Evidence That Large Granular Lymphocytes From B-CLL Patients With Hypogammaglobulinemia Down-regulate B-Cell Immunoglobulin Synthesis

By Neil E. Kay and Robert T. Perri

B-chronic lymphocytic leukemia (CLL) patients frequently suffer from moderate to severe hypogammaglobulinemia. This complication is a serious cause of morbidity and mortality in this disorder. There is recent evidence that natural killer (NK) cells modulate B-cell immunoglobulin (Ig) synthesis/secretion. The authors therefore evaluated the circulating NK cells from B-CLL patients on their ability to regulate mitogen-induced B-cell Ig synthesis. Blood, NK cells (CD16+, CD3−) from three B-CLL patients with hypogammaglobulinemia were able to clearly down-regulate the pokeweed mitogen (PWM)-induced–B-cell Ig secretion. In contrast, CD16+, CD3+ cells from age-sex–matched controls or B-CLL patients with normal Ig were either nonregulatory or enhanced mitogen-induced B-cell Ig secretion. An alternative explanation for hypogammaglobulinemia in B-CLL patients is the immunomodulation of B-cell Ig production/secretion by CD16+, CD3− blood cells.

In this disorder, there is recent evidence that mortality from hypogammaglobulinemia (LGL) is now believed to be necessary. Decreased serum Ig levels in B-CLL is believed to be from B-CLL patients on their ability to regulate mitogen-induced B-cell Ig synthesis. Blood. NK cells (CD16+, CD3−) from three B-CLL patients with hypogammaglobulinemia were able to clearly down-regulate the pokeweed mitogen (PWM)-induced–B-cell Ig secretion. In contrast, CD16+, CD3+ cells from age-sex–matched controls or B-CLL patients with normal Ig were either nonregulatory or enhanced mitogen-induced B-cell Ig secretion. An alternative explanation for hypogammaglobulinemia in B-CLL patients is the immunomodulation of B-cell Ig production/secretion by CD16+, CD3− blood cells.

Morbidity and mortality from hypogammaglobulinemia is noted in many B-chronic lymphocytic leukemia (B-CLL) patients. Clearly further understanding of the mechanism that results in this serious complication is necessary. Decreased serum Ig levels in B-CLL is believed to occur primarily through an expanding malignant B-cell clone that replaces normal B cells in marrow and nodes. B-CLL is, however, often accompanied by abnormal immunoregulatory T cell and natural killer (NK) cells. The NK cell or large granular lymphocyte (LGL) is now believed to have potent immunoregulatory function toward normal B cells in addition to cytotoxicity for viral-infected targets and tumor cells.

Indeed, the authors have recently shown that B-CLL blood LGL, purified from CLL patients with hypogammaglobulinemia, has excessive activity for down-regulation of normal B-cell proliferation. The authors therefore evaluated the function of circulating blood NK cells on B-cell Ig secretion from B-CLL patients with and without hypogammaglobulinemia.

MATERIALS AND METHODS

Preparation of large granular lymphocytes from B-CLL patients and normal subjects. Heparinized peripheral blood samples of 20 to 50 mL were obtained from three patients with B-CLL who had hypogammaglobulinemia, three patients with B-CLL who had normal serum Ig levels, and two age- and sex-matched normal subjects. First, mononuclear cells were separated from the blood samples by Ficoll-Hypaque (FH) centrifugation. The interface cells were harvested and monocytes removed by adherence to tissue culture flasks. The nonadherent peripheral blood lymphocyte (PBL) was then separated on a discontinuous density gradient of Percoll, as previously described. The two upper layers contain the majority of LGL. LGL harvested from these two layers is morphologically homogenous; however, there are two main phenotypic subgroups. LGL may be subdivided into either CD16+ , CD3− or CD16− , CD3− groups. The latter cells are non-major histocompatibility complex (MHC)-restricted cytotoxic lymphocytes (CTL), while the former cells contain most of the NK activity. To purify the Percoll fractions into CD16+ , CD3− and CD16− , CD3− cells, the authors dual stained the Percoll LGL with monoclonal antibodies (MoAbs) Leu-11 (CD16) and Leu-4 (CD3), then analyzed and sorted on an automated fluorescence-activated flowcytometer (FACS 440 Becton Dickinson Corp). In brief, cells (1 × 106/mL) were incubated for 30 minutes on ice with saturating concentrations of MoAbs (phycoerythrin-conjugated Leu-11 and fluorescein isothiocyanate (FITC)-conjugated Leu-4, Becton Dickinson Corp, Sunnyvale, CA). Cells were then washed twice with phosphate-buffered saline (PBS). The cells were then analyzed by the FACS 440. Populations of cells expressing either CD16+, CD3− or CD16− , CD3− phenotype were characterized by fluorescence to determine positive and negative gates (Fig 1). After appropriate gating the FACS 440 sorting was allowed to proceed to provide either CD16+, CD3+ or CD16− , CD3+ cell groups. The authors evaluated the quality of the sort by fluorescence microscopy. The cell sort was considered successful if greater than 99% of the cells in the positive fraction of the sort had visible fluorescence. The sorted cell fractions were then used immediately in the B-cell immunoglobulin assays (see enzyme-linked immunosorbent assay [ELISA] below).

Preparations of B and T cells from normal subjects. To obtain normal B and T cells required to generate pokeweed mitogen (PWM)-induced–Ig secretion (see below) the authors prepared purified B and T cells from normal donors. Peripheral blood mononuclear cells were isolated from venous blood by FH. Mononuclear cells were depleted by adherence to tissue culture flasks for 45 minutes at 37°C, 5% CO2. Nonadherent cells were harvested and then washed twice with phosphate-buffered saline (PBS). Cells were then rechallenged with pokeweed mitogen (PWM) in RPMI supplemented with 10% fetal bovine serum (FCS) for seven days in RPMI, 10% fetal calf serum (FCS; total

From the Department of Medicine, Section of Hematology/Oncology, Veterans Administration Medical Center, Minneapolis. Submitted April 19, 1988; accepted November 14, 1988. Address reprint requests to Neil E. Kay, MD, Section of Hematology/Oncology, Department of Medicine, Minneapolis Veterans Administration Medical Center, Minneapolis, MN 55417.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.

BLOOD, Vol 73, No 4 (March), 1988: pp 1016-1019
volume 2 mL) at 37°C in 5% CO₂. These cells were cultured in 5 mL wells of Co-star culture plates (Cambridge, MA). The culture supernatants were then harvested and assayed for IgG and IgM content by ELISA (see below). Only when PWM was added to these supernatants were IgG and IgM content determined by ELISA. IECA (plus or minus 120) was added to the plates. After incubation for 30 minutes, the plates were washed three times, and 100 μL of p-nitrophenylphosphate (1 mg/mL) and 1 mol/L diethanolamine buffer (pH 9.8) were added to each well. The enzymatic reaction was stopped after one hour by adding 50 μL of 3 mol/L NaOH. The absorbance at 405 nm was measured with a titertech multiscan Model 340 (Flow Laboratories, Inc, McLean, VA). In each experiment a standard curve was constructed for samples containing known concentrations of affinity-purified IgG and IgM. These reagents were purchased from TAGO. This curve was then used to calculate the IgG and IgM concentrations in the culture supernatants. Replicate samples of standard Ig reagents and Ig in culture supernatants have a standard error of mean that varies from 3% to 5%.

RESULTS AND DISCUSSION

Table 1 summarizes the pertinent clinical data of the six B-CLL patients and the two normal donors. All B-CLL patients and donors were male and either in their fifth, sixth, or eighth decade. The clinical stages were equivalent for the B-CLL patients with and without hypogammaglobulinemia (Table 1). The B-CLL patients studied were not on chemotherapy for at least 6 weeks prior to these studies. In addition, none of the three B-CLL patients with hypogammaglobulinemia were currently receiving replacement intramuscular (IM) or intravenous (IV) Ig. Table 2 illustrates the impact of the addition of CD16*, CD3* or CD16*, CD3* cell groups, purified from blood LGL (see “Methods”), of either the B-CLL patients or normal donors on normal B-cell Ig production.

First, suppression (ie, decreased at least 10% from control levels) of IgG or IgM culture supernatant levels was found only when LGL from B-CLL patients with low serum Ig were added to the B- and T-cell cultures (Table 2). In patients 1 through 3, purified CD16*, CD3+ cells were obviously effective at down-regulation of Ig synthesis or secretion of normal B cells. In all three B-CLL patients both IgG and IgM synthesis/secretion were clearly depressed from control levels by LGL with CD16*, CD3+ but not CD16*, CD3+ phenotype (Table 2). The data are shown for LGL added at 1 x 10⁶/mL. This LGL to B-cell concentra-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Stage</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>M</td>
<td>2</td>
<td>180</td>
<td>75</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>M</td>
<td>4</td>
<td>310</td>
<td>110</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>M</td>
<td>2</td>
<td>280</td>
<td>55</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>M</td>
<td>3</td>
<td>847</td>
<td>110</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>4</td>
<td>902</td>
<td>95</td>
<td>110</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>M</td>
<td>2</td>
<td>710</td>
<td>180</td>
<td>80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal donors</th>
<th>Serum Ig Levels†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>1,120</td>
</tr>
<tr>
<td>2</td>
<td>980</td>
</tr>
</tbody>
</table>

*†Disease stage of CLL using the Rai classification. IgG levels were determined immediately prior to in vitro studies and are presented as mg/dL normal values for serum Ig: IgG (600 to 1,500), IgA (60 to 380), and IgM (55 to 280).
were also capable of augmenting the PWM-induced B-cell Ig levels. This is not surprising, since CD16+, CD3+ LGL may be T-cell derived. Thus, the authors may simply have added T cells capable of providing help for PWM-directed B-cell activation. No augmentation of PWM-induced Ig synthesis was noted with CD16+, CD3+ LGL from the hypogammaglobulinemic B-CLL patients (Table 2). This finding is consistent with lack of T-cell assistance for B-cell proliferation or Ig synthesis previously noted in the T-cell population of B-CLL patients.

The down-regulation of Ig synthesis and/or secretion by a subset of blood LGL in B-CLL patients offers an additional explanation for the frequent hypogammaglobulinemia noted in B-CLL. These data add to an expanding array of abnormal immunoregulatory cell function in B-CLL. The etiology for the presence of a B-cell suppressor LGL in B-CLL is unclear. A potential explanation is that LGL have emerged in an attempt by the host to control the B-cell clone. If the B-suppressor LGL activity is directed against both malignant and normal B cells, there could be down-regulation of normal B-cell Ig synthesis. In this regard there is a recent finding that circulating myeloma protein may induce suppressor T cells capable of suppressing the messenger RNA (mRNA) of the clonal protein. It is tempting to speculate that an expanding B-cell clone with presentation of clonal membrane Ig may similarly induce immunoregulatory cells with B-suppressor function. Indeed, there is recent evidence that LGL may be induced to regulate class-specific antibody production. A human leukemic cell line (YT) with LGL properties and phenotype was shown to express class-specific Fc receptors and to release suppressive Ig-binding factors after exposure to purified Ig isotypes. This is similar to the Ig regulatory behavior observed for T-suppressor cells. Thus, in B-CLL the induction of B-suppressor LGL may result in down-regulation of B-cell populations capable of Ig synthesis/secreation and may ultimately play a role in the development of hypogammaglobulinemia.

REFERENCES


Evidence that large granular lymphocytes from B-CLL patients with hypogammaglobulinemia down-regulate B-cell immunoglobulin synthesis [published erratum appears in Blood 1989 Jun;73(8):2232]

NE Kay and RT Perri