Intracellular interleukin-2 expression by T-cell subsets in B-cell chronic lymphocytic leukemia

A three-color flow cytometry technique was used to determine interleukin-2 expression by T-cell subsets - $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cells in patients with newly diagnosed, untreated B-cell chronic lymphocytic leukemia (B-CLL) and in a control group. The detected statistically significant higher percentage of $CD3^+/CD8^+/IL2^+$ cells in patients than in controls may indicate the activation of T-lymphocytes, in particular $CD8^+$ cells, in early stages of B-CLL.

Changes in the cytokine network between malignant cells and T-lymphocytes may be responsible for accumulation of the malignant cell clone in B-cell chronic lymphocytic leukemia (B-CLL).[1] Among such cytokines, interleukin-2 (IL-2) acts as a pleiotropic mediator within the immune system having a variety of effects on proliferation of T- and B-cells, macrophages and large granular lymphocytes. It has been indicated that in B-CLL IL-2 may be involved in the induction and regulation of malignant cell proliferation,[2] and can also prevent malignant cells from programmed cell death.[3] To broaden knowledge about the role of this cytokine in B-CLL we tried to determine whether the IL-2 production capacity of T-cell subsets was altered in B-CLL patients in comparison within healthy individuals.

Twenty-two patients with newly diagnosed, untreated B-CLL and 12 healthy individuals were studied. Peripheral blood lymphocytes were isolated and stimulated by PMA and ionomycin in the presence of the intracellular protein transport inhibitor brefeldin A. Combined membrane and intracytoplasmic staining procedures were then performed using the following monoclonal antibodies (MoAbs): anti-CD3 PerCP, anti-CD4 FITC, anti-CD8 FITC, anti-IL-2 PE. A three-color flow cytometry technique was used to analyze labeled cells. The data were shown as the percentages among the T-cell subsets ($CD3^+/CD4^+$, $CD3^+/CD4^+$) and as the mean fluorescence intensity (MFI) measured from the upper limit of the negative control.

We detected a statistically significant higher percentage of $CD3^+/IL\text{-}2^+$ cells in the patient group than in the controls, 35.42±12.34% and 26.22±8.51% respectively. There was no difference in MFI between the two groups. There was not a statistically significant difference between patient and control groups in IL-2 expression by $CD3^+/CD4^+$ cells as far as the percentage of positive cells and MFI were concerned. There was a statistically significant higher percentage of $CD3^+/CD8^+$ cells expressing IL-2 in patients than in the control group, 18.90±10.30% and 10.36±3.65%, respectively. There were no differences in MFI between the two groups.

Flow cytometry is a new technique allowing cytokine expression to be identified at the level of the individual cell.[4] There are only a few reports regarding intracellular cytokine expression in B-CLL, in particular IL-4 and interferon-γ.[5-7] To the best of our knowledge this study is the first that uses a three-color flow cytometry technique to assess IL-2 expression in precisely identified T-cell subsets of interest. Using simultaneous labeling with anti-CD3 and anti-CD4 or anti-CD8 MoAb we were able to determine the subsets of interest: T-helper and T-cytotoxic/supressor cells (Figure 1).

The results of this study undoubtedly indicate a significant expansion of T-cells, in particular $CD3^+/CD8^+$ lymphocytes, that are capable of producing IL-2 in B-CLL patients. Thus the $CD8^+$ cells may be the main source of IL-2 in B-CLL patients. Nevertheless, the MFI of IL-2 expression, indicative of IL-2 production per cell, was not statistically significant different between patients and the control group in this study.

Our results are consistent with those of Rosi *et al.*[8] who detected much higher IL-2 production by PHA-stimulated PBMCs in patients than in controls. However, other authors have reported different results. Jansssen *et al.*,[9] using a limiting dilution culture approach, indicated that comparable numbers of IL-2-producing helper T-cells and cytotoxic T-cells were present in B-CLL patients and healthy controls. Reyes *et al.*,[10] who assessed IL-2 production by PHA-stimulated $CD2^+$ cells using an ELISA method, also did not detect differences in IL-2 production in B-CLL patients and controls.

Our data concerning intracellular expression of IL-2 do not provide information about IL-2 release by T-cells, that would need further investigation. They do, however, indicate the func-

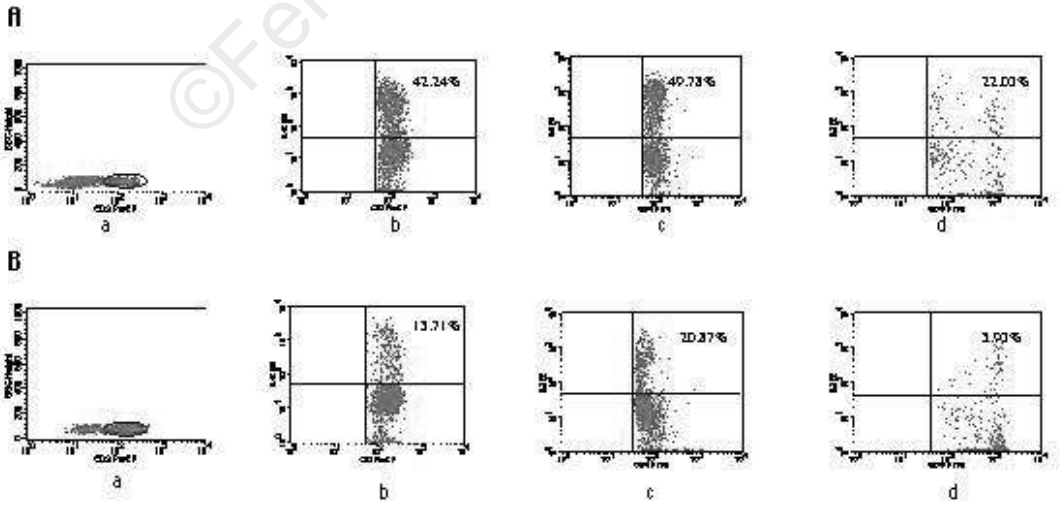

Figure 1. IL-2 expression by T-cell subsets in a B-CLL patient (A) and in a healthy individual (B); a - the dot plot shows SSC vs CD3PerCP pattern of peripheral blood lymphocytes; b,c,d - the dot plots show IL-2 expression by T-cells gated on CD3, CD3 / CD4, CD3 / CD8 lymphocytes, respectively.

tional capacity of T-lymphocytes to produce IL-2. In view of reports regarding T-cell abnormalities in B-CLL, that may be an indicator of the activation state of T-lymphocytes. It may suggest that the activated immune system in early stages of the disease tries to act against malignant cells (all analyzed patients were in stages 0-II). On the other hand, the higher number of T-cells expressing IL-2 in B-CLL patients may support the described role of IL-2 regulating proliferation and apoptosis of malignant cells.

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