Communication between B and T lymphocytes is mediated by cell surface receptors that engage other membrane-bound counterreceptors. One such pair of these molecules has received much attention: CD40, a glycoprotein belonging to the TNFR family that is expressed by normal and neoplastic B cells but not by T cells, and its physiological ligand (CD40L), a protein homologous to TNF that is expressed by activated T cells (mainly CD4+) but not by resting T cells or normal non-T lymphocytes. Stimulation of B cells by CD40L, together with appropriate cytokines, induces their activation, proliferation and differentiation and prevents apoptosis, enhancing the expression of the bcl-2 protein.1

In this study, expression of CD40L by purified B and activated CD4+ cells from patients with B-CLL was evaluated in order to provide insights into two aspects of the pathophysiology of this disease.

First aspect. Hypogammaglobulinemia occurs frequently in patients with B-CLL and specific antibody response is impaired, contributing to increased susceptibility to infections, a major cause of morbidity and mortality in this disease.2,3 The pathogenesis of hypogammaglobulinemia in B-CLL is poorly understood; since several studies have described a decreased T helper function, defective expression of CD40L by T-cells might possibly play a role.

Second aspect. B-CLL is regarded as an accumulative disease that results from decreased apoptosis rather than increased proliferation.4 Stimuli which inhibit the entry of neoplastic B cells into apoptosis may allow survival in situations where their normal counterparts would die.5 Since B cells from CLL can replace T cells and provide the contact-dependent signal necessary for the induction of B-cell proliferation and differentiation,6 the existence of a common surface determinant on B-CLL and normal T cells for this function (normally performed by CD40L) could be hypothesized. Therefore the aberrant presence of CD40L on B-CLL cells can be postulated as a mechanism by which leukemic cells stimulate themselves via CD40 to escape apoptosis.
Materials and Methods

Subjects

Eleven patients with B-CLL (see Table 1) and 15 adult healthy donors were studied.

Cell purification

CD4⁺ cells were purified using anti-CD4 coated magnetic beads (Dynabeads; Dynal, Oslo, Norway) as described elsewhere.⁷ After this procedure the number of positively-selected cells expressing CD4 was similar in patients and in controls (92.3±5.3 vs. 96.1±2.9; p: ns). B cells were purified by negative selection with anti-CD4 and anti-CD8 coated magnetic beads. More than 95% of the lymphoid cells were CD19⁺ after this procedure in leukemic patients.

Cell culture and flow cytometry analysis of CD40L expression

Purified CD4⁺ or B cells were cultured with or without PMA (Sigma Chemical, St. Louis; 5 ng/mL) plus ionomycin (Sigma Chemical; 500 ng/mL) for 16 hours to induce CD40L expression as previously described.⁸ This was then evaluated using a biotin-conjugated monoclonal antibody and streptavidin-PE. Data are expressed on a log scale. Dashed lines represent the fluorescence of cells stained with appropriate controls.

Table 1. Main clinical data of patients with B-CLL.

<table>
<thead>
<tr>
<th>Sex</th>
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<tbody>
<tr>
<td>Age (median)</td>
<td>67 (range 42-85)</td>
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<tr>
<td>Stage (according Rai)</td>
<td>0: 4 II: 3 III: 2 IV: 2</td>
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<tr>
<td>Treatment</td>
<td>8: no treatment 1: cyclophosphamide 1: chlorambucil+prednisone 1: prednisone+danazol</td>
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<tr>
<td>Serum Ig levels</td>
<td>4: normal 3: low IgM (&lt; 87 mg/dL) 1: low IgA (&lt; 123 mg/dL) 1: low IgM and IgG (&lt; 783 mg/dL) 1: low IgG and IgA 1: low IgG, IgM and IgA</td>
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Figure 1. CD40L expression in B-CLL is physiological. Purified B or CD4⁺ cells were cultured in complete medium (resting) or in the presence of PMA (5 ng/mL) + ionomycin (500 ng/mL) for 16h (activated). Cells were stained with TRAP1 monoclonal antibody or with CD40-Fc fusion protein. Data are expressed on a log scale. Dashed lines represent the fluorescence of cells stained with appropriate controls.
al antibody (TRAP1; R. Kroczek, Berlin) as already described, and (in some experiments), using a CD40Fc IgG fusion molecule (J-I. Bonnefoy, Geneva) followed by FITC-anti IgG.

Statistical analysis
Data are presented as the mean±SD. The Mann-Whitney rank sum test was used to determine the significance of the differences between groups.

Results
The percentage of purified CD4+ lymphocytes expressing CD40L after stimulation with PMA±Ionomycin was similar in B-CLL patients and controls (75.4±8.1 vs 80.5±9.3; p: ns). The mean channel of fluorescence of CD40L+ cells was also similar (138±11 vs 143±12), indicating that the density of expression of the molecule on positive cells did not differ in the two groups (Figure 1).

No significant difference was observed when patients were divided according to stage (0 vs. II-IV), Ig levels (low IgG and/or IgA vs normal IgG and IgA), treatment or no treatment. In contrast, no expression of CD40L was detectable on fresh or stimulated purified B cells either from patients or controls.

Discussion
This study demonstrates that expression of the CD40L molecule is essentially normal in B-CLL; it is present on purified CD4+ cells, at least after optimal in vitro stimulation, and absent from fresh B cells. Although it cannot be excluded that the CD40/CD40L system is affected in vivo by other mechanisms (e.g. via soluble CD40), these data suggest that the genesis of hypogammaglobulinemia in B-CLL is different from that observed in other states of primary and acquired hypogammaglobulinemia where defective expression of CD40L plays a role, such as X-linked immunodeficiency with hyper-IgM, a subset of common variable immunodeficiency, and in normal newborns. On the other hand, CD40 is normally expressed on leukemic B cells and activation through CD40 is effective on B cells from CLL patients as it is on normal resting B cells, indicating therefore that this pathway is intact in B-CLL. The question of why leukemic cells accumulate in B-CLL is still unanswered. Many studies have focused on the possibility that abnormal auto- or paracrine cytokine activity could be responsible for this phenomenon. The present study has considered the possibility that aberrant expression of CD40L by B-CLL cells might provide a pathway by which leukemic cells could autostimulate themselves. Although the evidence from our investigation is negative, analysis of membrane receptors involved in cell-to-cell contact signalling is an interesting new field of investigation in the pathophysiology of lymphoid neoplasms.

References