CD39 Expression on T Lymphocytes Correlates With Severity of Disease in Patients With Chronic Lymphocytic Leukemia

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Abstract

Introduction: Chronic lymphocytic leukemia (CLL) is a B-cell disorder, but it is also associated with abnormalities in T-lymphocyte function. In this study we examine changes in T-lymphocyte CD39 and CD73 expression in patients with CLL. **Methods:** Blood samples were drawn from 34 patients with CLL and 31 controls. The cells were stained for CD3, CD4, CD8, CD19, CD39, and CD73 and analyzed by flow cytometry. **Results:** Overall, patients with CLL had a higher percentage of $CD39^+$ T lymphocytes than did controls. The percentage of cells expressing CD39 was higher in both $CD4^+$ cells and $CD8^+$ cells. Higher CD3/CD39 expression was associated with a later disease stage. No correlations between T-lymphocyte CD39 levels and CD38 or Zap-70 expression were observed. In contrast, the percentage of T lymphocytes and B lymphocytes that expressed CD73 was decreased in patients with CLL. Average B-lymphocyte CD73 expression was decreased in CLL because the majority of CLL clones were CD73. However a minority of CLL clones were $CD73^+$, and patients with $CD73^+$ clones tended to have earlier stage disease. **Conclusion:** T-lymphocyte CD39 and CD73 expression may be useful prognostic markers in patients with CLL. Expression of CD73 on the malignant cell population in CLL may be a marker of better prognosis.

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Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common leukemias in North America, representing 25%-30% of all leukemias.[1] An estimated 14,570 new cases and 4380 deaths from CLL are expected in 2011.[2] The leukemia is characterized by a clonal expansion of long-lived, mature-appearing B lymphocytes that coexpress the CD5, CD19, and CD23 surface antigens.[3] In contrast to many other malignancies, CLL is not known to be associated with an increased risk of thrombosis in the absence of secondary reasons for thrombosis.[4]

Abnormalities in T-cell expression and function have been reported previously in CLL.[5,6] The absolute number of T lymphocytes is often increased in CLL, largely caused by increases in the $CD8^+$ population,[7] although the relative number is usually reduced because of the large number of malignant B lymphocytes that accumulate. However the T lymphocytes induce lower proliferation and activation responses to mitogen and may not be able to stimulate normal B lymphocytes to produce immunoglobulin (Ig).[5] Increased, decreased, or normal expression of T-lymphocyte activation markers has been reported by various investigators.[5] An increase in T-regulatory (T-reg) cells has been reported as well.[6]

CD39 (ectonucleotidase, NTDPase1) is an ADPase and ATPase found on the surface of endothelial cells, normal lymphocytes, and other leukocytes.[8] It is strongly expressed on peripheral B lympho-

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cytes, weakly expressed on marginal zone B lymphocytes, and is not expressed on B lymphocytes in germinal centers.[9,10] Its principal function on the endothelial cell surface is to decrease platelet activation and recruitment by metabolizing platelet-released adenosine diphosphate (ADP). In leukocytes the enzyme has a variety of other direct or indirect effects as well, including modulation of cytokine expression and the inflammatory response.[11-13] CD39 will quickly metabolize the ADP produced by its metabolism of adenosine triphosphate (ATP) to adenosine monophosphate (AMP). In normal controls, CD39 is expressed on about 6%-8% of T lymphocytes and most B lymphocytes.[14] Expression on T lymphocytes is higher on activated and memory/activated T lymphocytes than on naive T lymphocytes, with 30%-35% of $CD25^+$ T lymphocytes (vs. $< 5\%$ $CD25^-$ lymphocytes) and about 10%-12% of $CD4^+/CD45RO^+$ T lymphocytes (vs. about 4% $CD4^+/CD45RO^-$ lymphocytes) expressing CD39.[14] Another study of CD39 expression in T lymphocytes has suggested that CD39 is expressed primarily in T-reg cells.[14] However in this study, not all T-reg cells were $CD39^+$, suggesting that $CD39^+$ T-reg cells may be a specific subset that could be of particular interest. In CLL, CD39 is almost always expressed on the malignant clone, although activity is slightly higher than normal in early disease and may decrease to subnormal activity in later disease.[15]

CD73 (5′ nucleotidase—an ectonucleotidase not structurally related to CD39) is an extracellular enzyme that catalyzes the dephosphorylation of purine and pyrimidine ribophosphate and deoxyribonucleoside monophosphates to their corresponding nucleosides, with a preference for AMP.[16] It is inhibited by ADP and ATP. Adenosine and other metabolites produced by CD73 stimulate T-cell proliferation and may mediate cell adhesion.[16] CD73 is expressed on subsets of both T lymphocytes and B lymphocytes. CD73 expression and function are generally reported to be low in CLL.[16,17]

Together CD39 and CD73 are responsible for metabolizing nucleoside triphosphatases, diphosphatases, and monophosphatases to their base equivalents. The preferred substrate of CD39 and CD73 is adenosine phosphate. However other nucleoside phosphates can be metabolized by these molecules. Fludarabine, a chemotherapeutic agent commonly used in CLL, is supplied clinically as fludarabine monophosphate and must be dephosphorylated in order to enter the cell. Thus CD73 expression on the malignant cells in CLL or on bystander cells may influence sensitivity to fludarabine. Additionally both ATP and adenosine have been implicated in alterations in the immune response, and thus changes in extracellular concentration of these molecules mediated by CD39 and CD73 may influence the immune response to CLL. In general, high levels of ATP are thought to stimulate the immune system, whereas high levels of adenosine are immunosuppressive. Thus higher levels of CD39 and lower levels of CD73 might be expected to correlate with less immune resistance to malignancy and worse outcomes in CLL as well as possibly greater vulnerability to opportunistic infections or secondary malignancies.

We previously examined abnormalities in CD39 expression and activity on the neoplastic B lymphocytes in patients with CLL.[15] CD39 abnormalities have not been reported previously on the T lymphocytes of these patients. In this study we examined CD39 and CD73 expression on the nonmalignant T lymphocytes in CLL. CD73 levels on the malignant B-cell population in CLL were examined as well to confirm the observations of other investigators that showed a general decrease in CLL and further describe its role in this disease.

Methods

Patient Population

Patients were recruited from the Hematology Clinic and inpatient Hematology/Oncology Service at the New York Veterans Affairs (VA) Harbor Healthcare System and Weill Cornell Medical College. The protocol was approved by the institutional review boards at both institutions. The diagnosis of CLL was made by the presence of the characteristic immunophenotype ($CD5^+$, $CD19^+$, $CD23^+$, sIg dim), and all patients had absolute lymphocytosis with a clonal B-lymphocyte population of $> 5 \times 10^9$. Patients with CLL were asked to participate in the study if they were able to give informed consent, had a hemoglobin value of > 8 g/dL, and had no contraindications to blood donation. After informed consent was obtained, blood was drawn into a heparinized tube. Blood was also drawn from a subset of healthy volunteers participating in the THrombophilia In Cryptogenic stroke (THICK) study, which served as the control group. Patient data collected included Rai stage, complete blood count results, chemotherapy history, and Zap-70, CD38, and IgVH mutational status when available.

Fluorescence Activated Cell Sorter Analysis

Cells were single-stained with the following antibodies: CD3-APC/Cy7, CD19-FITC, CD39-PE, CD73-PE, CD4-PERCP, CD5-APC, CD69-PE/Cy7, and CD8-FITC (CD19-FITC). Antibodies were obtained as follows: all antibodies were obtained from BD Biosciences (San Jose, CA) except for the following: CD39-PE (Ancell Corporation, Bayport, MN), CD3-APC/Cy7 and CD5-APC (BioLegend, San Diego, CA). Cells were also double- or triple-stained for CD3/CD39/CD69, CD4/CD39, CD8/CD39, CD5/CD19/CD39, CD3/CD73, CD4/CD73, CD8/CD73, and CD5/CD19/CD73. Cells were incubated with antibody for 45-90 minutes at room temperature in the dark with gentle shaking. Acquisition and analysis of fluorescence activated cell sorter (FACS) data were performed on a FACSCanto using FACSDiva software (BD Biosciences). Percentage positivity and geometric mean fluorescence for normal and malignant lymphocytes were examined. Geometric mean fluorescence was measured only for cells that were considered to be positive by FACS.

Statistics

A Student 2-sided *t* test with unequal variance was used to generate *P* values when comparing groups, except for analysis of CD69 activity in $CD39^+$ vs. $CD39^-$ cells, for which a Student paired 2-sided *t* test was used.

Results

Clinical Data of Participants

Data were available for 34 patients and 31 controls. Patients ranged from 35 to 86 years of age, with a median of 67 years. Eleven patients were women, and 23 were men. Among controls, the age range was 22-70 years with a median of 39 years. Sixteen controls were men and 15 were women. Seven patients were Rai stage 0, 12 were Rai stage 1, 5 were Rai stage 2, and 10 were Rai stage 3-4.