CD69 expression in B-cell chronic lymphocytic leukemia: a new prognostic marker?

A highly variable clinical course of disease characterizes B-cell chronic lymphocytic leukemia (B-CLL). In fact, some patients do not require any treatment for many years and have a long-standing disease, while others may die within a few months of diagnosis because of B-CLL itself or disease-related complications. In an attempt to identify subgroups of B-CLL patients with peculiar features predictive of the clinical behavior of the disease, several clinical and laboratory parameters have already been tested. Indeed, various immunophenotypic markers have been proposed as having prognostic relevance, such as the intensity of CD20 expression, the expression of CD23 and CD21, and, more recently, the expression of CD38.

CD69 identifies a type II integral membrane protein with a single transmembrane domain belonging to the C-type lectin family of surface receptors. Initially described as an antigen expressed early in the activation of lymphoid cells, CD69 was considered restricted to activated lymphocytes. As a matter of fact, resting peripheral blood lymphoid cells do not express CD69. However, the stimulation of the T-cell receptor/CD3 complex in T-cells quickly induces expression of CD69. In addition, CD69 expression is inducible by immature thymocytes, B-cells (through crosslinking of surface immunoglobulin), natural killer cells, monocytes, neutrophils and eosinophils. Although a specific ligand has not yet been identified, CD69 generates intracellular signals with various cellular responses.

We analyzed CD69 (clone L78, Becton Dickinson Immunocytometry Systems, BDIS, San José, CA, USA) expression on neoplastic cells by means of flow cytometry (FACSCalibur, BDIS) in peripheral blood samples from 92 immunologically typical (CD5−CD23+) untreated B-CLL patients. An additional panel of fluorescein (FITC) and phycoerythrin (PE) directly-conjugated monoclonal antibodies including CD19 (Leu-12), CD20 (Leu-16), CD22 (Leu-14), CD23 (Leu-19), CD5 (Leu-1), C1 (Leu-17), FMC7, κ/λ light chains, all purchased from BDIS, and CD79b (CB3-1, Immunotech, Marseill, France), was used. Finally, the number of CD20 and CD22 molecules per cell, evaluated as antibody-binding capacity (ABC), was measured by means of Quantibrite technology (BDIS), as described elsewhere.

Forty-eight (52%) patients expressed CD69 in more than 30% of CD19-positive cells. Table 1 reports the clinico-biological features of B-CLL patients according to the expression of CD69. As shown, no differences by age, gender, typical or atypical morphology (FAB criteria), expression of FMC7, CD79b and CD38, as well as density of CD20, CD22 and surface membrane immunoglobulins were observed between the two groups of patients. However, peripheral blood lymphocytosis, Binet stages B and C, and diffuse pattern of bone marrow infiltration were found to be closely associated with CD69 expression. In addition, trisomy 12 (a cytogenetic marker of poor prognosis) was significantly more represented in the CD69-positive group, while del 13q14 (usually correlated with a better prognosis) was detected more frequently in the CD69-negative group. Ten patients (5 in the CD69-positive and 5 in the CD69-negative group) did not carry any of these abnormalities. As a result, median overall survival of CD69-positive B-CLL patients was 98 months, while it is still not reached at 150 months in the CD69-negative B-CLL patients (Figure 1). Finally, multivariate analysis (Cox model) confirmed the independent positive prognostic weight of CD69 expression at diagnosis in B-CLL (p = 0.015) (data not shown).
Thus in our hands, CD69 was found to be expressed on neoplastic B-cells of more than half of our B-CLL patients. Since this expression correlated with worse clinico-biological findings as well as a shorter survival than did CD69-negative forms, CD69 could be considered as a new promising immunologic prognostic parameter in B-CLL. However, the exact role of CD69 in the pathogenesis and clinical behavior of B-CLL remains to be better established, needing further investigations.

Department of Onco-Hematology, "San Giovanni di Dio e Ruggi d’Aragona" Hospital, Salerno; *Division of Hematology, IRCCS “Casa Sollievo della Sofferenza” Hospital, San Giovanni Rotondo, Italy

Key words: chronic lymphocytic leukemia, CD69, flow cytometry, prognostic factors.

Correspondence: Giovanni D’Arena, MD, Department of Onco-Hematology, "San Giovanni di Dio e Ruggi d’Aragona" Hospital, 84100 Salerno, Italy. Phone: international +39.089.672485. Fax: international +39.089.672491. E-mail: gdarena@altavista.it

References