Human leukocyte antigens HLA DRB1 influence clinical outcome of chronic lymphocytic leukemia

We investigated HLA DRB1 correlations with chronic lymphocytic leukemia (B-CLL) outcome in 90 patients. Neither of the alleles was associated with B-CLL clinical characteristics or mortality. HLA DRB1*01 and HLA DRB1*02-null were associated with shorter overall survival (p=0.007, p=0.002). Our results suggest that HLA-restricted adaptive immunity influences CLL outcome.

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B-cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia in Western countries and North America. It is characterized by the clonal accumulation of $CD5^+$ B-cells expressing CD19, CD23 and low surface IgM.[1] Its origins still remains largely unknown, although genetic susceptibility appears to play an important role.[2] The Human Leukocyte Antigen complex (HLA) is one of the inherited factors that influences the development of B-CLL and clinical outcome of diffuse large B-cell lymphoma.[3-5] This led us to examine whether HLA antigens contribute to the clinical course of CLL.

Allelic frequencies of HLA DRB1 were systematically examined in 90 B-CLL patients seen in the Department of Hematology during 2003-2004 for control visits, and in 94 ethnically-matched, healthy controls. Patients' confidentiality was maintained in accordance with Polish regulations for studies on human subjects. Clinical characteristics of CLL patients are presented in Table 1. Of 90 patients enrolled in the study, 58 required treatment and were available for an estimation of treatment response. Forty patients (69%) achieved complete or partial remission and 18 (31%) did not. Twenty-one patients (36%) experienced disease progression or relapse and 25 patients died. HLA DRB1 genotyping was performed using PCR-based reverse blot technology (RELI SSO; Dynal, Oslo, Norway) according to the manufacturer's protocol. Statistical tests were two-tailed with the level of significance $p<0.05$. Survival was estimated according to the Kaplan-Meier method. Comparison of survival was based on log-rank testing as previously described, with death for any cause as a censoring variable.[5]

The HLA DRB1 allelic frequencies and distributions were consistent with the Hardy-Weinberg equilibrium, and did not differ significantly between CLL patients and the control group. We assessed HLA DRB1 allele frequency using low (two-digit) typing resolution. We were therefore unable to detect previously reported associations of HLA DRB1*0401 and DRB1*0403 with CLL incidence, or DRB3 (DR52) and DRB4 (DR53) supertypical loci with age-at-onset of CLL.[3,6]

There were no associations between HLA DRB1 alleles and clinical characteristics of CLL patients at diagnosis, including age, clinical stage according to Rai classification, surface CD38 expression, serum levels of lactate dehydrogenase (LDH) and β2-microglobulin (*data not shown*). In patients with HLA DRB1*01 allele, there was a trend towards a shorter time from diagnosis to treatment (log-rank test, p=0.07, Figure 1A.) Neither of assessed HLA DRB1 alleles was associated with response to first-line treatment or mortality. With a median follow-up of surviving patients of 49 months (range 2.5-105 months), the subgroups of patients with HLA DRB1*01 and HLA DRB1*02 null allele had a significantly shorter overall survival (OS), (log–rank test, p=0.007 and p=0.002, respectively; Figure 1B and C). To further characterize HLA DRB1 linkages with survival in CLL patients, we analyzed both DRB1*02 splits (DRB1*15 and DRB1*16) and found that only DRB1*15 null allele remained significantly associated with shorter OS (p=0.015; Figure 1D). However, neither of these genetic markers was found to be associated with freedom from progression in CLL patients. Clinical stage according to Rai classification as a unique parameter (Rai 0-1 vs 2-4), with HLA DRB1* 01, HLA DRB1*02 null alleles and CD38 surface expression was included for analysis. In a multivariate Cox regression model, the HLA DRB1*01 remained an independent factor predicting for shorter OS (p=0.005, relative risk [RR]= 3.84).

Overall, our studies suggest that HLA-DRB1 alleles influence CLL outcome. Although T cells from patients with CLL contain a number of defects, they are capable of initiating autologous cytotoxic and proliferative responses directed against the CLL cells *in vitro*. These can be further increased with professional antigen-presenting cells in an HLA–I and –II restricted fashion.[7,8] Our data suggest that natural T-cell responses against CLL antigens are also likely to depend on certain inherited HLA alleles and their ability to efficiently present tumor-related antigens. These observations may explain the differences in overall survival in CLL patients carrying HLA-DRB*01 and -DRB*02 null allele. Alternatively, we cannot exclude that HLA-DRB1 alleles are markers of other pathologically relevant genes remaining in linkage disequilibrium with these loci. It has been recently reported that HLA-G, a non-classical, immunosuppressive HLA class I molecule remaining in linkage disequilibrium with certain DRB1 alleles, is an independent factor predicting CLL progression.[9,10]

In conclusion, we suggest that HLA-DRB1 alleles influence CLL outcome. These observations may have profound implications for understanding DC-based immunotherapies of CLL, implying that patients with certain HLA alleles are less likely to respond to DC vaccinations. Passive immunomodulation with HLA DR

Table 1. Clinical characteristics of 90 CLL patients included in the study.

Characteristics (N=90)	*Number and distribution*
Age (median, range)	66 (39-84%)
Sex (n, %)	
M	51 (56%)
F	39(44%)
Rai stage at diagnosis (n, %)	
0	43 (48%)
I+II	28 (31%)
III+IV	19 (21%)
Lymphocyte count ($\times10^9$/L)	25 (5.8-410%)
Granulocytes ($\times10^9$/L)	4.1 (0.9-13%)
Hemoglobin (g/dL)	13.5 (4.2-16.7%)
Platelets ($\times10^9$/L)	187 (36-476%)
Disease duration (months)	49 (2.5-105%)
Patients treated (n,%)	58 (64%)
Purine analog containing regimen (n,%)	29 (32%)

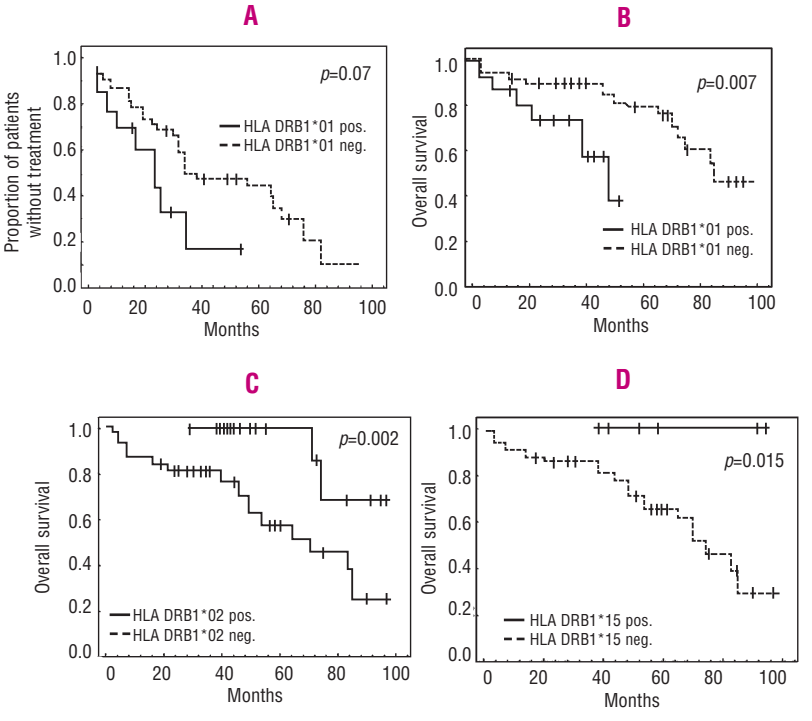

Figure 1. HLA DRB1 alleles and clinical course of chronic lymphocytic leukemia. A. Time to treatment in HLA DRB1*01 positive and negative patients. B. Overall survival in HLA DRB1*01 positive and negative patients. C. Overall survival in HLA DRB1*02 positive and negative patients. D. Overall survival in HLA DRB1*15 positive and negative patients.

monoclonal antibodies may help those with a poor prognosis to achieve a stable remission.

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