RELATIONSHIP BETWEEN HLA A*02 A*03 ASSOCIATION AND THE RISK OF CHILDHOOD ACUTE LEUKEMIA

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Abstract: In relation with the increasing incidence of childhood leukemia, the present study aims to investigate correlations between A*02 human leukocyte antigen (HLA) genotype and immuno-hematological malignant pathology. HLA alleles were genotyped by PCR with sequence-specific oligonucleotides (PCR-SSO) technique utilizing an automatic DNA extractor. SSO profile utilizes a PCR amplification with biotinylated primers and oligonucleotide specific sequences, which hybridize with polymorphic sequences of the HLA target locus. This study found that A*02 A*03 HLA association confers a higher susceptibility for a specific forms of leukemia (acute lymphoblastic leukemia (ALL)), but does not change significantly the risk for acute myeloid leukemia (AML).

INTRODUCTION

Hematopoietic stem cell transplantation is a curative treatment for a various hematological disorders, as malignant diseases (acute and chronic leukemia, myelodysplasic syndromes), aplastic anemia or autoimmune diseases. Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy in developed countries, where it constitutes over 30% of childhood cancers (Stiller et al, 1995, Taylor et al, 2008). During the last few years, stem cells are frequently collected from peripheral blood or even from ombilical cord, but the main source of stem cells still remains the bone marrow in 75% cases. HLA typing is utilized to identify HLA allele. The existence of a donor-receptor compatibility is the best chance for successful transplantation and reintegration in the social life of the individual.

The HLA loci of the human Major Histocompatibility Complex (MHC) located on the short arm of human chromosome 6 (Francke and Pellegrino, 1977, Trowsdale et al, 1985) encode two distinct classes of highly polymorphic cell surface molecules that bind and present processed antigens in the form of peptides to T lymphocytes.

The class I molecules, such as HLA-A, are found on most nucleated cells. They are cell surface glycoprotein’s that bind and present processed peptides derived from endogenously synthesized proteins to $CD8^+$T cells. These heterodimers consist of an HLA-encoded alpha chain associated with the non-MHC-encoded polypeptide, β_2-microglobulin. While β_2-microglobulin is monomorphic, the alpha-chain genes are extremely polymorphic. This variability is localized primarily in exons 2 and 3 (Marsh, 1998), which encode the amino-terminal extracellular domains that function as the peptide-binding site. Within these two exons, the polymorphism is concentrated into discrete clusters that lie within a relatively conserved framework region. Analysis of HLA class I crystal structures, has shown that these polymorphic residues line the peptide-binding cleft and interact directly with peptide and/or the T-cell receptor (Bjorkman et al, 1987, Garrett et al, 1989, Madden et al, 1992).

In relation with the increasing incidence of childhood leukemia, the present study aims to investigate correlations between A*02 A*03 HLA genotype and immuno-hematological malignant pathology.

MATERIALS AND METHODS

We extracted data on 59 patients diagnosed with two different types of leukemia, and 65 healthy subjects. Informed consent was obtained from all subjects and the experiments performed for this investigation comply with current guidelines and ethics. Blood sample collection and HLA molecular typing were carried out with national and local ethical consent (Feltbower et al, 2005). Blood was collected on in order to establish HLA genotype for medular transplant.

The genotyping for the *HLA-A* was performed using PCR-SSO technique utilizing an automatic DNA extractor MagNA Pure LC DNA isolation with a specific kit. The Dynal RELI SSO HLA-A test is based on three processes, as PCR-target amplification (Saiki et al, 1985, Mullis and Faloona, 1987), hybridisation of the amplified products to an array of immobilized sequence-specific oligonucleotide probes, and detection of the probe-bound amplified product by colour formation (Saiki et al, 1989).

The reagent mixture for the PCR contains the DNA specimen which is heated to 95ºC, in order to separate the double-stranded DNA and to expose the specific primer target sequences. The mixture is cooled and the biotinylated primers

anneal to their targets. The thermostable recombinant *Thermus aquaticus* (Taq) DNA polymerase in the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of deoxythymidine), extends the annealed primers along the target templates to produce a biotinylated DNA sequence termed an amplicon. This process is repeated for a number of cycles, each cycle effectively doubling the amount of target DNA. For this test, the required number of cycles has been determined to be 35, theoretically yielding more than a billion-fold amplification.
After the PCR amplification process, the amplicons are chemically denatured to form a single-stranded DNA, these are added to a nylon membrane which contains an array of immobilized, sequence-specific oligonucleotide (SSO) probes. The biotin-labelled amplicons bind (hybridise) to those SSO probes that contain a complementary target sequence and thus are „captured" onto the membrane strip.
A wash step after hybridisation ensures the specificity of the reaction and removes all unbound amplicon.
The amplicon-probe complex is visualised using a colourimetric reaction. Streptavidin-horseradish peroxidase (SA-HRP) conjugate is added to the membrane and binds to the biotin-labelled amplicons captured by the SSO probe. Addition of hydrogen peroxide (H_2O_2) and tetramethylbenzidine (TMB) substrate, results in the formation of a blue colour complex in the presence of SA-HRP. The resulting probe signals are compared to the control probe intensity and the samples hit pattern recorded for interpretation.
The AutoRELI 48 Instrument automates the hybridisation reaction and detection reaction.
Statistical analysis were made using a version of Microsoft Office Excel 2003.
Was also calculated odds ratio (OR) as per Haldane modified Woolf's formula: (Haldane, 1955, Gopalkrishnan et al, 2006), $OR = [(a+0.5)(d+0.5)/(b+0.5)(c+0.5)]$, where, a and b are the number of patients and controls positive for a given allele, respectively; c and d represent the number of patients and controls negative for the allele, respectively.

RESULTS AND DISCUSSIONS

There are some of the associations HLA-diseases are: insulin dependent diabetes mellitus and HLA-DR3 and –DR4, ankylosing spondylitis and HLA-B27, rheumatoid polyartritis and HLA-DR1 and –DR4 (Paunescu and Homberg, 1999). Acute lymphoblastic leukemia is a distinct morphologic form of leukemia, albeit with a diversity of molecular subsets, and in children, common (precursor B-cell) acute lymphoblastic leukemia is the predominant subtype of leukemia, comprising the largest subgroup of malignant disease in this age group (Feltbower et al, 2005, MacKenzie et al, 2006). There is now a compelling evidence that chromosome translocations are often the first or initiating events in leukemia, occuring prenatally during fetal development (Greaves, 2002).
We extracted data on 59 patients diagnosed with two different types of leukemia: 38 patients with acute lymphoblastic leukemia (ALL), 21 patients with acute myeloid leukemia (AML), and 65 healthy controls. The results included information about patients, which were included in transplant programme, registered in the Regional Centre for Transplant Immunology of the West, South-West, and North-West part of Romania between 2008 and 2010.
In figure 1 is presented the percent distribution of patients with ALL and AML compared to the healthy controls.

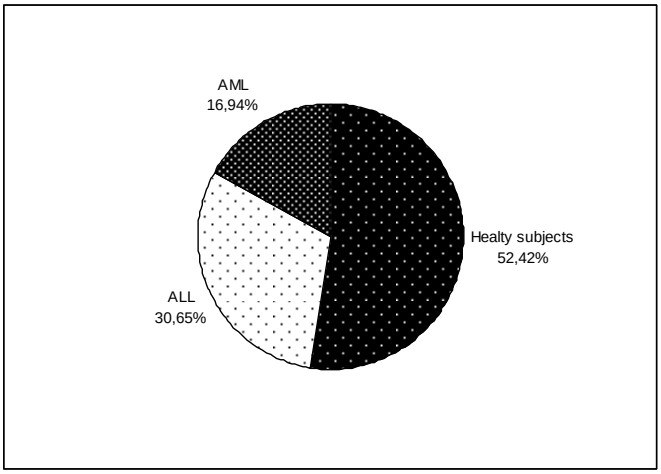

Figure 1. AML and ALL patients, and healthy controls percent distribution.

One HLA-A02*A03 allele was detected in healthy controls as in the patients with AML, while in patients with ALL were detected five. The frequency of HLA-A02*A03 in patients with ALL (13.16%) was higher than the controls (1.54%) and the patients with AML (4.76%). Also a higher frequency of this allele was found in patients with AML (4.76%) than the controls (1.54%) (Table 1).

Table 1. Frequencies of HLA-A*02*A*03 allele (%) in patients with ALL and AML and healthy controls.

	Healty controls (N=65)	ALL (N=38)	AML (N=21)
A*02*A*03 (%)	1,54	13,16	4,76

Alleles of HLA-A02*A03 significantly associated with the susceptibility to ALL identified by a P-value lower than 0.01. The differences between AML patients, with A02*A03 allele and healthy controls are not significantly important (P-value higher than 0.5).
A striking difference was observed for HLA-A02*A03 allele in patients with ALL as against healthy controls (OR – 6.30; P = 0.008). In patients with AML was found an odds ratio of 1.72, meaning that the presence of HLA-A02*A03 allele does not represent a risk factor to develop the disease.

CONCLUSIONS

This study found that A*02 A*03 HLA association confers a higher susceptibility for a specific forms of leukemia (ALL) but does not change significantly the risk for AML.

REFERENCES

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L., Wiley, D.C., (1987): *The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens*. Nature, 329, 512-518

Feltbower, R.G., Manda, S.O.M., Gilthorpe, M.S., Greaves, M.F., Parslow, R.C., Kinsey, S.E., Bodansky, H.J., McKinney, P.A., (2005): *Detecting Small-Area Similarities in the Epidemiology of Childhood Acute Lymphoblastic Leukemia and Diabetes Mellitus, Type 1: A Bayesian Approach*. Am J Epidemiol., 161, 1168–1180
Francke, U., Pellegrino, M.A., (1977): *Assignment of the major histocompatibility complex to a region of the short arm of chromosome 6*. Proc.Natl.Acad.Sci USA, 74, 1147-1151
Garrett, T.P.J., Saper, M.A., Bjorkman, P.J., Strominger, J.L., Wiley, D.C., (1989): *Specificity pockets for the side chains of peptide antigens in HLA-Bw68*. Nature, 342, 692-696
Gopalkrishnan, L., Patil, S., Chhaya, S., Badwe, R., Joshi, N., (2006): *HLA alleles in pre-menopausal breast cancer patients from western India*. Indian J Med Res, 124, p. 305-312
Greaves, M., (2002): *Childhood leukemia*. BMJ, 324, p. 283-287
Haldane, J.B.S., (1955): *The estimation and significance of the logarithm of the ratio of frequencies.* Ann Hum Gent, *20* : 309-14
MacKenzie, J., Greaves, M.F., Eden, T.O.B., Clayton, R.A., Perry, J., Wilson, K.S., Jarrett, R.F., (2006): *The putative role of transforming viruses in childhood acute lymphoblastic leukemia*. Haematologica, 91, 240-243
Madden, D.R., Gorga, J.C., Strominger, J.L., Wiley, D.C., (1992): *The three-dimensional structure of HLA-B27 at 2.1a resolution suggests a general mechanism for tight peptide binding to the MHC*. Cell, 70, 1035-1048
Marsh, S.G.E., (1998) Anthony Nolan Bone Marrow Trust Website, http://www.anthonynolan.org.uk
Mullis, K.B., Faloona, F.A., (1987): *Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction*. Methods in Enzymology, 155, 335-350
Paunescu, V., Homberg, J.C., (1999): *Immunology*, Ed. Orizonturi Universitare
Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., Arnheim, N., (1985): *Enzymatic amplification of beta-globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia*. Science, 230, 1350-1354
Saiki, R.K., Walsh, P.S., Levenson, C.H., Erlich, H.A., (1989): *Genetic analysis of amplified DNA with immobilised sequence-specific oligonucleotide probes*. Proceedings of the National Academy of Sciences USA, 86, 6230-6238
Stiller, C.A., Allen, M.B., Eatock, E.M., (1995): *Childhood cancer in Britain: the National Registry of Childhood Tumours and incidence rates 1978-1987*, Eur J Cancer., 31A, 2028-2034
Taylor, G.M., Hussain, A., Lightfoot, T.J., Birch, J.M., Eden, T.O.B., Greaves, M.F., (2008): *HLA-associated susceptibility to childhood B-cell precursor ALL: definition and role of HLA-DPB1 supertypes*. British Journal of Cancer, 98, 1125 – 1131
Trowsdale. J., Young, J.A., Kelly, A.P., Austin, P.J., Carson, S., Meunier, H., So, A., Erlich, H.A., Spielman, R.S., Bodmer, J., (1985): *Structure, sequence and polymorphism in the HLA-D region*. Immunol.Rev., 85, 5-43

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