotic protein BIM, is essential both early in lymphoid development and later on in the maintenance of mature lymphocytes (37). Compared with controls, an altered stromal cytokine production was observed in CVID patients, characterized by decreased IL-7 levels. This pattern suggests a critical role of BM accessory cells in the altered cytokine secretion in CVID patients and raises the possibility that diminished availability of IL-7 might contribute to accelerated death for apoptosis of stem cell precursors and to the development of CD4+ T cell depletion in CVID patients.

In conclusion, a reduced content of in vitro committed and primitive progenitors was observed in BMMCs of CVID patients, associated with phenotypic abnormalities, and an altered stromal cell composition and cytokine production. These abnormalities may be due, at least in part, to the increased TNF-α production by inflammatory cells in the BM and to the decreased IL-2 levels. We hypothesize that the damage of T cell compartment may be at least partially due to an altered generation of new T cells starting from the hemopoietic stem/progenitor cells. Based on these results, therapeutic administration of some cytokines (i.e., IL-2 plus IL-7) and the use of antiapoptotic factors as a future strategy may be useful for the treatment of some patients affected by CVID syndrome.

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