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Molecular identification of the HLA-DRB1-DQB1 for diagnosis and follow-up of acute leukemias

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ABSTRACT

We analyzed a group of 45 Brazilian individuals, 30 with acute myeloid leukemia (AML), 15 with acute lymphoid leukemia (ALL) and 100 healthy controls to assess genetic factor risk and HLA association contribution to the disease. Patient rates were compared with age and sex-matched control groups by directly typing the HLA-DRB1/3/4/5 and -DQB1 loci by PCR analysis. We observed significantly increased allelic distribution of HLA-DRB1*07 in AML patients and of HLA-DRB1*03 in ALL patients, which suggests that individuals in both groups are susceptible to the disease. We also found significantly decreased allelic distribution of HLA-DQB1*04 in AML patients and of HLA-DRB1*04 and -DQB1*03 in ALL patients, which suggests protection against the disease.
We further found increased HLA-DRB1*07 and -DQB1*02 haplotypes in AML patients, which suggests susceptibility to the disease and decreased HLA-DRB1*04 and -DQB1*03 haplotypes in ALL patients, which also suggests protection against the disease.
Future studies with larger and/or multicentric samples will be required for better comprehension of the HLA role in acute leukemia pathogenesis.

Introduction

Leukemia is a blood cancer that starts in the bone marrow, characterized as a cancerous change in the early cells from which mature blood cells develop. The precursor stem cells are usually found in the bone marrow and normally develop into lymphocytes or myeloid precursors. It is rare for leukemia to arise simultaneously from both lymphoid and myeloid precursors and the malignant change rarely occurs in hematopoietic stem cells prior to its commitment to either the lymphoid or myeloid lineage. On the other hand, growing evidence exists challenging these lineage models, since as a result of chemotherapy ALL may convert to AML; furthermore, a substantial portion of leukemia cases shows simultaneous expression of both myeloid and lymphoid antigens [1].

Acute leukemias are divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). ALL is a malignant disorder of lymphoid progenitor cells in the marrow and other organs, particularly the spleen and liver, affects both children and adults, with peak prevalence between the ages of 2 and 5 years [2,3]. AML is the most common form of acute leukemia in adults, accounting for over 80% of all acute leukemias [3]. AML is a heterogeneous group of leukemias that arise in precursors of myeloid, erytroid, megakaryocytic, and monocytic cell lineages [4]. ALL and AML are divided into a number of different subtypes and the correct diagnosis is based upon a wide range of clinical, morphologic and new developments of immunophenotyping, cytogenetics and molecular biology [2,5–7].

In humans, molecules HLA-DR and -DQ are class II cell surface glycoproteins encoded by the major histocompatibility complex -MHC. The polymorphic HLA-DR and -DQ antigens are encoded by DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1 genes in human chromosome 6 [8]. Strong linkage disequilibrium has been observed between HLA-DR and -DQ alleles and there are many stable class II haplotypes that are inherited in a Mendelian fashion [9]. Several associations between HLA and human leukemia have been quoted; however, the HLA-DR53 molecule has been one of the most consistently observed in several populations [10–13] and is codified by the haplotype HLA-DRB4, -DRB1 composed of the alleles * 04, *07 e *09 [14–17]. Significant advances have occurred in understanding the pathogenesis of human leukemia and epidemiologic studies strongly suggest that these diseases have an inherited basis; in that way, more reports have described an increase in homozygosis of HLA antigens [18] and an HLA identity with maternal alleles in case

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parents shared an HLA-DR antigen [11,19] as well as the recessive MHC nature which is probably related to the HLA-DR locus [15,17]. Through the hard work of a large number of investigators, the biology of acute leukemia is becoming increasingly known.

We report here a comparison of HLA-DRBI and -DQBI frequencies in a group of 45 Brazilian acute leukemia patients and 100 Brazilian healthy controls. Marker frequencies and homozygosity, as well as linkage analysis for selected haplotypes was performed. Rates in these patients were compared with those from age- and sex-matched local control groups by directly typing the HLA-DRB1/3/4/5 and -DQB1 loci by PCR analysis, to evaluate the genetic risk factor and contribution of HLA association with those diseases.

Study design

Selection of patients

The subject population consisted of 45 patients from Campinas, State of São Paulo, in South-eastern Brazil who attended the Hematology Outpatient Clinic of the Teaching Hospital, School of Medical Science, State University of Campinas. 30 patients showed adult AML and 15 showed adult ALL, which was diagnosed by immunophenotyping. Patients with adult AML ($n=30$) and those with ALL ($n=15$) were consecutively diagnosed in a single institution, between 2001 and 2002. A study was carried out and samples were obtained with informed consent and ethical review board approval.

Controls selection

The control group consisted of 100 unrelated healthy adults. Blood samples were collected from blood donors and volunteers. The control panel can be considered representative for the population of Campinas, in Southeast Brazil and reasonably corresponds to the regional distribution of the patients' panel.

HLA class II typing

Blood samples were collected in EDTA. Genomic DNA of 45 patients and 100 controls was extracted from peripheral blood leucocytes by the salting-out method [20]. All class II genes from patients and controls were typed, HLA-DRB1, -DQB1 genotyping was carried out by the PCR and sequence-specific primers (PCRSSP) method [21,22] Kits (One Lambda Inc. Ca-EUA). These Kits allow medium-resolution DNA-typing of all expressed HLA-DRB1, -DRB3, -DRB4, -DRB5 and HLA-DQB1 loci for all samples [21]. PCR was performed on M J-PTC-100, with appropriate cycling conditions [21].

Typing for the HLA alleles was performed at the Immunogenetics Laboratory of Transplantation, Department of Clinical Pathology, School of Medical Science, UNICAMP which is accredited for clinical HLA typing by the "Serviço Unico De Saude—SUS"[1] and the Brazilian Association of Histocompatibility.

Statistical analysis

Associations between each allele and genotype in patients and controls were compared by means of Chi-square or a two-tailed Fisher's exact test. Level of significance was set to p value<0.05; corrected p values were obtained by multiplying the number of alleles tested for each locus. Relative Risk (RR) was calculated using the method of Woolf with Haldanes' continuity correction [23]. Haplotype frequencies and linkage disequilibrium for two-loci haplotypes were calculated by applying the formula estimated from population data [24].

Results

45 patients with leukemia and 100 control individuals were used for the association analysis. In LMA patients, 97 HLA-DRB1/3/4/5 alleles and 53 HLA-DQB1 alleles were found, while in patients with acute lymphoblastic leukemia, 49 HLA-DRB1/3/4/5 alleles and 25 HLA-DQB1 alleles were detected. 340 HLA-DRB1/3/4/5 alleles and 183 HLA-DQB1 alleles were found in the control group (Figs. 1 and 2). Association of HLA-DRB1 and DQB1 alleles with ALL resulted in a positive association (RR = 3.0588) of the allele HLA- DRB1*07 with the disease, with a RR = 6.633.

For ALL, an increase of the following alleles was frequently found when compared with the control group: DRB1*03 ($p=0.0086$) and DQB1*02 ($p=0.01$). Further, a decrease of the following alleles was often found when compared with the control group: -DRB1*04 ($p=0.02$) and -DQB1*03 ($p=0.003$) (Tables 1 and 2).

The allele HLA-DQB1*03 showed a reduced frequency in comparison with the control group, both for AML ($p=0.0015$) and for ALL, which suggests a possible association for protection from acute leukemias (Table 2).

Association of HLA-DRB1 and DQB1 alleles in patients with acute myeloid leukemia and acute lymphoblastic leukemia

In the case of acute myeloid leukemia, the HLA-DRB1*07 allele showed a positive association (RR = 3.0588) with the disease, with $\chi^2=6.633$ and $p=0.01$. On the other hand, the HLA-DQB1*03 allele showed a negative association (RR = 0.2576) with the illness, with $\chi^2=10.1407$ and $p=0.0015$.

Regarding acute lymphoblastic leukemia, the HLA-DRB*03 (RR = 4.4615; $\chi^2=6.8943$ and $p=0.008$) and the HLA-DQB1*02 (RR = 4.0606; $\chi^2=6.3152$ and $p=0.01$) alleles showed a positive association with the disease, while the HLA-DRB1*04 allele (RR = 0.1327; $\chi^2=4.8692$ and $p=0.02$) and the HLA-DQB1*03 allele (RR = 0.1873; $\chi^2=8.4723$ and $p=0.003$) showed a negative association with the disease (Table 2). Corrected p was carried out for all of the significant values.

The positive and negative associations for the alleles are represented in AML and ALL.

Association with HLA-DRB1 and DQB1 haplotypes

A comparison of HLA-DR/DQ haplotype frequencies in patients with acute myeloid leukemia, acute lymphoblastic leukemia and the control group was carried out. Regarding acute myeloid leukemia, DRB1*07 DQB1*02 haplotype (RR = 2.84, $p=0.01$) was found to be positively associated with the disease.

As for acute lymphoblastic leukemia, DRB1*04 DQB1*03 haplotype (RR = 0.000, $p=0.01$) was found to be negatively associated with the disease. Haplotype analysis detected increased frequency of HLA-DRB1*07/DQB1*02 ($p=0.01$, RR = 2.84) in AML and in ALL patients; lower frequency was found concerning the controls HLA-DRB1*04/DQB1*03 haplotype ($p=0.01$, RR = 0.000) (Fig. 3).

Discussion

The biological importance of HLA association in leukemia is emphasized by the fact that HLA may be involved in the identification of candidate genes, which confer the advantage of resistance and the disadvantage of disease susceptibility.

As the initial studies on mouse susceptibility to leukemia showed that the H-2k haplotype caused increased susceptibility to both virus-induced and spontaneous leukemia and the H-2b haplotype seemed

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