CD38 Expression Distinguishes Two Groups of B-Cell Chronic Lymphocytic Leukemias With Different Responses to Anti-IgM Antibodies and Propensity to Apoptosis

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The expression of CD38 by B cells of chronic lymphocytic leukemia (B-CLL) was studied in 20 untreated patients. The cells expressed abundant CD38 (relative fluorescence intensity range, 6 to 15) in 6 cases (group I patients), whereas CD38 expression was low to absent (relative fluorescence intensity range, 0 to 3) in the remaining cases (group II patients). Exposure of the cells from group I patients to goat antihuman μ chain antibodies (Gαμ-αb) resulted in the elevation of intracellular free Ca²⁺ concentration ([Ca²⁺]i) followed by apoptosis. In contrast, exposure of group II cells to Gαμ-αb was not followed by increased levels of [Ca²⁺]i, programmed cell death or cell proliferation. No differences in the expression of surface IgM were noted in the two groups of B-CLL cells. Normal peripheral blood B cells, which expressed low to absent CD38, were capable of mobilizing [Ca²⁺], and of proliferating after exposure to Gαμ-αb. The collected data suggest that, although group I B-CLL cells were able to transduce the signals delivered by IgM cross-linking, this pathway was severely impaired in group II B-CLL cells. However, unlike that observed in normal circulating B cells, stimulation of group I cells with Gαμ-αb resulted in apoptosis rather than proliferation. CD38 did not appear to be directly involved in [Ca²⁺]i mobilization induced by Gαμ-αb in group I B-CLL cells because their exposure to anti-CD38 monoclonal antibodies failed to cause [Ca²⁺]i mobilization or to block the [Ca²⁺]i response induced by Gαμ-αb. These data indicate that CD38 expression identifies a particular subset of B-CLL cells with defined functional properties, including the propensity to undergo apoptosis.

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antibodies (Southern Biotechnologies, Birmingham, AL) as second antiamune Ig reagents. 

For two-color immunofluorescence, MoAbs of different isotypes and the corresponding FITC- or PE-conjugated GAM-Ig isotypes were used. In control tests, cells were treated with an irrelevant MoAb followed by the conjugated antibody. Stained cells were analyzed with a FACScan (Becton Dickinson). Data were expressed as histograms of the fluorescence intensity versus cell number or as relative fluorescence intensity (RFI) calculated according to the formula: (Mean Fluorescence Intensity of Cells Stained With the MoAb)/(Mean Fluorescence Intensity of Control Cells) = RFI.

Calcium measurements. Measurements of intracellular free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{i}\)) were performed as described by Cassatella et al. \(^{2}\) Briefly, \(^{10^7}\) cells were loaded with 3 \(\mu\)mol/L of FURA 2/AM (Molecular probes, Eugene, OR) in 4 mL of Hank’s balanced salt solution supplemented with glucose (1 mg/mL) and CaCl\(_2\) (1 mmol/L) for 30 minutes at 37°C. Cells were then washed three times, resuspended in 2 mL of the same buffer, and exposed to the appropriate stimuli (see below). [Ca\(^{2+}\)]\(_{i}\) was detected by excitation of the probe at two alternative wavelengths (340 and 380 nm) and by measuring the emitted fluorescence at 510 nm with a spectrophotometer (Perkin-Elmer Cetus, Norwalk, CT). A separate calibration for each sample was performed. When indicated, the cells were exposed to stimuli in the presence of 1 mmol/L EDTA (Sigma Chemical Co, Milan, Italy). Stimuli added during the test were polyclonal goat antimouse Ig \(\mu\) chain antibodies (GAM-\(\mu\)ab) or normal goat globulins (NGG) (all used from 10 ng/mL to 100 \(\mu\)g/mL (both from Southern Biotechnology Associates). A rabbit Fab\(_2\):antihuman IgM (Accu-specs, Westbury, NY; range of concentrations used from 10 ng/mL to 100 \(\mu\)g/mL), a murine monoclonal antihuman Ig \(\mu\) chain antibody (anti-\(\mu\)-MoAb, 5 \(\mu\)g/mL; Becton Dickinson) followed by a goat antimouse Ig antiseraum (GAM-Ig; Southern Biotechnology), anti-CD39 MoAb (5 \(\mu\)g/mL) followed by GAM-Ig, and one of the three different anti-CD38 MoAbs described above (1 \(\mu\)g/mL) followed by GAM-Ig. The concentrations of the MoAbs were selected based on the results of preliminary titration experiments.

Apoptosis. Cells (1 \(\times\) 10\(^7\)/mL) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, both from GIBCO, Paisley, UK) for various time intervals. The cells were exposed to the following stimuli in various combinations: Gapab, rabbit Fab\(_2\); antihuman IgM, and NGG (all used from 10 ng/mL to 100 \(\mu\)g/mL); one of the three different anti-CD38 MoAbs (IB4) described above (5 \(\mu\)g/mL); anti-CD40 MoAb (ascites used at the dilution of 1:500), recombinant interleukin-4 (rIL-4; 100 U/mL; Genzyme Corp, Boston, MA). The optimal concentrations of the stimuli used were selected after titration experiments.

At the end of culture, the cells were recovered, and apoptosis was measured by three different methods. First, permeabilized cells were stained with propidium iodide (PI; 50 \(\mu\)g/mL; Sigma Chemical Co) and the amount of DNA fragmentation was calculated by flow cytometer analysis, as previously described. \(^{21}\) The results were expressed as the percentage ratio between fragmented and total DNA. Second, Giemsa-stained cytospin preparations were analyzed by light microscopy and the percentage of cells with morphologic features of apoptosis was calculated out of the total number of 200 cells. Third, DNA laddering in purified low molecular weight DNA was measured according to the method of Facchinetti et al. \(^{2}\) Briefly, \(^{10^7}\) cells were resuspended in 5 mL of ice-cold lysing buffer (150 mmol/L NaCl, 50 mmol/L Tris [pH 7.6], 1% Triton X100, 10 mmol/L EDTA) containing 10 \(\mu\)g DNase-free RNase (Boehringer Mannheim Biochemicals, Indianapolis, IN); after 10 minutes on ice, the sample was centrifuged for 5 minutes at 2,000 rpm, and the supernatant containing DNA fragments was recovered. After phenol extraction and ethanol precipitation, the samples were resuspended in 50 \(\mu\)L TE (Tris-EDTA). After 5 minutes at 60°C, the fragments were loaded on a 1.5% agarose gel and run at 7.5 V/cm for 3 hours. DNA laddering was detected by staining with ethidium bromide.

Cell proliferation. Cells were cultured as described above. At the end of culture, cell proliferation was measured by PI staining of the cells and analysis of cell cycle phases by flow cytometry. The percentage of the cells present in each cell cycle phase was calculated by cellfit program. \(^{22}\) Alternatively, 5-Bromo-deoxyuridine (BrdUrd) incorporation in DNA was measured by flow cytometry. \(^{24}\) Briefly, cells were pulsed with BrdUrd (Sigma) during the last 20 minutes of the culture. Subsequently, cells were stained with anti-BrdUrd MoAb (BioCell Consulting, Grellingen, Switzerland) and FITC-conjugated GAM Ig, followed by incubation with PI (1 \(\mu\)g/mL). Two-color fluorescences (FL1, BrdUrd incorporation; FL2, DNA content) were analyzed on FACScan.

RESULTS

CD38 expression distinguishes two groups of B-CLL cells with different [Ca\(^{2+}\)]\(_{i}\), response to IgM cross-linking. The expression of CD38 by the malignant cells of 20 B-CLL patients was investigated by flow cytometry. CD38 was expressed at high density in 6 cases, whereas, in the remaining cases, CD38 was absent (10 cases) or expressed at low levels (4 cases). These results were confirmed by observations with three different anti-CD38 MoAbs. The patients were divided into two groups: the first, group I, included patients with leukemic cells with high CD38 expression; and the other, group II, was composed of cases with low to absent levels of CD38 (Fig 1A). Further analysis failed to disclose differences in surface marker expression between the malignant cells from group I and group II patients. In all cases, more than 95% of the malignant cells expressed CD19, CD20, CD21, CD23, CD39, CD40, and CD5. Although variations existed among the different cases, slgM and slgD were generally expressed in low quantities. All the B-CLL cases expressed Ig-associated protein \(\beta\) chain (CD79b; Fig 1B).

The cells from 5 patients (A, B, C, D, and E) of group I and from 5 patients of group II (F and G with low CD38 expression and H, I, and L with absent CD38 expression) were exposed to Gau-ab (10 \(\mu\)g/mL) and studied for [Ca\(^{2+}\)]\(_{i}\) mobilization. The RFI of slgM stainings did not differ significantly in the two groups (RFI ranges were 3 to 20 and 4 to 24, respectively). In the B-CLL cells from group I, Gau-ab induced a [Ca\(^{2+}\)]\(_{i}\) increase that reached a peak in approximately 60 to 80 seconds. The [Ca\(^{2+}\)]\(_{i}\) level then declined for the next 8 minutes, although it remained higher than the basal level. In the cells from group II, the level of [Ca\(^{2+}\)]\(_{i}\) was unchanged or only marginally increased on exposure to Gau-ab compared with control cells exposed to NGG. [Ca\(^{2+}\)]\(_{i}\) mobilization was not observed over a wide range of Gau-ab concentration (from 10 ng/mL to 100 \(\mu\)g/mL) and not even when the measurements were extended for up 15 minutes (data not shown). Representative profiles of [Ca\(^{2+}\)]\(_{i}\), mobilization (1 from each group of patients) are reported in Fig 2A. Figure 2A also shows the mean values \(\pm\) SD determined in all of the patients studied.

In another set of experiments, the cells from the group I or group II cases studied above were exposed to a rabbit Fab\(_{2}\):antibody against human IgM (10 \(\mu\)g/mL; Fig 2B). The values of [Ca\(^{2+}\)]\(_{i}\), mobilization were virtually identical to those observed with undigested Gau-ab in group I cells. In contrast, no [Ca\(^{2+}\)]\(_{i}\) increase could ever be observed in
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Fig 1. CD38 expression by B-CLL cells. (A) Typical flow cytometry profiles of B-CLL cells stained with anti-CD38 MoAb: group I B-CLL cells are characterized by a high expression and group II B-CLL cells by a low to absent expression of CD38. The ranges of RFI are indicated. (B) Typical phenotypic profile of B-CLL cells analyzed with the indicated MoAbs. This pattern of staining was observed in both group I and group II B-CLL cells. Dotted lines indicate the control fluorescence obtained with an irrelevant MoAb. Group I B-CLL cells were exposed to anti-CD38 MoAb (IB4) for the times indicated in Fig 3B and stimulated with Gaμ-αb. An early and rapid increase of [Ca\(^{2+}\)] was observed after exposure to Gaμ-αb, irrespective of whether the cells were pretreated with anti-CD38 MoAb or an unrelated MoAb (anti-CD39 MoAb). Cross-linking of anti-CD38 (or of control anti-CD39) MoAb with GAM-Ig did not alter the results. These findings indicate that anti-CD38 MoAb did not block or synergize with the [Ca\(^{2+}\)] mobilization induced by Gaμ-αb.

Gaμ-αb induces apoptosis in group I B-CLL cells. Apoptosis is often dependent on a sustained cytosolic calcium increase. Therefore, it was of interest to investigate whether the [Ca\(^{2+}\)] mobilization caused by exposure to Gaμ-αb also resulted in programmed cell death. Purified B-CLL cells were cultured with Gaμ-αb (10 μg/mL) for 24 hours and evaluated for apoptosis by PI staining and flow cytometry. When B-CLL cells were cultured in medium alone (data not shown) or in the presence of N~G, low or absent DNA fragmentation was observed in all of the sample tested (Fig 4A). Exposure to Gaμ-αb resulted in a substantial amount of apoptosis in group I B-CLL cells, whereas apoptosis was low (case L; Fig 4A) or absent (cases F, G, H, and I; Fig 4A) in group II B-CLL cells. The occurrence of apoptosis in Gaμ-αb-stimulated cells from group I B-CLL was confirmed by morphologic studies on Giemsa-stained cytospin preparations (Fig 4B). Condensed and fragmented nuclei were present in group I B-CLL cells, but not in group II B-CLL cells treated in the same manner. Gel electrophoresis disclosed DNA laddering typical of apoptosis in group I B-CLL cells cultured with Gaμ-αb for 24 hours (Fig 4C). DNA fragmentation was barely visible when the same B-CLL cells were cultured in medium alone (Fig 4C). Virtually no DNA fragmentation was detected in group II B-CLL cells irrespective of whether they were exposed to Gaμ-αb (Fig 4C). In this regard, it is of interest that apoptosis of group II B-CLL cells was not
observed with a wide range of doses of Gaα-aβ (from 10 ng/mL to 100 µg/mL). Moreover, exposure to a rabbit F(ab')2 antihuman IgM either in free solution (from 10 ng/mL to 100 µg/mL) or immobilized onto plastic plates failed to induce apoptosis of group II B-CLL cells (data not shown).

The cells from three B-CLL cases from each group were exposed to Gaα-aβ or NGG for different times and DNA fragmentation was examined by PI staining. Gaα-aβ induced 45% ± 15% apoptosis of group I B-CLL cells by 18 to 24 hours. The values observed remained constant for the following period (Fig 5). In the cultures that contained NGG, there was also cell death by apoptosis. However, this was substantially inferior to that observed in the cultures exposed to Gaα-aβ. In group II B-CLL cells, there was a small DNA fragmentation during the first 72 hours, irrespective of whether the cells were exposed to Gaα-aβ or NGG. This fragmentation increased, albeit marginally, in the late culture times both in the presence and in the absence of the stimulus (Fig 5). Notably, spontaneous
Fig 4. Apoptosis of group I B-CLL upon exposure to Gaμ-ab. (A) Cells from 5 group I (A through E) and 5 group II (I through L) B-CLL patients were exposed to Gaμ-ab or NGG for 24 hours, stained with PI, and analyzed for the presence of fragmented DNA by flow cytometry. (B) Morphologic analysis of Giemsa-stained preparations of Gaμ-ab–stimulated cells from 1 group I (top) and from 1 group II (bottom) B-CLL patient (patients B and F, respectively). (C) Agarose gel electrophoresis to detect DNA laddering. DNA laddering is observed only in group I B-CLL cells exposed to Gaμ-ab. Experiments were performed with cells from patients B and F.

Apoptosis was consistently greater in group I than in group II B-CLL cells.

Gaμ-ab–induced apoptosis occurs in cells at the G0-G1 phase of the cell cycle. Cells from group I and group II patients were exposed to Gaμ-ab (10 μg/mL) for different times, stained with PI, and analyzed for the cell cycle by flow cytometry. As shown in Fig 6A, cells both group I and group II patients consistently remained in the G0-G1 phase of the cell cycle after Gaμ-ab stimulation. A substantial proportion of cells in S phase of cell cycle could be observed in control LAM BL cell line studied with the same method.

Cells from group I and group II patients were analyzed for BrdUrd incorporation and DNA content. As shown in
Fig 6B, the cells that incorporated BrdUrd and entered in the S phase of the cell cycle, upon exposure to Gau-αB, were negligible or virtually absent in both group I and group II B-CLL (Fig 6B, box 3). Again, BrdUrd incorporation together with a substantial proportion of cells that progressed through the S-G2/M phases was observed in control LAM BL cell line (Fig 6B, box 3). Notably, apoptosis was evident in the group I and not in the group II cells exposed to Gau-αB (Fig 6B, box 1), although the values recorded were somewhat lower than those obtained in the experiments reported in Fig

Fig 6. Cell cycle analysis and BrdUrd labeling of group I and group II B-CLL cells exposed to Gau-αB. (A) Percentage of cells entering the S phase of cell cycle in group I and group II B-CLL cells. Three cell samples from each group of patients were exposed to Gau-αB and analyzed for cell cycle by PI staining at different intervals. Data are expressed as the mean ± SD. The values observed for the two groups were not different from the control values obtained by culturing the cells with NGG. The LAM BL cell line, used as control, was cultured in the absence of stimuli. (B) BrdUrd incorporation and analysis of DNA content in 2 representative B-CLL cases (1 from group I [patient E] and 1 from group II [patient F]) and LAM BL control cell line. B-CLL cells that had been exposed to NGG or Gau-αB for 18 hours and unstimulated LAM BL cell line were incubated with BrdUrd for 20 minutes and stained with anti-BrdUrd MoAb and PI. Two-color immunofluorescence was analyzed by flow cytometry. FL1 indicates the values of BrdUrd incorporated by the cells and FL2 indicates the cellular DNA content. Box 1 comprises the cells containing less than 2N DNA (apoptotic cells). In box 2, the cells with 2N DNA content and negative for BrdUrd incorporation are gated (cells in G0/G1 phase of cell cycle). Box 3 comprises cells containing >2N DNA and incorporating BrdUrd (S phase). In box 4, there are cells that have completed their cell cycle (4N DNA) and have negative values of BrdUrd incorporation (G2/M phase). The unmarked box below box 3 contains the cells undergoing apoptosis after BrdUrd incorporation. These cells are virtually absent in all of the experiments. Identical results were obtained exposing the cells to Gau-αB at 2, 8, and 36 hours.
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4A due to the different sensitivity of the methods used. The cells undergoing apoptosis did not incorporate BrdUrd, as shown by the absence of BrdUrd-stained cells in the unmarked box below box 3 (in Fig 6B). This is consistent with the concept that group I B-CLL cells underwent apoptosis without entering into the cell cycle.

rIL-4 but not anti-CD38 MoAb protects group I B-CLL cells from apoptosis. Previously, it was shown that anti-CD38 MoAb (IB4) could inhibit the spontaneous apoptosis of normal germinal center (GC) B cells in vitro. Therefore, it was of interest to determine whether anti-CD38 MoAb could inhibit apoptosis of group I B-CLL cells. Three different samples of group I B-CLL cells were incubated with Gaμ-α (10 μg/mL) in the presence or in the absence of an anti-CD38 MoAb (IB4; 1 μg/mL). The cells were also exposed to Gaμ-α and rIL-4 or anti-CD40 MoAb, which are known to prevent B-cell apoptosis in various experimental conditions. The cells were cultured for 36 hours and examined for DNA fragmentation by PI staining. As shown in Fig 7, anti-CD38 MoAb did not prevent Gaμ-α--induced apoptosis, even when its concentration was increased 10-fold (data not shown). rIL-4 substantially reduced the Gaμ-α--induced apoptosis, whereas anti-CD40 MoAb failed to prevent apoptosis.

Normal peripheral blood B cells express low levels of CD38 and do not undergo apoptosis upon exposure to Gaμ-α. Next, we investigated the expression of CD38 by normal PB B cells. These PB B cells expressed low levels of CD38, thus resembling group II B-CLL cells (Fig 8A). They also expressed the same markers as both group I and group II B-CLL cells. CD5 was detected on some but not all of the cells. When PB B cells were exposed to anti-μ MoAb, a significant [Ca^{2+}] mobilization was observed and was substantially increased by the addition of GAM-Ig (Fig 8B). The same values of [Ca^{2+}], mobilization were observed when PB B cells were stimulated with Gaμ-α (data not shown). Moreover, exposure to Gaμ-α for 18 hours failed to induce apoptosis of PB B cells (Fig 8C); instead, the cells were induced into the cell cycle, as shown by the appropriate studies on cells that were incubated with Gaμ-α for 18, 36, and 48 hours. Figure 8D shows a typical experiment performed on PB B cells incubated with Gaμ-α for 18 hours.

DISCUSSION

The present study identified two discrete groups of B-CLL cells. The first group (group I) expressed CD38, was apoptosis-prone, and was characterized by a rapid [Ca^{2+}] mobilization after cross-linking of slg. The second group (group II) included cells expressing low to nil CD38 that failed to respond to stimulation with anti-IgM antibodies and were apoptosis-resistant. These observations raise a number of issues regarding the role of CD38 in B-cell activation, calcium mobilization, and apoptosis. In addition, they might provide new tools for the possible subclassification of B-CLL cells.

The present study shows a correlation between the process of apoptosis and CD38 expression by B-CLL cells. CD38+, but not CD38− B-CLL cells underwent apoptosis upon exposure to Gaμ-α. Moreover, spontaneous apoptosis was observed in a substantial proportion of group I cells when kept in culture without stimuli (Fig 5). In these conditions, group II cells displayed little, if any, apoptosis. Finally, Gaμ-α failed to induce apoptosis of normal B cells that expressed low levels of CD38. Previous studies showed that B cells that are prone to apoptosis express CD38. Thus, the cells of certain Burkitt’s lymphoma cell lines express abundant CD38 and undergo apoptosis in vitro after the removal of FCS from the culture media or exposure to Gaμ-α. In contrast, the cells from Burkitt’s lymphoma cell lines that do not express CD38 are resistant to apoptosis. Moreover, when transfected with c-myc carrying vectors in vitro, B cells of normal lymphoblastoid cell lines that are normally apoptosis-resistant acquire both the propensity to apoptosis and expression of CD38. Finally, GC B cells that undergo apoptosis spontaneously in vitro express abundant surface CD38. Whether CD38 has a physiologic role in the process of apoptosis remains to be established.

Expression of CD38 and the propensity to apoptosis suggest similarities between GC B cells and group I B-CLL cells. Because of this, it is tempting to speculate that group I B-CLL cells are in the process of differentiating into GC B cells and thus represent a more mature stage of B cells than group II B-CLL cells. This hypothesis would also be supported by the observation that normal B cells, whereas maturing into GC B cells acquire the capacity to express CD38. However, there are several differences between GC B cells and group I B-CLL cells. For example, apoptosis of group I B-CLL cells was prevented by rIL-4 and not by exposure to anti-CD40 or CD38 MoAb. Instead, these agents were found to be very efficient at inhibiting apoptosis of GC B cells. Finally, GC B cells are actively cycling cells and may conceivably enter the apoptotic pathway after the commitment point in late GIphase; in group I B-CLL cells, programmed cell death occurs before this stage (Fig 6B). Apoptosis can be initiated at several phases of the cell cycle. Taken together, the considerations mentioned above suggest that group I B-CLL may be committed to entering the GC B-cell differentiation pathway, but may have lesions...
that prevent their complete maturation. Alternatively, group I B-CLL cells may represent a special maturation stage of CD5+ B cells, a subset of cells that is not unanimously thought capable of entering into the germinal center.46

Most of the findings described here suggest that the CD38 expressed by group I B-CLL cells was not directly involved in the process of signal transduction. First, anti-CD38 MoAb failed to cause [Ca²⁺], mobilization in B-CLL cells. Second, anti-CD38 MoAb did not appear to enhance or block the stimulating signals delivered through surface Ig. Third, anti-CD38 MoAb did not induce apoptosis of B-CLL cells. Finally, anti-μ MoAbs were found to be capable of stimulating normal recirculating B cells that expressed low to absent levels of CD38. These observations were somewhat surprising, especially in light of previous data concerning the CD38 molecule.37 In humans, anti-CD38 MoAbs prevent spontaneous apoptosis of GC B cells32 and block lymphopoiesis,46 although they do not appear to be capable of inducing proliferation of normal B cells or enhancing the proliferation promoted by exposure to Gaμ-αb (our unpublished data). However, in the mouse, agonistic anti-CD38 MoAbs induce [Ca²⁺], mobilization in B cells and stimulate their proliferation.49,50 CD38 was shown to have a bifunctional enzymatic activity and to regulate the synthesis of cADP ribose,51-53 which in turn upregulates the ryanodine receptor-mediated [Ca²⁺], mobilization.49-51 Based on these observations, it has been suggested that CD38 may participate in cell activation through this particular pathway.46 At present, it is difficult to provide explanations for the different effects of anti-CD38 MoAb on the various human B-cell subsets. Moreover, the situation becomes even more complicated when the data in humans and mice are compared, although several different hypotheses have been proposed.46

The inability of group II B-CLL cells to mobilize [Ca²⁺], upon cross-linking of surface IgM raises the possibility that their signal transduction pathway is blocked. This block does not appear to be related to faulty expression of signal transducing molecules associated to Ig such as CD19, CD20, CD21, and CD79b (Fig 1B). Nevertheless, it may involve the early steps of the signal transducing pathways because neither [Ca²⁺], mobilization nor tyrosine phosphorylation (data not shown) induced by exposure to Gaμ-αb seems to be operative. Blocks at various levels of the signal transducing pathway have previously been reported in B-CLL cells,34,49 although it is not clear whether they represent specific lesions of the leukemic cells or a physiologic feature of normal cells at the same maturation stage as B-CLL cells. In connection with this, it is of note that several studies in experimental animals have documented blocks in the signal transduction pathway of normal B cells at relatively early maturation stages.45-49

A final issue to be discussed is the relationship between group I and group II B-CLL. They may be two subtypes of B-CLL or represent different maturation stages of B-CLL cells that can convert from one type into the other. Support to the latter hypothesis is provided by some preliminary observations. For example, when CD38 expression by 20 untreated B-CLL patients was compared with that of 20 patients under treatment with steroids and alkylating agents, it was found that, although the proportion of cases expressing CD38 in the untreated group was similar to that observed in this study (30%), none of the treated cases expressed CD38. Moreover, a longitudinal study in 1 patient with high CD38 expression before and after therapy with chlorambucil and steroids recorded a decrease in CD38 expression after the beginning of therapy. Finally, we have recently observed
that group II B-CLL cells become CD38+ and prone to apoptosis on prolonged culture in vitro (at least 4 days; data not shown). Although not formally proven, these data may suggest that therapy could eliminate more mature (CD38+) B cells preferentially and that CD38- cells may mature into CD38+ cells upon culture in vitro.

Previous studies showed that B-CLL cases can be subdivided into subgroups depending on the capacity of the malignant cells to express CD11b. Although we have confirmed such heterogeneity for CD11b expression, no correlation was found with that of CD38 in the single cases. Similar studies are currently in progress for CD11c and APO-1 (CD95) expression.

The identification of different subsets of B-CLL characterized by different phenotypic and functional features may offer new possibilities in the clinical approach to the disease, leading to more specialized treatment of patients with different clinical courses and/or response to therapy. If therapy eliminates preferentially group I (thus converting group I cases into group II cases), then the question arises of whether this conversion may represent a progression step into a more aggressive form of the disease. Apoptosis induced by physiologic signals or even by chemotherapeutic treatments may be poorly operative or not operative in group II cells, thus facilitating the survival of the malignant clone.

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