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ORIGINAL ARTICLE

# Role of Decreased Production of Interleukin-10 and Interferon-Gamma in Spontaneous Apoptosis of B-Chronic Lymphocytic Leukemia Lymphocytes *In Vitro*

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*Background and Aims.* B-chronic lymphocytic leukemia (B-CLL) is characterized by the progressive accumulation of small B lymphocytes that do not undergo apoptosis due to an underlying defect. One potential mechanism of defective apoptosis may be irregular cytokine production. The goal of our investigation was to determine *in vitro* production of relevant cytokines by lymphocytes of B-CLL patients.

*Methods.* Thirty untreated (stage A) B-CLL patients, as well as 20 stage B and C patients and 30 healthy volunteers as a control group were examined. Interleukin-4 (IL-4), interferon-gamma (IFN-γ), interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA) in supernatants of lymphocyte cultures of all three investigated groups. The method applied for detecting apoptosis was fluorescence microscopic analysis using acridine orange/ethidium bromide (AO/EB) double staining.

*Results.* Investigation showed that *in vitro* lymphocyte production of IL-10 and IFN-γ were significantly decreased in B-CLL patients, whereas there were no statistically significantly differences of IL-4 and TNF-α production among the tested groups. Compared with the spontaneous apoptosis observed in control subjects' lymphocytes, B-CLL lymphocytes showed increased percentages of apoptotic cells after incubation for 24 h. Interestingly, increased spontaneous apoptosis of B-CLL lymphocytes was followed by decreased IL-10 and IFN-γ production. Stage of disease did not influence B-CLL lymphocyte spontaneous apoptosis in *vitro*.

*Conclusions.* These changes in cytokine production in cultures of B-CLL lymphocytes may be one of the potential mechanisms in the pathogenesis of abnormal apoptosis. © 2009 IMSS. Published by Elsevier Inc.

*Key Words:* Apoptosis, Chronic lymphocytic leukemia, Interleukin-4, Interferon-gamma, Interleukin-10, Tumor necrosis factor-alpha.

## Introduction

B-chronic lymphocytic leukemia is a clonal expansion of relatively mature $CD5^+$ B lymphocytes (1). The progressive increase of lymphocyte count coupled with the very low proportion of proliferating cells has led to the notion that B-CLL may be originated by defective apoptosis (2,3). By an unknown mechanism, molecular defects are likely inducing the constitutive activation of numerous signaling pathways that regulate the differentiation, proliferation and apoptosis of B lymphocytes.

Apoptosis is the physiological process accompanied by many morphological and biochemical changes whereby

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