*short report*
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# STUDY OF CD40 LIGAND EXPRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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**ABSTRACT**

**CD40 ligand (CD40L) is a membrane molecule that plays a key role in T cell-B cell cooperation, providing B cells the *helper* signals needed for activation, proliferation, differentiation and prevention of apoptosis. Patients with B-cell chronic lymphocytic leukemia (B-CLL) were studied to verify the following hypotheses: a) whether defective CD40L expression on activated T cells could account for deficient *helper* signals and therefore for hypogammaglobulinemia; b) whether aberrant CD40L expression on B cells could be a mechanism by which leukemic cells stimulate themselves via CD40 to escape apoptosis. Results showed physiological expression of CD40L on *in vitro* activated $CD4^+$ cells, while this expression was absent on fresh and activated B cells. Abnormalities in CD40/CD40L interaction do not seem to play a role either in the pathogenesis of hypogammaglobulinemia or in lymphocyte accumulation in B-CLL.**

*Key words: CD40 ligand, B-CLL, hypogammaglobulinemia, apoptosis*



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.

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## *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 monoclon-

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

| | |
|---|---|
| Sex | 4 F; 7 M |
| Age (median) | 67 (range 42-85) |
| Stage (according Rai) | 0: 4 |
| | II: 3 |
| | III: 2 |
| | IV: 2 |
| Treatment | 8: no treatment |
| | 1: cyclophosphamide |
| | 1: chlorambucil+prednisone |
| | 1: prednisone+danazol |
| Serum Ig levels | 4: normal |
| | 3: low IgM (< 87 mg/dL) |
| | 1: low IgA (< 123 mg/dL) |
| | 1: low IgM and IgG (< 783 mg/dL) |
| | 1: low IgG and IgA |
| | 1: low IgG, IgM and IgA |

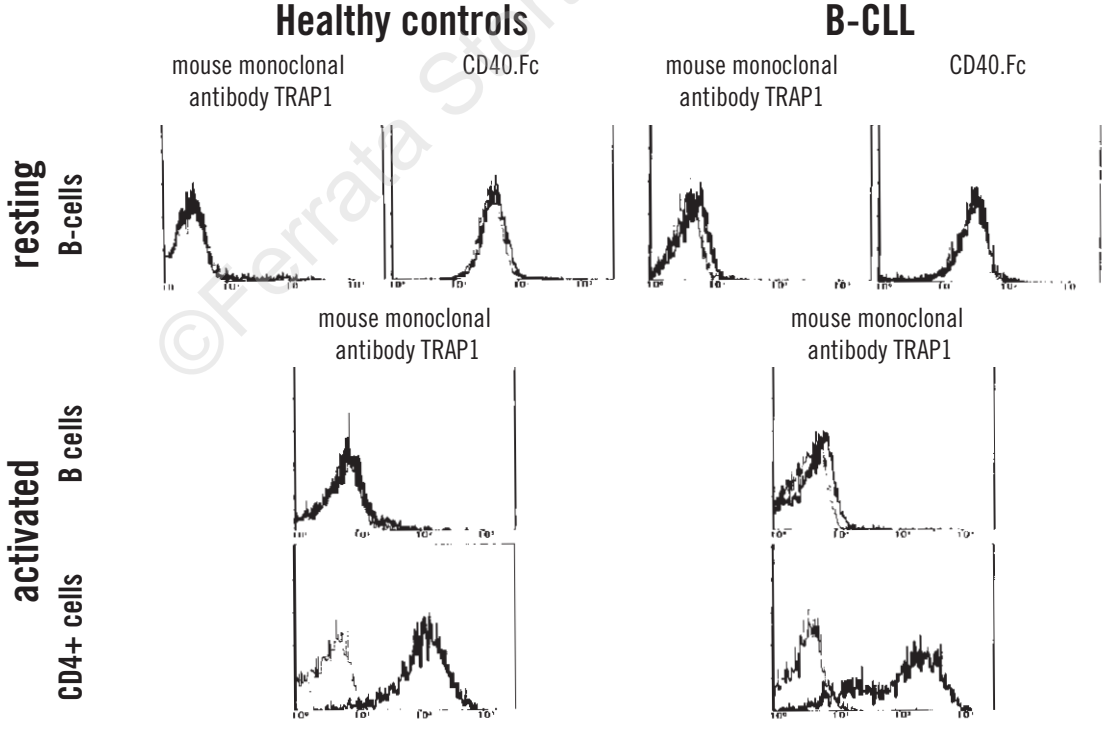


Figure 1. CD40L expression in B-CLL is physiological. Purified B or CD4+ cells were cultured in complete medium (resting) or in the presence of PMA (5 ng/mL) + ionomycin (500 ng/mL) for 16h (activated). Cells were stained with TRAP1 monoclonal antibody or with CD40-Fc fusion protein. Data are expressed on a log scale. Dashed lines represent the fluorescence of cells stained with appropriate controls.

al antibody (TRAP1; R.Kroczek, Berlin) as already described,[7] and (in some experiments), using a CD40.Fc 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.[8-9]

On the other hand, CD40 is normally expressed on leukemic B cells[1] and activation through CD40 is effective on B cells from CLL patients as it is on normal resting B cells,[10] 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.[4] 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*


1. Bancherau J, Bazan F, Blanchard D, et al. The CD40 and its ligand. Annu Rev Immunol 1994; 12:881-922.
2. Griffiths H, Lea J, Bunch C, Lee M, Chapel H. Predictors of infections in chronic lymphocytic leukemia. Clin Exp Immunol 1992; 89:374-7.
3. Molica S, Levato D, Levato L. Infections in chronic lymphocytic leukemia. Analysis of incidence as a function of length of follow-up. Haematologica 1993; 78:374-7.
4. Caligaris-Cappio F, Bertero MT, Bergui L. Autoimmunity, autoimmune diseases and lymphoproliferative disorders. Haematologica 1994; 79:487-92.
5. Bergamaschi G, Rosti V, Danova M, Lucotti C, Cazzola M. Apoptosis: biological and clinical aspects. Haematologica 1994; 79:86-93.
6. Nusslein HG, Dietz A, Burger R, Trag T, Kalden JR, Gramatzki M. Chronic lymphocytic leukemia cells induce non-T cells to produce IgE in the presence of Interleukin-4. J Clin Immunol 1993; 13:397-405.
7. Brugnoni D, Prati E, Airò P, Castelli F, Cattaneo R. The ability of CD4+ cells from HIV+ individuals to express CD40 ligand after in vitro stimulation is not impaired. Clinical Immunol Immunopathol 1995; 74:112-4.
8. Brugnoni D, Airò P, Graf D, et al. Ineffective expression of CD40 ligand on cord blood T cells may contribute to poor immunoglobulin production in the newborn. Eur J Immunol 1994; 24:1919-24.
9. Laman JD, Claassen E, Noelle RJ. Immunodeficiency due to a faulty interaction between T cells and B cells. Curr Opin Immunol 1994; 6:636-41.
10. Ranheim EA, Kipps TJ. Activated T cells induce expression of B7/BB1 on normal or leukemic cells through a CD40-dependent signal. J Exp Med 1993; 177:925-35.