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Cytoplasmic Interleukin-4 (IL-4) and Surface IL-4 Receptor Expression in Patients With B-Cell Lymphocytic Leukemia

To the Editor:

B-cell chronic lymphocytic leukemia (B-CLL) is a malignancy characterized by the accumulation of long-lived CD5⁺ cells¹ in which cytokines might be involved in the proliferation and survival of malignant B cells.²⁻⁶

In particular, interleukin-4 (IL-4) prevents B-CLL cell clones from entering spontaneous apoptosis by increasing the expression of bcl-2⁵ and protects B-CLL cells against anti-APO1-induced apoptosis.⁶ Only one report has analyzed the intracellular expression of IL-4 in T cells of patients with B-CLL.⁷ We examined the expression of IL-4 and IL-4 receptor (IL-4R) in unstimulated leukemic B cells and T cells from 10 patients with untreated stage A B-CLL and compared them with 10 normal controls using flow cytometric analyses.

We found that the proportion of CD19⁺ cells expressing cytoplasmic IL-4 was significantly higher in B-CLL patients than in controls ($P < .002$; Table 1). The proportion of CD3⁺ cells expressing cytoplasmic IL-4 was significantly higher in B-CLL patients than in controls ($P < .02$). Although the proportion of CD19⁺ cells expressing IL-4R

was similar, the proportion of CD3⁺ cells expressing the IL-4R in B-CLL patients was significantly higher than in normal controls ($P < .02$). IL-4 could not be detected in the supernatant after the in vitro culture of the cells from both patients and controls. After stimulation with the mitogen PWM, six of the seven control samples and none of the patient cells had detectable supernatant IL-4 ($P < .03$; Table 2).

The demonstration that T cells from B-CLL patients display a greater percentage of IL-4R expression from normal individuals is novel. It is unclear whether the T cells from B-CLL patients express both the IL-4R together with IL-4 or this aberrant expression occurs on different T-cell populations in these patients. It is intriguing that no increase in IL-4R expression could be found on malignant B-CLL cells despite the expression of cytoplasmic IL-4. It is possible that the IL-4 from the B-CLL B cells is released and taken up rapidly by the T cells, and this leads to the aberrant expression of the IL-4R. Alternatively, the interaction between the malignant B cells and T cells may occur in a microenvironment in which local concentrations of IL-4 may be high, but these are not reflected in the blood. The disproportionate relationship between IL-4 and its receptor in the normal T-cell compartment compared with the malignant B-cell compartment in B-CLL is presum-

Table 1. Comparison of Cytoplasmic IL-4 and IL-4R Expression in B-CLL Patients Versus Controls

Patient No.	CD19/IL-4 Unst*	CD3/IL-4 Unst*	CD19/IL-4R*	CD3/IL-4R*	Controls	CD19/IL-4 Unst*	CD3/IL-4 Unst*	CD19/IL-4R*	CD3/IL-4R*
1	30.00	21.57	97.13	2.67	1	5.14	2.17	52.97	1.06
2	21.57	26.09	42.91	3.74	2	4.71	2.96	61.47	0.83
3	28.78	13.52	28.22	0.58	3	5.35	1.82	82.53	0.63
4	18.30	11.17	11.22	1.52	4	10.13	1.67	74.76	2.48
5	53.80	5.35	70.50	6.75	5	9.89	4.18	64.85	0.78
6	21.64	2.62	56.26	3.22	6	1.45	3.83	88.37	3.29
7	14.81	7.23	69.97	3.47	7	6.76	3.36	69.13	1.33
8	6.36	2.62	83.41	6.08	8	8.16	0.41	69.63	1.76
9	17.39	3.17	20.50	5.16	9	11.93	0.96	82.16	3.70
10	11.29	2.19	96.87	7.80	10	8.32	0.93	41.55	0.98

Abbreviation: unst, unstimulated.

*The data are presented as a percentage of CD19 and CD3⁺ cells expressing cytoplasmic IL-4 and surface IL-4R. The proportion of CD3⁺ cells expressing IL-4R in B-CLL patients was significantly higher in patients than in controls ($P < .01$). The proportion of CD19 and CD3⁺ cells expressing cytoplasmic IL-4 was also significantly higher in B-CLL patients than in controls ($P < .03$ and $P < .01$, respectively).

ably important in the pathogenesis of B-CLL and merits further investigation.

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Table 2. IL-4 Secretion by Lymphocytes From B-CLL Patients and Normal Controls

Patient No.	Spon (pg/mL)	PWM (pg/mL)	Controls	Spon (pg/mL)	PWM (pg/mL)
1	ND	ND	1	ND	0.23
2	ND	ND	2	ND	3.27
3	ND	ND	3	ND	1.02
4	ND	ND	4	ND	0.15
5	ND	ND	5	ND	2.37
6	ND	ND	6	ND	4.78
7	ND	ND	7	ND	ND

IL-4 secretion by lymphocytes from normal controls was significantly increased compared with that from the patients ($P < .01$).

Abbreviations: Spon, spontaneous secretion; PHA, phytohemagglutinin-stimulated secretion; PWM, pokeweed mitogen-stimulated secretion; ND, not detected.

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Primary Role of the Liver in Thrombopoietin Production Shown by Tissue-Specific Knockout

To the Editor:

Maintenance of an appropriate number of circulating platelets is required for hemostasis and blood coagulation. The control of platelet production is mediated by thrombopoietin (Tpo) through its effect on the proliferation of megakaryocyte progenitors and on megakaryocyte ploidy.^{1,2} The levels of Tpo available to stimulate platelet production are thought to be directly regulated by the platelet mass itself.³ In this model, Tpo is constitutively produced and released in the circulation, where it is being cleared by platelets through c-mpl-mediated binding.⁴ But, to date, the site(s) of Tpo production remains to be defined. Using Northern blot analysis, the liver and kidneys appear to be the major site of Tpo mRNA expression.⁵ However, using more sensitive methods,

such as reverse transcription-polymerase chain reaction (RT-PCR), Tpo transcripts can be detected in most tissues, including the bone marrow stroma, which could produce Tpo directly in the megakaryocyte microenvironment. To evaluate the contribution of the liver to the Tpo production required to maintain a normal platelet count, we have generated tissue-specific knockout mice by transplanting the liver of Tpo-deficient mice⁶ into wild-type recipients.⁷ As shown in Fig 1A, RT-PCR analysis of wild-type mice shows the presence of Tpo transcripts in all tissue analyzed, whereas there is no detectable transcript in any tissue from Tpo-KO mice. In wild-type mice transplanted with a Tpo-KO liver, the Tpo transcripts were absent from the liver only. Circulating Tpo levels were assessed by a c-Mpl-dependent proliferation assay⁵ and were below the detection limit in both wild-type