stimulating factor, blood and platelet support, and empiric antibiotic treatment, the patient died of pneumonia in September 1996.

No specific association is known between primary myelofibrosis and lymphoma, despite a few reported cases of myelofibrosis complicated by concomitant[4] or subsequent lymphoma.[5-7] On the other hand, lymphoma is a recognized, albeit uncommon, cause of myelofibrosis.[6] Severe myelofibrosis in the setting of follicular NHL is exceedingly rare, and its occurrence following fludarabine administration suggests an etiopathogenic link. The role that fludarabine played in our patient's myelofibrosis can only be speculated, but given the short experience with this drug we alert physicians to be aware of this potential severe complication of purine analog administration.

Key words

Myelofibrosis, fludarabine, indolent lymphoma

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In vitro modulation of bcl-2 protein expression, drug-induced apoptosis and cytotoxicity by interleukin-10 in chronic lymphocytic leukemia

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Interleukin-10 failed to modify either the percentage of bcl-2$^+$ cells or the number of bcl-2 molecules, or to reduce 2-chlorodeoxyadenosine- and fludarabine-induced apoptosis. The cytokine at 0.1 ng/mL induced an increase of cell survival both in the absence or in the presence of 2-chlorodeoxyadenosine, while no difference was appreciated with fludarabine.

Controversial results have been recently reported on interleukin-10 (IL-10) as an apoptosis inducer in chronic lymphocytic leukemia (CLL) cells.[1-3] In this regard, we would like to report our experience on its effect on 22 previously untreated CLL patients.

IL-10 (Schering-Plough, Milan, Italy) at 0.1 ng/mL induced a statistically significant increase of cell survival, as measured by the MTT assay,[4] compared to cytokine-free cultures (p=0.024). Similarly, IL-10 0.1 ng/mL significantly raised the 2-chlorodeoxyadenosine- (2-CDA) (Leustatin, Ortho Biotech, USA) LD_{50} values (p=0.009), while no significant difference was appreciated in the fludarabine (FAMP) (Fludara, Schering AR, Germany) group (Figure 1A). IL-10 also failed to reduce significantly the percentage of either 2-CDA- or FAMP-induced apoptotic nuclei (Figure 1B) as evaluated by flow-cytometry analysis.[5] Finally, the percentage of bcl-2$^+$ cells, which significantly reduced after a 4 days of culture, remained unmodified in the presence of IL-10 (Figure 2A). In 11 cases, the number of bcl-2 molecules was also analyzed by DAKO QUIFIKIT assay (Figure 2B). The number of bcl-2 molecules decreased spontaneously after 4 days of culture (14,746 versus 6,689 ABC units, p=0.0044). We failed to document that IL-10 had an effect on reducing bcl-2 antigen density (6,689 versus 6,130 ABC units, p=not significant).

Our data are in line with the results of Jurlander *et al.*[3] regarding the effect of IL-10 in modulating both cell viability and apoptosis of CLL cells. Our experiments, like those performed by Jurlander, were carried out on freshly isolated cells; this is a key point to explain why our and Jurlander results differ from those of Fluckiger.[1] It is worth noting that other authors demonstrated that IL-10 prevents CLL cells from undergoing apoptosis.[2] In our study *in vitro* culture of CLL cells significantly reduced both the percentage of cells expressing the bcl-2 protein content and the antigen density. The addition of IL-10, which has been claimed to down-regulate the bcl-2 product in CLL,[1] slightly, but not significantly, lowered both the percentage and the amount of molecules of the bcl-2 positive-cells. Likewise, a more recent report showed that IL-10 enhanced the survival of CLL cells by inhibiting the process of apoptotic cell death, without increasing bcl-2 expression.[2] The finding that IL-10 mRNA expression is firmly associated with non-progressive disease[6] gives a speculative indication for immunotherapy with rh-IL-10 in CLL patients to prevent disease progression. On the other hand, the fact that the growth of the B-1 malignancy, a murine counterpart of CLL, is dependent on autocrine production of IL-

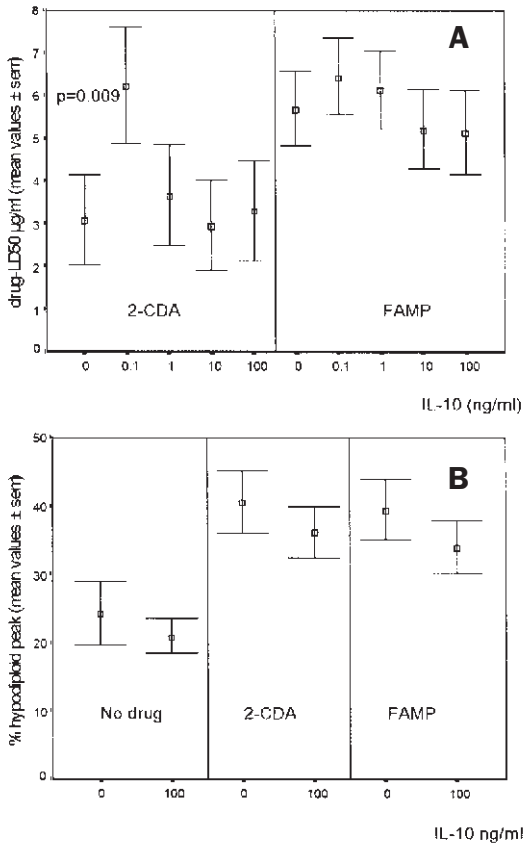

Figure 1. A) *In vitro* effect of several IL-10 concentrations on 2-CDA- and FAMP-LD_{50} values. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test: the comparison of the mean values was significant only between IL-10=0 and 0.1 ng/mL in the presence of 2-CDA (p=0.009). B) Effect of IL-10 100 ng/mL on flow cytometry analysis of 2-CDA- (1.25 µg/mL) or FAMP- (1 µg/mL) induced apoptotic nuclei on CLL cells after 4 days of incubation. Statistical analysis revealed no significant modification caused by the addition of IL-10.

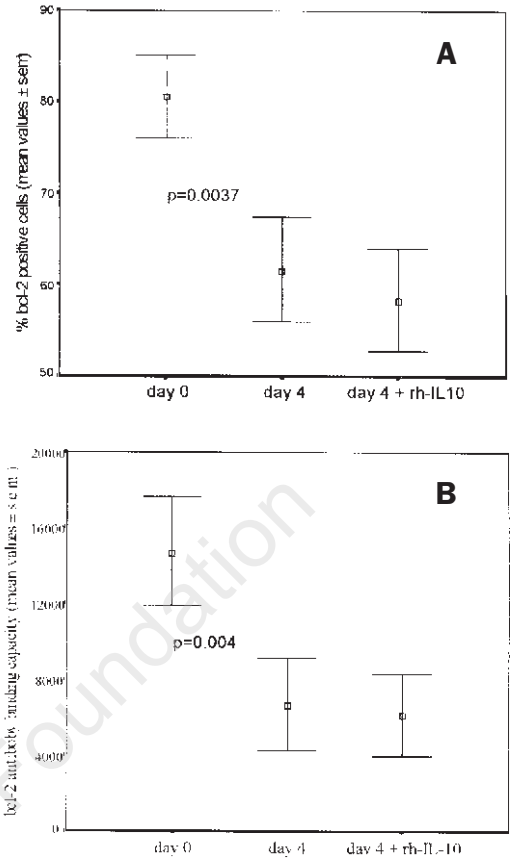

Figure 2. A) Flow-cytometry evaluation of bcl-2 percentage on freshly isolated cells (day 0), and cells after 4 days of culture with or without IL-10 100 ng/mL. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test: day 0 versus day 4, p=0.0037; day 0 versus day 4 + IL-10, p=0.0006; day 4 versus day 4 + IL-10, p= not significant. B) Flow cytometry determination of bcl-2 antigen density, expressed as antigen binding capacity units, on freshly isolated cells (day 0) and after 4 days of culture without or with IL-10 100 ng/mL. Statistical analysis was performed by Wilcoxon matched pairs signed-ranks test: day 0 versus day 4, p= 0.0044; day 4 vs. day 4 + IL-10, p = not significant.

10^7 is in line with our results showing a significant increase of cell viability after 4 days of culture in the presence of a low concentration of IL-10.

In conclusion, these data suggest that IL-10 has no *in vitro* relevance in giving a death signal either by down-regulating bcl-2 cellular content, or by enhancing apoptosis or by improving the effect of either 2-CDA or FAMP in inducing cell cytotoxicity in CLL cells. However, clinical approaches are required to analyze the magnitude of its potential *in vivo* effect.

Key words

bcl-2 protein, apoptosis, interleukin-10, CLL

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Blastogenic response of activated human umbilical cord blood T-lymphocytes

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Donor T-lymphocytes are thought to play a crucial role in both acute and chronic graft-versus-host disease (GvHD), pathological conditions that frequently complicate allogeneic bone marrow transplantation.[1] These diseases are described as occurring with a lower incidence and lesser severity when human umbilical cord blood (HUCB) cells, which have recently emerged as a potential source of hematopoietic progenitors, are used for transplantation.[2-6] This condition is probably related to the immaturity of neonatal HUCB T cells.[7,8] Lymphocyte blastogenic response to phytohemagglutinin (PHA), evaluated by means of flow cytometry, is a useful tool for testing the functional ability of T-cells to display an immune response against allo-antigens, reproducing *in vitro* the *in vivo* mechanism of activation. This study was designed to verify whether an impairment in HUCB T-cell ability to undergo an *in vitro* blastogenic response to mitogens could explain their reduced *in vivo* ability to induce GvHD.

Lectins, such as PHA, are proteins and glycoproteins able to bind specifically to certain carbohydrate structures on cell surfaces.[9] In lectin-mediated lymphocyte activation the first step is probably the cross-linking of membrane proteins such as T3 complex which is now recognized as the probable transducer of antigenic signals in T-cell activation.[10,11] Cellular DNA staining with intercalating dyes of stimulated peripheral blood (PB) lymphocytes has commonly been used to test cell-mediated immunity.[12] PHA predominantly stimulates T-lymphocytes. Lymphocyte blastogenesis is induced in cultures and evaluated by flow cytometric cell-cycle analysis after propidium iodide (PI) staining of the nuclei. PI binds to DNA in cells at all stages of the cell cycle, and the nucleus light emission intensity is directly proportional to its DNA content. Stimulated and activated cells entering the cell cycle pass through the S-phase and synthesize DNA. Furthermore, activated T-cells co-express HLA-DR and CD25 glycoproteins. On these bases, in our study we used flow cytometry as a tool to verify whether differences in allo-activation ability of T-lymphocytes of cultured HUCB and PB samples exist.

PB mononuclear cells were obtained from 20 HUCB and 20 healthy adult PB heparinized samples by Lymphoprep (Nycomed Pharma AS, Oslo, Norway) gradient centrifugation. Eight aliquots of 200 µL each containing 2×10^9 cells were obtained from the pellet. Six aliquots were cultured in 6 flasks containing 5 mL of RPMI 1640 medium with 100 µg/mL PHA. Cultures were placed in a 37°C incubator gassed with 5% CO_2 in air. From the seventh aliquot, 50 µL were stained with CD3-phycoerythrin (PE)/HLA-DR-fluorescein isothiocyanate (FITC), and 50 µL with CD3-PE/CD25-FITC monoclonal antibody combinations, respectively, and evaluated by means of flow cytometry following incubation for 30 minutes at 4°C in the dark. Aliquot number 8 was immediately stained with PI, as follows: to 2×10^6 cells, 2 mL of PI solution (50 µg/mL) + 25 µL of RNAse solution (0.5 mg/mL: 100 U) + 25 µL of Nonidet P40 0.1% solution were added. Samples were incubated at room temperature in the dark for 30 minutes. These reagents are able to isolate and stain the cell nuclei from blood cells. Samples were then filtered through a 40 µm nylon mesh just before analysis. For each sample a minimum of 50,000 events were acquired in list mode data files on a FACSort flow cytometer (Becton Dickinson Immunocytometry Systems, BDIS, San José, CA, USA) equipped with a 15

Table 1. Stimulation of mononucleated HUCB and PB samples with PHA.

Hours	*HUCB*	*PB*	*p*
$CD3^+HLA\text{-}DR^+$ cells (%)			
0	3.6±0.5	3.6±0,9	ns
24	34.6±12.7	38.6±10.3	ns
48	40.3±10.2	42.1±5.5	ns
72	58.2±12.1	59.4±5.6	ns
$CD3^+CD25^+$ cells (%)			
0	3.9±0.5	5.1±0.3	ns
24	45.2±9.6	42.4±14.9	ns
48	44.3±15.8	55.8±16.5	ns
72	64.6±7.4	70.1±14.6	ns
S-phase cell number (%)			
0	1.7±1.2	1.4±0.4	ns
24	6.1±1	4.4±1.9	ns
48	10.9±9	9.2±5.3	ns
72	24±12.8	25.2±9.8	ns

Data are expressed as mean percentage cell number±standard deviations. Statistical comparisons were carried out using Student's t-test.