Interleukin 4 Protects Chronic Lymphocytic Leukemic B Cells from Death by Apoptosis and Upregulates Bcl-2 Expression

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Summary

B chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of slow-dividing and long-lived monoclonal B cells arrested at the intermediate stage of their differentiation. We previously showed that interleukin 4 (IL-4) not only inhibits but also prevents the proliferation of B-CLL cells. We report here that IL-4 protects the B-CLL cells from death by apoptosis (programmed cell death [PCD]). IL-4 inhibits spontaneous and hydrocortisone (HC)-induced PCD of highly purified B cells from 12 unselected CLL patients, as shown by sustained cell viability and lack of DNA fragmentation. IL-1, -2, -3, -5, -6, -7, tumor necrosis factor α, and transforming growth factor β have no protective effect. The in vitro rescue from apoptosis by IL-4 is reflected by an increased expression of Bcl-2 protein, a proto-oncogene directly involved in the prolongation of cell survival in vivo and in vitro. Hence, IL-4-treated B-CLL cells express significantly more Bcl-2 than unstimulated, HC-treated, or fresh B-CLL cells. Furthermore, subcutaneous injection of IL-4 into one CLL patient enhances Bcl-2 protein expression in the leukemic B cells. These data may suggest that IL-4 prevents apoptosis of B-CLL cells using a Bcl-2-dependent pathway. Given our recent observations that fresh T cells from B-CLL patients express IL-4 mRNA, we propose that IL-4 has an essential role in the pathogenesis of CLL disease, by preventing both the death and the proliferation of the malignant B cells.

Programmed cell death (PCD;[1] or apoptosis) is a common form of cell deletion that can be activated in different cell types in response to a number of physiologically relevant stimuli. This process is usually accompanied by the cleavage of host chromatin into nucleosome-size fragments of ~200 bp appearing as a typical ladder pattern (1). To date, a specific endonuclease or internucleosomal activity has not been purified. Triggering of T cells within the thymus with anti-CD3 antibodies (2), superantigens (3), or specific peptides (4), or exposure of the thymocytes to glucocorticoids (5), leads to apoptosis. In contrast, in the germinal centers of lymph nodes, the selection of B lymphocytes that produce high-affinity antibodies is achieved by prevention from apoptosis for those cells that receive a positive signal after antigen recognition by their Ig receptors (6). Similarly, factor-dependent cell lines undergo apoptosis after disappearance of the stimulus, such as removal of growth factors from hematopoietic precursors (7).

Bcl-2 is a unique proto-oncogene that plays an important role in cell survival. It was first isolated from the breakpoint of the translocation between chromosomes 14 and 18 frequently found in the neoplastic cells of patients with indolent follicular B cell lymphoma (8). In normal tissues, Bcl-2 expression is topographically confined to the zones of surviving B cells in germinal centers or surviving mature thymocytes in the medulla (9). Infection with Bcl-2 retrovirus prevents PCD occurring in some pro-B cell lines upon IL-3 deprivation (10). Gene transfer into several cell types has confirmed that Bcl-2 acts by inhibiting cell loss rather than by stimulating cell proliferation (11).

Bcl-2 cooperates with c-*myc* to promote proliferation of B cell precursor (10), and doubly transgenic mice ($\epsilon\mu$-Bcl-2/*myc*) develop tumors much faster than $\epsilon\mu$-*myc* mice, suggesting that prolonged B cell life may increase tumor incidence (12). A similar synergy between Bcl-2 and c-*myc* has been extended to T cells in which combined transfer of the two genes enhances tumorogenicity of human T lymphoid cell line into athymic mice (13).

It was recently demonstrated that Bcl-2 enhances T cell survival using Bcl-2 transgenic mice expressing the transgene in the T cell lineage (14, 15). Thymocytes of Bcl-2 transgenic strains not only exhibit an increased survival when

[1] *Abbreviations used in this paper:* HC, hydrocortisone; PCD, programmed cell death.

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cultured in the absence of growth factors but also resist multiple forms of apoptosis, including treatment with glucocorticoids, anti-CD3, and γ radiation. Interestingly, clonal deletion of T cells that recognize endogenous superantigens is still occurring, suggesting the existence of a Bcl-2-independent pathway.

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of monoclonal long-lived B-CLL cells arrested at the intermediate stage of their differentiation (16). B-CLL cells enter into apoptosis either spontaneously when cultured in vitro (17) or after incubation with glucocorticoids, Ca^{2+} ionophore, and anti-Ig antibodies (18). We previously reported that glucocorticoid hormone (hydrocortisone [HC]) is absolutely required with IL-4 to induce the differentiation of unfractionated CLL cells into IgE-producing cells (19). We therefore examined whether IL-4 would prevent HC-induced PCD in these leukemic cells. Here we report that IL-4 inhibits spontaneous and HC-induced apoptosis in highly purified B cells from unselected CLL patients and upregulates their Bcl-2 expression.

Materials and Methods

CLL Samples. 13 CLL patients were examined in the present study. Their age range was from 39 to 70 yr. Their peripheral blood contained 40–90 $\times$ 10^9 leukocytes/mm^3, of which 60–90% were lymphocytes. Flow cytometry analysis indicated that their B-CLL cells coexpressed CD20, CD5, and CD23; nine patients were IgM λ and four patients IgM κ. Patients 1–7 were at clinical stage Rai 0 (n = 2), Rai I (n = 1), and Rai III (n = 4), and patients 8–12 were at Rai 0 (n = 2) and Rai III (n = 3). 10 patients were not receiving therapy at the time of the study. Patients 7 and 10 were administered oral prednisone for a consecutive 5 d before the study. Patient 13 (Rai IV) belonged to the preclinical study initiated to assess the antiproliferative effect of IL-4 in CLL disease (20). He received subcutaneous injections of rIL-4 (100μg/m^2) for a consecutive 5 d with no significant side effects.

Cell Separation and Culture Conditions. PBMC from CLL patients (>90% CD5 and CD20 positive) were isolated with Lympho Prep (Cederlane Laboratories, Ontario, Canada). Highly purified B cells (no detectable CD3- or CD14-positive cells) were negatively selected by a combination of rosetting (twice) with aminoethylisothiouronium bromide–treated SRBC, adherence to plastic, and treatment with L-leucine ester. Highly purified B-CLL cells were incubated at 2.5 $\times$ 10^6/ml (48-well flat-bottomed plate) in HB101 serum-free medium for a culture period time of 24–48 h. HC (Sigma Chemical Co., St. Louis, MO), was used at 10^{-5} M or 5 $\times$ 10^{-4} M according to dose-response curves (Fig. 1) and previously reported data (18). Recombinant IL-4 was obtained from either Genzyme Corp. (Boston, MA) or from Dr. H. Hofstetter (CIBA-GEIGY, Basel, Switzerland) and was used at 10 ng/ml final concentration. Recombinant IL-1, -2, -3, and -6 were purchased from Genzyme Corp. IL-5 and TNF-α were from Dr. W. Fiers (State University, Ghent, Belgium), IL-7 was obtained from Immunex Corp. (Seattle, WA), and TGF-β2 was from Dr. D. Cox (CIBA-GEIGY, Basel). Recombinant soluble CD23 (sCD23) was prepared in our laboratory. Cell viability was determined by trypan blue dye exclusion or by staining the cells with propidium iodide (2 μg/ml) and analysis by flow cytometry (FACscan®; Becton Dickinson & Co., Mountain View, CA).

DNA Fragmentation Assay. Cells (2.5 $\times$ 10^6/ml) were washed twice with PBS and pelleted by centrifugation at 200 g for 5 min at room temperature. DNA was isolated using a slightly modified procedure as described (21). Briefly, cell pellets (2.5 $\times$ 10^6) were resuspended in cell lysis buffer (10 mM EDTA, 50 mM Tris [pH 8], containing 0.5% [wt/vol] N-laurylsarcosine and 0.5 μg/ml proteinase K) and incubated for 1 h in a 50°C water bath. RNase A was then added to a concentration of 0.25 mg/ml and incubation was prolonged for 1 h at 50°C. The DNA preparations were then extracted (twice) with buffered phenol (Sigma Chemical Co.), followed by two chlorophorm/isoamyl alcohol (24:1) extractions for removal of protein and residual traces of phenol. DNA preparations were then brought to 1.5 vol by the addition of 10 mM Tris-HCL, pH 8, 1 mM EDTA (TE buffer), and were centrifuged at 14,000 g to separate intact from fragmented chromatin. The supernatants, containing fragmented DNA, were placed in separate tubes and DNA was precipitated with 0.5-vol ammonium acetate (2 M) and 2 vol of ethanol at −70°C for 24 h. The DNA precipitates were recovered by centrifugation at 13,000 g for 15 min, air dried for 1 h at room temperature, and resuspended in TE buffer. Samples were supplemented with loading buffer (0.25% bromophenol blue [wt/vol], 50% glycerol [wt/vol], and 10 mM EDTA) at a 5:1 ratio and then heated at 65°C for 10 min. Electrophoresis was carried out in 1% gel at 6 V/cm of gel using TBE buffer (2 mM EDTA [pH 8], 89 mM Tris, 89 mM boric acid). A $BgI1^+$ and Hinf1 digest of pBR328 DNA (Boehringer Mannheim Biochemicals, Indianapolis, IN) was applied to each gel to provide size markers. After electrophoresis, DNA was visualized by soaking the gel for 30 min in TBE containing 1 μg/ml ethidium bromide and destained for 30 min in TBE buffer.

Western Blot Analysis of Bcl-2 Protein Expression. Western blots were performed as described (22). Briefly, cell lysates were obtained by sonicating cells for 20 s and boiling for 5 min in 50 mM Tris buffer, pH 6.8 with 2% SDS, 2% 2-ME, 10% glycerol, and 0.1% bromophenol blue. Samples containing lysates of 4, 2, or 1 $\times$ 10^6 cells in 20 μl were separated by discontinuous electrophoresis on a 10% acrylamide gel and blotted onto nitrocellulose filter. Blotted filters were blocked for 2 h using a 5% suspension of dried skimmed milk in Tris buffer, pH 7.5 (milk TBS). The filter was then incubated at 4°C with anti-Bcl-2 mAb (10% supernatant) (kindly given to us by Dr. D. Y. Mason, Headington, Oxford) in 5% milk TBS. After overnight incubation, the filter was washed and further incubated for 1 h at 20°C with 1:5,000 rabbit anti–mouse Ig (Dako Corp., Santa Barbara, CA) in 5% milk TBS, washed, and incubated for 1 h with ^{125}I-labeled protein A (Amersham Corp., Arlington Heights, IL) diluted at 0.1 μCi/ml in 5% milk TBS. After further washing, the filter was exposed from 4 to 48 h. Quantitative analysis was performed by either cutting the bands from the filter and counting in a gamma counter (in cpm) or by scanning densitometry (GS300; Hoefer Scientific, San Francisco, CA) (relative intensity units). Protein molecular weight standards (Rainbow TM; Amersham Corp.) were run with each gel.

Electron Microscopy. For the morphological studies, the cells were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer, post-fixed with 1% osmium tetroxide, and embedded in Epon according to routine techniques. For electron microscopy, the thin sections were mounted on nickel grids and examined with an electron microscope (410; Philips Electronics Instruments, Inc., Piscataway, NJ) staining with uranyl acetate and lead citrate.

Results

IL-4 Rescues B-CLL Cells from Their Entry into Apoptosis. We first show that HC significantly decreases cell via-

Table 1. *IL-4 Prevents HC-induced Apoptosis of B-CLL Cells*

Patient	Percent viability from:			
	Medium	IL-4	HC	IL-4 + HC
1	96.3	98.5	57.2	95.9
2	93.6	97.3	51.6	90.5
3	95.6	98.3	67.7	92.4
4	83.0	86.0	41.0	81.4
5	94.4	97.2	40.4	96.7
6	77.5	ND	57.0	76.5
7	84.0	82.8	63.0	81.0

B-CLL cells were cultured (2.5×10^6/ml) in the absence or presence of IL-4 (10 ng/ml) supplemented or not with HC (10^{-5} M). After 24 h (patients 1–3) or 48 h (patients 4–7), cell viability (%) was determined by trypan blue dye exclusion.

bility of highly purified B cells isolated from unselected CLL patients ($n = 7$), as determined by trypan blue dye exclusion, and that this effect is completely prevented by IL-4 (Table 1). Maximal HC-induced cell death is obtained after a 2-d culture (Fig. 1 *A*), and IL-4 exerts its protective effect from day 1 to 3. This protective effect may be prolonged until day 5 (not shown). The concentrations of HC (5×10^{-4} M) and IL-4 (10 ng/ml) used throughout the study have been selected on the basis of dose-response curves (Fig. 1, *B* and *C*) and of our previous report indicating that B-CLL cells differentiate into IgE-secreting cells under these conditions (19). Morphological examination of the HC-treated B-CLL cells at the electron microscope (Fig. 2) demonstrates the occurrence of apoptotic cells at different stages. Also, the typical "ladder" DNA degradation observed during the process of apoptosis upon exposure to HC totally disappears in the presence of IL-4 (Fig. 3), indicating that IL-4 rescues B-CLL cells from apoptotic death. B-CLL cells are next stained with propidium iodide to quantitate cell death. As shown in Fig. 4, brightly stained cells (FL3 positive) are dead cells, while cells with reduced forward scatter likely represent those entering apoptosis that have not started to take up trypan blue. Our results clearly show that IL-4 treated cultures (in the presence or absence of HC) contain virtually no dead cells and a low proportion of cells with reduced forward scatter, further demonstrating that IL-4 completely abrogates spontaneous or HC-induced PCD. Hence, some B-CLL clones may undergo spontaneous PCD after in vitro culture (17). IL-4 sustains cell viability of these particular B-CLL cells ($n = 5$) (Table 2, and Figs. 1 and 4) without inducing cell division and proliferation (M. Sarfati, personal observations). IL-4 prevents the spontaneous DNA fragmentation occurring in these B-CLL clones (not shown). We next show that IL-1, -2, -3, -5, -6, -7, TNF-α, and TGF-β have no protective effect on HC-induced PCD in B-CLL cells (Fig. 5). In contrast to normal germinal center B cells (23), CD23 in the presence or absence of IL-1α does not significantly rescue B-CLL cells from apoptosis. Finally, the antiapoptotic effect of IL-4 is abolished by neutralizing anti-IL-4 mAb (not shown).

IL-4 Upregulates Bcl-2 Protein Expression in B-CLL Cells. B-CLL cells from the great majority of patients express Bcl-2

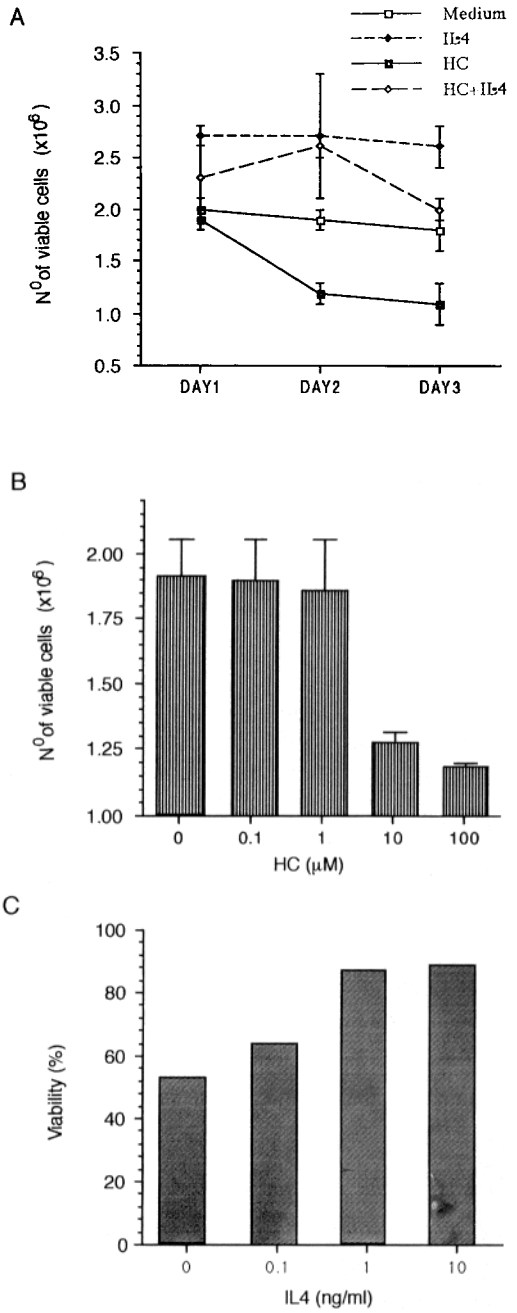

Figure 1. Kinetic studies of HC-induced cell death in B-CLL cells (*A*) and dose-related effects of HC (*B*) and IL-4 (*C*). B-CLL cells from patient 11 were cultured (2.5×10^6/ml) for 1, 2, or 3 d in the absence or presence of HC (5×10^{-4} M) with or without IL-4 (10 ng/ml) (*A*); in the absence or presence of various concentrations of HC for 2 d (*B*); with HC (5×10^{-4} M) for 2 d in the absence or presence of various concentrations of IL-4 (*C*). At the end of the culture, the number of viable cells was counted in duplicate using trypan blue dye exclusion method. Shown is one representative patient out of two.

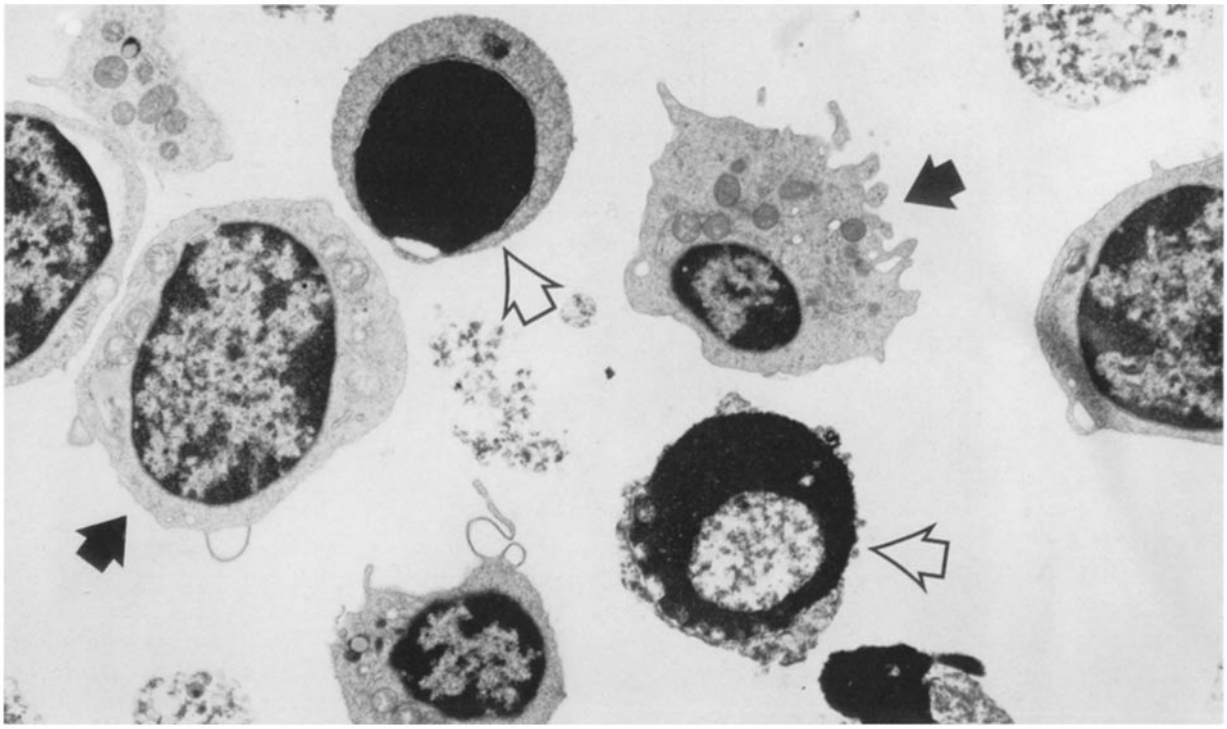

Figure 2. Electron microscopy of apoptic cells in HC-treated B-CLL cell cultures. B-CLL cells (2.5×10^6/ml) from patient 5 were cultured with HC (5×10^{-4} M) for 2 d. Morphological view of the cells at electron microscope (×15,000) demonstrating the presence of normal cells (*filled arrows*) together with cells at different stages of apoptosis (*open arrows*).

(24, 25), a proto-oncogene encoding an inner mitochondrial membrane protein directly involved in the prevention of apoptotic cell death (26). As shown in Fig. 6, Bcl-2 expression drastically decreases in B-CLL cells incubated with HC but not in IL-4- and HC-treated cultures. Also, IL-4-treated B-CLL cells express more Bcl-2 than unstimulated B-CLL cells, suggesting that the rescue from apoptosis by IL-4 is reflected by a sustained Bcl-2 expression.

We next examine whether IL-4 is capable of upregulating Bcl-2 protein in the leukemic cells by comparing Bcl-2 expression of freshly isolated B-CLL cells with that of B-CLL cells incubated for 24 h in the presence of IL-4. Quantitative analysis indicates that IL-4 significantly upregulates the level of Bcl-2 protein expression in B-CLL cells when gels are loaded with lysates of 1, 2, and 4×10^6 B-CLL cells (Fig. 7) (mean ± SEM for two CLL patients: 11,270 ± 3,416 cpm [fresh B-CLLs]; 14,208 ± 3,849 cpm [IL-4-treated B-CLLs]; $p = 0.003$, paired Student's t test). Finally, and most interestingly, subcutaneous administration of IL-4 (100 $\mu g/m^2$) into one B-CLL patient (Rai IV) augments Bcl-2 protein expression

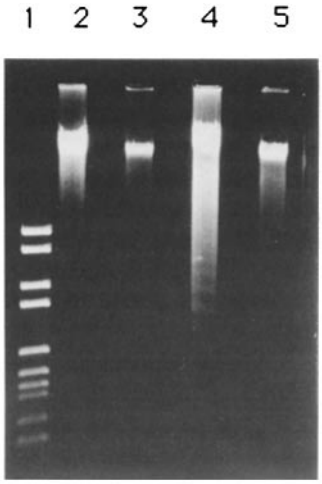

Figure 3. IL-4 inhibits HC-induced DNA fragmentation in B-CLL cells. B-CLL cells from patient 5 were cultured for 24 h. (Lane *1*) Molecular size markers; (lane *2*) unstimulated cultures; (lane *3*) IL-4-treated cultures; (lane *4*) HC-treated cultures; and (lane *5*) IL-4 and HC-treated cultures. Shown is one representative patient out of three.

Table 2. *IL-4 Prevents Spontaneous Apoptosis of B-CLL Cells*

	Percent viability	
Patient	Medium	IL-4
8	69.9	95.3
9	59.0	82.0
10	33.7 (0.8)	89.7 (2.6)
11	74.3 (1.7)	98.6 (2.5)
12	77.0 (1.3)	97.0 (2.1)

B-CLL cells were cultured (2.5×10^6/ml) in the absence or presence of IL-4 (10 ng/ml). After 48 h, cell viability (%) was determined by trypan blue dye exclusion. Number of viable cells ($\times 10^6$) is shown in parenthesis.

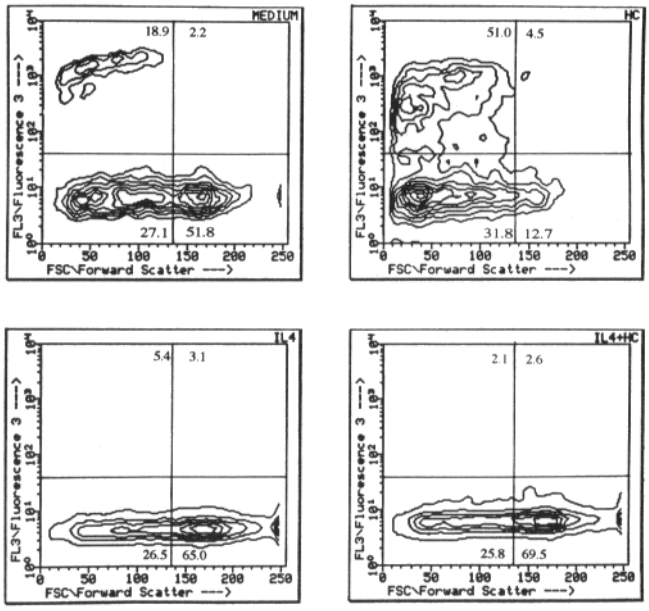

Figure 4. Flow cytometry analysis of HC-induced apoptosis in B-CLL cells and of IL-4 protective effect. B-CLL cells (2.5×10^6/ml) from patient 11 were cultured in the absence or presence of HC (5×10^{-4} M) with or without IL-4 (10 ng/ml). After 2 d, cells were stained with propidium iodide (2 μg/ml) to show dead cells (FL3 fluorescence). Shown is one representative experiment out of five.

in their B cells, strongly supporting the view that IL-4 is indeed capable of upregulating Bcl-2 expression in the malignant B cells (Fig. 8). Of note, the efficacy of IL-4 administration is substantiated by a parallel increase in surface CD23 expression (from 23 to 98% CD23-positive cells) in the same leukemic B cells.

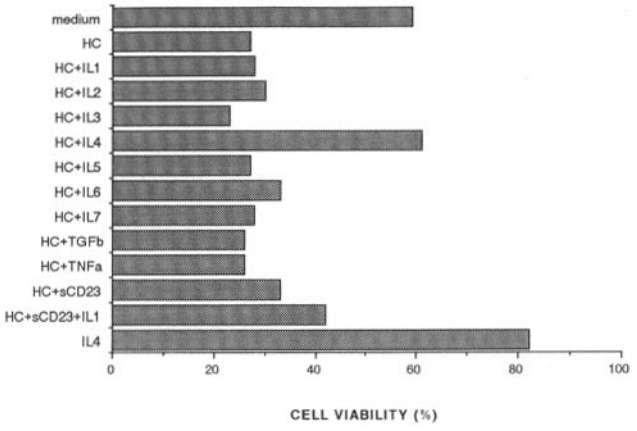

Figure 5. The rescue of B-CLL cells from apoptic death is restricted to IL-4. B-CLL cells from patient 9 were cultured for 48 h in the absence or presence of HC (5×10^{-4} M) with or without IL-1α (1 ng/ml), IL-2 (20 U/ml), IL-3 (10 ng/ml), IL-4 (10 ng/ml), IL-5 (1/500), IL-6 (500 U/ml), IL-7 (1,000 U/ml), TGF-β (2 ng/ml), TNF-α (25 ng/ml), sCD23 (25 ng/ml), or sCD23 and IL-1α. B-CLL cells were also incubated with IL-4 in the absence of HC. Shown is one representative patient out of two.

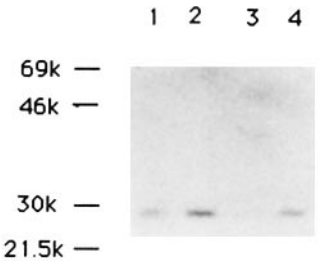

Figure 6. IL-4 sustains Bcl-2 expression in B-CLL cells. B-CLL cells from patient 4 were cultured (2.5×10^6/ml) in medium (lane *1*) supplemented with IL-4 (lane *2*), HC (10^{-5} M) lane *3*), or IL-4 and HC (lane *4*). After 24 h, equal amounts of cell lysates (2×10^6 cells) were separated on a 10% SDS-PAGE gel. Immunoblot for the detection of Bcl-2 protein (25 K) followed by autoradiography (48-h exposure) was performed as described in Materials and Methods. Shown is one representative patient out of three.

Discussion

IL-4 is a T cell–derived multifunctional cytokine, originally described as B cell stimulatory factor (BSF-1), for normal preactivated human and mouse B cells (27). We and others recently reported that IL-4 is a very potent antiproliferative agent for B-CLL cells by showing that IL-4 not only blocked but also prevented the proliferation of the leukemic B cells to various stimuli (i.e., IL-2, IFN-α, TNF-α, and low molecular weight B cell growth factor) (28, 29). In this report, we present a novel function for IL-4 in CLL, in that IL-4 protects B-CLL cells from programmed cell death. IL-4 prevents both glucocorticoid-induced and spontaneous apoptosis in B-CLL cells, as shown by sustained cell viability (trypan blue exclusion and flow cytometry) and absence of DNA fragmentation in B-CLL cells cultured in the presence of IL-4. The high degree of variability in the amount of spontaneous or HC-induced cell death observed in different CLL samples is in keeping with previous reports (17, 18) and is not correlated with the phenotype of the B-CLL clones (not shown). The effect of IL-4 is specific as it is overcome by anti-IL-4-

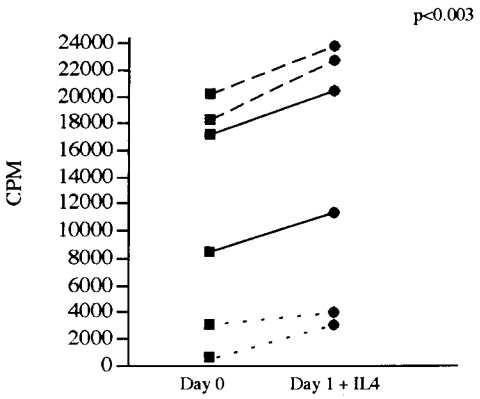

Figure 7. IL-4 upregulates Bcl-2 protein in B-CLL cells in vitro. Immunoblot for the detection of Bcl-2 protein was carried out with B-CLL cells from patients 1 and 2. Cell lysates of 1×10^6 (· · · · ·), 2×10^6 (——), and 4×10^6 (- - - -) cells were prepared from freshly isolated B-CLL cells (day 0) or B-CLL cells incubated with IL-4 (10 ng/ml) for 24 h (day 1 + IL-4), and separated on a 10% SDS-PAGE. After immunoblotting and hybridization, bands were cut and counted in a gamma counter.

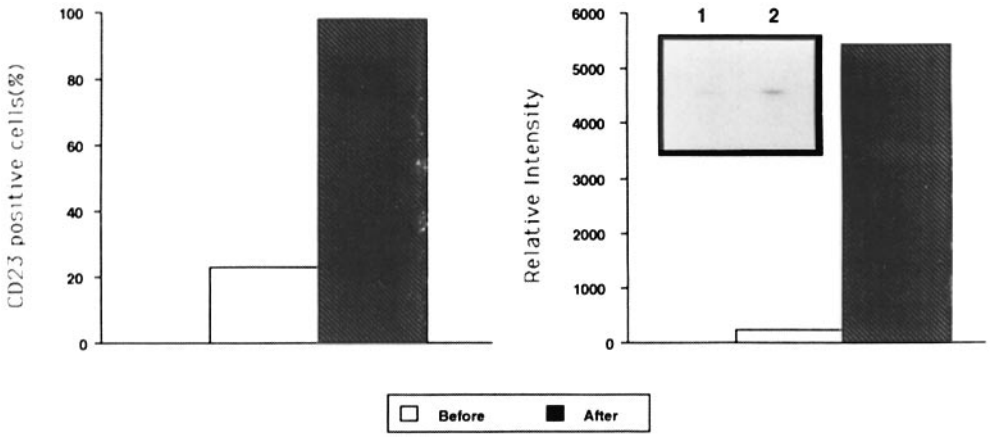

Figure 8. IL-4 upregulates Bcl-2 protein in B-CLL cells in vivo. B-CLL cells were isolated from patient 13, before and 5 d after subcutaneous administration of IL-4. B-CLL cells were stained with anti-CD23-PE mAb (Becton Dickinson & Co.) for CD23 expression (*left*). Detection of Bcl-2 protein by immunoblotting and hybridization was carried out with 2×10^6 cell lysates (lane *1*, before treatment; lane *2*, 5 d after treatment). Autoradiograph was scanned by densitometry, and results are expressed as units of relative intensity (*right*).

neutralizing mAb (not detailed). Moreover, IL-1, -2, -3, -5, -6, -7, TNF-α, and TGF-β have no protective effect.

The ability of IL-4 to rescue B-CLL cells from apoptosis contrasts with its lack of protective effect on spontaneous PCD of germinal center B cells (6), on ionomycin-induced apoptosis of tonsillar B cells (30), or on apoptosis of group I Burkitt lymphoma cells (31). It is relevant to note that IL-4 also inhibits ionomycin-induced PCD in B-CLL cells as well as in umbilical cord blood B cells (M. Sarfati, personal observations), strongly suggesting that the protective effect of IL-4 is more related to the biology of the B-CLL cell or its normal cellular counterpart rather than to the pathways used to induce its apoptosis. In opposition to B-CLL cells, IL-2 blocks ionomycin-induced apoptosis in normal B cells (30). Similarly, CD23 in combination with IL-1α rescue germinal center B cells (centrocytes) from their entry into apoptosis (23), whereas they have only a slight effect on B-CLL cells, suggesting that IL-4 does not mediate its protective effect via the upregulation of CD23. Supporting this view, anti-CD23-neutralizing mAb does not inhibit the IL-4 rescue pathway (M. Sarfati, personal observations).

Occupancy of the surface Ig receptor for antigen protects centrocytes from PCD (6), whereas it induces PCD in normal tonsillar B cells, B-CLL cells (18), and in B lymphoma cell lines (32), leading to the notion that the ability to induce apoptotic cell death may depend on the differentiation stage of the B cell. Signaling through CD40 antigen also prevents cell death in both centrocytes and Burkitt lymphoma cell lines (6, 23). However, anti-CD40 has no protective effect in B-CLL cells (not shown). So far, the only common agent described, capable of preventing PCD in both normal and leukemic CLL B cells, is PMA, an activator of protein kinase C (PKC) (18, 30). These observations suggest that activation of PKC is a common pathway to desensitize the cells to PCD. Hence, PMA also prevents apoptotic cell death in human thymocytes incubated with anti-CD3 antibodies (33). Finally, activation of EBV-latent genes protects human B cells from apoptotic death (31). This protective effect has been recently attributed to the latent membrane protein (LMP 1) (34). Most interestingly, LMP 1 prevents PCD by directly inducing Bcl-2 protein.

Bcl-2 is unique among proto-oncogenes, being localized in the inner mitochondrial membrane and extending cell survival by inhibiting programmed cell death (26). The Bcl-2 gene was first discovered at the breakpoint of the translocation between chromosomes 14 and 18 (t [14;18]) found in most follicular B cell lymphomas and some diffuse large cell lymphomas (8). It was also reported that the rearrangement of the Bcl-2 gene occurs in a significant fraction (10%) of B-CLLs, with a preferential linkage to the Ig light chain gene (35). However, recent reports indicate that most follicular and diffuse lymphomas, as well as all CLL cases lacking Bcl-2 rearrangement, display intense to intermediate Bcl-2 staining (24, 25), suggesting that mechanisms other than classic translocation may be deregulating Bcl-2 expression.

Our present study shows that the protective effect of IL-4 is associated with an enhanced Bcl-2 expression in the leukemic B cells. Of note, activation of centrocytes by signals that prevent their entry into apoptosis (i.e., anti-CD40 mAb, or sCD23 and IL-1) also induces Bcl-2 expression (22). Our data indicate that various levels of Bcl-2 expression are observed in fresh B cells from all CLL cases examined. Nevertheless, IL-4-treated B-CLL cells express significantly more Bcl-2 protein than freshly isolated, unstimulated, or HC-treated B-CLL cells, indicating that IL-4 upregulates Bcl-2 in vitro. Interestingly enough, subcutaneous injection of IL-4 into one B-CLL patient (Rai IV) upregulates Bcl-2 expression in the leukemic B cells. Recent studies have shown that glucocorticoid-induced apoptosis in the thymus is Bcl-2 dependent, while clonal deletion appears to be Bcl-2 independent (14, 15). We therefore propose that IL-4 inhibits HC-induced PCD of B-CLL cells via their upregulation of Bcl-2 without ruling out the existence of an independent pathway.

Given our recent observations that freshly isolated T CLL cells, which are comprised of a very low ratio of $CD4^+/CD8^+$ cells, express the IL-4 gene (36) and that HC suppresses IL-4 production by activated normal T cells (37), the glucocorticoid-induced PCD in CLL may be explained by

a direct effect of HC on B-CLL cells combined with its ability to suppress the IL-4 gene in T-CLL cells. Finally, with CLL being characterized by the accumulation of long-lived abnormal B cells with a very low proliferative index, it is tempting to speculate that IL-4 plays an important role in the biology of the B-CLL cell by preventing both its proliferation and death.

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Note added in proof: Since the admission of our manuscript, supporting data have been reported indicating that IL-4 inhibits glucocorticoids-induced apoptosis of Th_2 clones (38).

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