CONCISE REPORT

T-Cell Subpopulations in Chronic Lymphocytic Leukemia: Abnormalities in Distribution and in In Vitro Receptor Maturation

By N. E. Kay, J. D. Johnson, R. Stanek, and Steven D. Douglas

Purified human thymus-derived (T) lymphocytes were analyzed by detection of Fc receptors for either IgG or IgM in healthy controls and in patients with chronic lymphocytic leukemia (CLL). There was a significant and persistent increase in the numbers of T cells bearing receptors for IgG (Fcγ) in CLL patients in comparison to the controls. After an in vitro culture period, there was a significantly decreased appearance of cells with IgM receptors (Fcμ) in CLL patients in comparison to the control group. These results indicate an imbalance in circulating T-cell subpopulations for CLL patients. In addition, an in vitro defect in CLL T-cell membrane receptor appearance is present.

Chronic lymphocytic leukemia (CLL) has been characterized predominantly as a B-cell-related disorder. There are, however, residual T cells in the peripheral blood, and these T cells are believed to be functionally normal. CLL is frequently accompanied by abnormalities in immunoglobulin (Ig) production, such as hypogammaglobulinemia or monoclonal immunoglobulins. Although serum protein disorders may be a consequence of B-cell dysfunction, B–T cell interaction is critical for differentiation of a mature Ig-producing cell. That is, subsets of T cells may exert positive or negative effects on the proliferation and activation of bone-marrow-derived (B) cells. In man, the relevance of normal T-cell–B-cell interaction for protein synthesis is suggested by the report of imbalance in thymic cell function in both congenital and acquired hypogammaglobulinemias. Recent advances in techniques have permitted a more detailed analysis of human T-cell populations. The thymus-derived (T) lymphocytes have been subdivided both functionally and by receptor or antigenic markers in mice and man. In man, Fc receptors for IgG (Fcγ) and IgM (Fcμ) have been detected on T cells, and following immune complex exposure, T cells with receptors for IgG may convert to cells with Fc receptors for IgM over a prolonged in vitro culture period. In an effort to more completely characterize CLL T cells, we studied the proportion, number, and in vitro Fc receptor transitions of peripheral blood T cells from patients with CLL in comparison to T cells for healthy individuals.
MATERIALS AND METHODS

Peripheral blood samples from 10 healthy volunteers and 9 patients with CLL were obtained in heparinized syringes. No patient had received chemotherapy within 4 wk of this investigation. Eight patients had stage III or IV disease and one had stage I, with age ranges of 41–75 yr.

Cell Isolation and Identification

Peripheral blood mononuclear cells were isolated from venous blood by Ficoll-Hypaque (F/H) centrifugation. To deplete monocytes, the intact mononuclear cells were then placed in tissue culture flasks for 30 min at 37°C in 5% CO₂, and nonadherent cells were harvested. This procedure resulted in less than 2% contaminating monocytes in the nonadherent population as judged by latex ingestion and nonspecific esterase staining. T lymphocytes were then isolated from the nonadherent cells by incubating 2-aminoethylisothiouroniumbromide hydrobromide (AET) (Aldrich Chemical, Milwaukee, Wisc.) treated sheep erythrocytes (Colorado Serum, Denver, Colo.) with the nonadherent cells for 30 min at 37°C, then pelleting this mixture as previously described. A subsequent F/H centrifugation of this lymphocyte-erythrocyte mixture resulted in a pellet of T cells and erythrocytes. This procedure resulted in a T-cell purity of 96%–99% for the control cells and 93%–98% for the patient cells. The total T-cell recovery was 70%–85% and 65%–75% for controls and patients, respectively.

Aliquots of the purified T cells (10⁶) were placed in 1 ml of medium 199 (Gibco) with 20% fetal calf serum (FCS) for overnight incubation at 37°C in 5% CO₂ in order to serially evaluate the proportions of T-Fc-µ and T-Fc-γ cells during an in vitro culture period.

Cell Identification

T cells were identified by E-rosette formation as previously described. B cells were evaluated by an immunofluorescent technique for surface Ig (Slg). Fc-µ and Fc-γ receptors were detected by a modification of a technique previously described. Ox red blood cells (ORBC) (Colorado Serum) were washed three times in medium 199. Then, a 5% ORBC suspension was sensitized with an anti-ORBC IgG fraction (Cappel Labs; protein concentration 0.25 mg/ml) or with an anti-ORBC IgM fraction (Cappel Labs; protein concentration 0.25 mg/ml). The cells were washed twice and resuspended in media 199 with 20% FCS to yield 1% and 0.5% suspensions of EA-IgG and EA-IgM, respectively. The purity of the IgG and IgM fractions was tested and confirmed by Ouchterlony immunodiffusion using goat anti-rabbit IgG or IgM. The EA-IgG or EA-IgM (0.5 ml) were then mixed with 0.5 ml of cells (2 x 10⁶). EA-IgG cell mixture was incubated at 37°C for 30 min, spun down at 200 g for 5 min, and then incubated at 4°C for 2 hr. The EA-IgM cell mixture was incubated at 37°C for 30 min and spun down at 200 g for 5 min. The cell pellets were then very gently resuspended, a drop placed on a slide, and 200 cells counted for rosette formation by light microscopy. The binding of three or more erythrocytes per cell was considered a positive rosette, and trypan blue exclusion was used concurrently to test viability. Uncoated ORBC were used as controls. T-Fc-µ and T-Fc-γ markers were performed on freshly isolated T cells (day 0) and after 24 hr of in vitro culture. Statistical analysis of the results were performed by the use of Student’s t test.

RESULTS

T-Cell-Enriched Fractions

The purity of the isolated T-cell populations is shown in Table 1. Viability of the T-cell-enriched fractions for the patients and controls was 95%–97%. This corresponded to 6–8 hr after initial venous sampling from the patients and controls. There was no difference in viability in either patient or control cells over the in vitro culture period.

Fc-µ and Fc-γ Receptors on T-Cell-Enriched Fractions

Figure 1 shows the Fc-µ and Fc-γ receptors percentages for the controls and patients on day 0 (i.e., 6–8 hr of in vitro handling) and after 24 hr of in vitro culture. The increase of Fc-γ receptors (Fig. 1A) for CLL patients is evident at day...
Table 1. Surface Markers of the Isolated T-Cell Populations

<table>
<thead>
<tr>
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<th>E-rosette*</th>
<th>Sig*</th>
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<tbody>
<tr>
<td>Controls (n = 10)</td>
<td>98.2% ± 0.7%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>(96-99)</td>
<td></td>
<td>(0.5-1.2)</td>
</tr>
<tr>
<td>CLL (n = 9)</td>
<td>95.6% ± 0.8%</td>
<td>2.4% ± 1.1%</td>
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<tr>
<td>(93-98)</td>
<td></td>
<td>(1.5-3.3)</td>
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*Means ± SEM with range in parentheses (200 cells counted).

0 (< 0.001), and this increase persisted after a 24-hr cultivation period (< 0.01). There were detectable Fcμ cells (Fig. 1B) in both the controls and patients (8.0% ± 3.1% and 6.4% ± 2.8%, respectively) and then a marked increase in Fcμ receptors in the controls (to 65% ± 5.7%) and a less dramatic increase in Fcμ receptors in CLL to 21.4% ± 4.8% (< 0.01). These proportions of Fc receptors were found in all eight patients with stage III or IV and in the one patient with stage I disease. In two patients, these studies have been repeated with similar findings in the proportion of Fc receptors and reduced Fcμ after in vitro culture periods of 48 hr (see Table 2).

DISCUSSION

Fc receptor subpopulations in the purified T cells from CLL patients have been shown to be altered dramatically from a control T-cell population. This alteration was manifested as an initial increase in Fcγ receptor-bearing cells and a reduced appearance of Fcμ receptor cells during in vitro culture. Previous functional evaluation of T cells in CLL has suggested that they are normal, though their effects are diluted by the poorly or nonresponsive leukemic lymphocytes.1,14 T cells from patients with CLL are capable of responding to mitogen, albeit in a somewhat reduced fashion. In one study, isolated T cells from CLL patients responded normally to both pokeweed mitogen and phytohemagglutinin (PHA).1 In a small

![Fig. 1](image-url)
Table 2. Fc Receptors on Isolated T Cells of Two CLL Patients

<table>
<thead>
<tr>
<th></th>
<th>Patient A</th>
<th>Patient B</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>First study</td>
<td>Second study</td>
<td>First study</td>
</tr>
<tr>
<td>EA-IgG* Day 0</td>
<td>43 ± 2.1</td>
<td>47 ± 3.5</td>
<td>68 ± 4.4</td>
</tr>
<tr>
<td>EA-IgG* 24 hr</td>
<td>31 ± 2.8</td>
<td>35 ± 4.1</td>
<td>51 ± 3.7</td>
</tr>
<tr>
<td>EA-IgM* Day 0</td>
<td>1.8 ± 0.8</td>
<td>2.4 ± 1.1</td>
<td>2.4 ± 1.9</td>
</tr>
<tr>
<td>EA-IgM* 24 hr</td>
<td>15.6 ± 2.8</td>
<td>18.1 ± 3.4</td>
<td>17.4 ± 2.1</td>
</tr>
</tbody>
</table>

*Percent rosettes expressed as mean ± SEM.

percentage of patients with CLL, T cells may be the major cell identified in the peripheral blood.12,15 Alternatively, the number of T cells may fluctuate greatly in some patients with CLL who have the more usual B-cell predominance at diagnosis.16,17 The functional capacity and quantity of T cells has not been particularly helpful in assessing the clinical status of a patient with CLL. While it is important to emphasize that the exact significance of these Fc receptors bearing T cells is still not completely known, the finding of an imbalance between Fcγ and Fcμ cells in CLL suggests the acquisition of excessive numbers of suppressor cells in this disease. The presence of suppressor cell activity has been reported in many disease processes, including multiple myeloma and congenital or acquired immunodeficiencies.5,18 These cells may have apparently either a primary or secondary role in the evolution of various diseases. The relationship of T cells in CLL to the malignant B-cell line has been suggested by recent observations in which anti-human T-cell antisera detected a group of antigens common to B cells and T cells in CLL.18 This study supports the observations that the defective response of CLL lymphocytes to PHA appears to be mediated in part by the presence of excessive suppressor cell function.20 Since it is now possible not only to identify these T-cell subsets but also to isolate them, future in vitro functional evaluation of CLL T cells should provide further information about the role of these cells in the pathophysiology and complications of CLL.

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American Society of Hematology
Twenty-Second Annual Meeting
Phoenix, Arizona
December 1-4, 1979

The twenty-second annual meeting of the
American Society of Hematology will be
held at the Phoenix Civic Plaza, Phoenix,
Arizona, December 1-4, 1979. Programs by
the Education Committee and the Scientific
Subcommittees will be held on Saturday,
December 1 and Sunday, December 2. The
Presidential Symposium will be held Tues-
day, December 4. Contributed papers will be
presented Monday, December 3 and Tues-
day, December 4.

The abstract deadline for contributed
papers is September 6, 1979. Abstract forms
may be obtained from the Charles B. Slack
Company, address below.

Subsequent issues of Blood will contain
details of the education and subcommittee
programs and the Presidential Symposium
and information about registration for the
meeting. Preregistration material will be
sent early this fall to members. Nonmem-
ers wishing to receive preregistration infor-
mation and forms should write to Charles B.
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