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HLA-DR2 Frequency Increase in Severe Aplastic Anemia Patients is Mainly Attributed to the Prevalence of DR15 Subtype

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The association between severe aplastic anemia (AA) and DR2 antigen seems to be well established. However, since discrimination between two DR2-associated splits, namely DR15 and DR16, rarely was performed, it remains unclear whether one or both of these subvariants are responsible for AA susceptibility. In this study, we have analyzed the HLA-DR allelic distribution in a group of 37 AA patients of slavic origin from North-Western Russia. The experimental design included PCR-based amplification of DRB-specific sequences, followed by reverse dot-blot hybridization of the biotinylated PCR-product with the set of sequence-specific oligonucleotide probes. HLA-DRB alleles

were identified by non-radioactive enzymatic reaction, then standard serological specificities of HLA-DR antigen were estimated according to the WHO nomenclature. Whereas DR15 subtype occurred more often in the patients (23.0% vs. 13.3%, $p < 0.05$), DR16 split did not show the same tendency. The results, show the overall predominance of HLA-DR2 specificity (DR15+DR16) did not reach statistical significance (24.4% vs. 17.5%, $p < 0.2$). Thus, we conclude that repeatedly reported DR2 frequency increase in AA patients is mainly attributed to the prevalence of DR15 subtype. (Pathology Oncology Research Vol 3, No 2, 106-108, 1997)

Key words: aplastic anemia; HLA-DR antigens; DNA extraction

Introduction

Aplastic anemia (AA) is a rare disorder of haematopoiesis resulting in the reduction of peripheral blood cellularity and hypoplasia of bone marrow. The etiology of AA is rather unclear. Many investigations reported the trigger role of viral infections,^{9,23,24,26} or chemical compounds^{6,25} in pathogenesis of AA. However, infrequent occurrence of AA in exposed groups implies an involvement of other mechanisms in disease development. In particular, the autoimmune component seems to contribute into AA manifestation.² For instance, Torok-Storb et al. observed the suppression of granulocyte colony formation by acti-

vated T-cells from AA patients.²¹ Later, Hanada et al. found the inhibitory effect of autologous T-cells from AA individuals (measured on erythroid colony formation), which was reversible by AA-specific treatment.⁷

In addition, unusual distribution in HLA profiles, being very typical for nearly all autoimmune diseases,²⁰ has been repeatedly demonstrated for AA cohorts.^{1,4,5,8,11,14,15} Several research teams revealed some AA-associated HLA class I phenotypes,^{1,5} but others failed to confirm these data.¹⁹ More consistent results were obtained for HLA class II molecules. For instance, increased prevalence of HLA-DR2 specificity in AA patients appears to be a reproducible phenomenon.^{4,8,11,14,15} Moreover, the presence of DR2 antigen was supposed to predict the efficiency of immunosuppressive therapy.^{8,11,15}

Noteworthy, HLA-DR antigen determination was usually performed by means of serological or cellular typing in most of these studies.^{4,8,11,14,15} However, during the last decade, these traditional procedures have often

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Table 1. The HLA-DR allelic distribution among AA patients of slavic origin from North-Western Russia.

DR antigen specificity	HLA-DRB allele	healthy donors		AA patients	
		number of alleles	frequency (%)	number of alleles	frequency (%)
DR1	DRB1*01	49	12.3	6	8.1
DR2(15)	DRB1*15	53	13.3	17	23.0
DR2(16)	DRB1*16	17	4.2	1	1.4
DR3	DRB1*03	38	9.5	6	8.1
DR4	DRB1*04	56	14.1	16	21.6
DR11	DRB1*11	43	10.8	6	8.1
DR12	DRB1*12	11	2.8	1	1.4
DR13	DRB1*13	50	12.5	12	16.2
DR14	DRB1*14	9	2.3	0	0.0
DR7	DRB1*07	48	12.1	8	10.7
DR8	DRB1*08	15	3.8	1	1.4
DR9	DRB1*09	5	1.3	0	0.0
DR10	DRB1*10	4	1.0	0	0.0
Total number of alleles		398	100	74	100
Total number of individuals		199	100	37	100

become replaced by polymerase chain reaction (PCR)¹⁶ based techniques. Re-evaluation of the reported HLA-disease associations at the DNA level has a special importance for DR2-linked pathologies: whereas, routine typing antisera identify DR2-positive individuals as a homogenous group, DNA analysis allows to discriminate 2 different antigenic variants (DR15 and DR16) within DR2 specificity.

Taking also into account the existence of interethnic variations for many of the HLA-disease correlations reported,²² we were able to analyze the HLA-DR allelic distribution in the group of AA patients from North-Western Russia by PCR-based genotyping.

Materials and methods

DNA isolation

Thirty seven AA patients of slavic origin from North-Western Russia were studied. 199 unrelated healthy persons of the same ethnic origin were taken as a control group. Genomic DNA from peripheral leukocytes was extracted by simple salting-out procedure.¹⁰ Briefly, 5 to 7 ml of unfractionated EDTA-stabilized peripheral blood were frozen at -20°C for 2 hours or more. Prior to DNA extraction, the collected blood was thawed at 37°C and then thoroughly mixed with 2 volumes of cold double distilled water for complete lysis of erythrocytes. Further centrifugation at 2000g for 10 min was followed by stan-

dard proteinase K treatment of the pellet. After performing the salting-out procedure, DNA was precipitated by 2 volumes of ethanol.

HLA-DRB genotyping

Polymorphic sequences of HLA-DRB genes were amplified by the set of biotinylated primers¹⁸ in 20 µl of standard PCR mixture: 67 mM Tris-HCl, pH 8.8; 16.6 mM ammonium sulfate; 0.01% Tween-20; 1.5 mM MgCl₂; 200 µM each of dNTPs; 500 nM primers; 1 unit of Taq-polymerase (Biomaster, Moscow) and 100 ng of template DNA. 35 PCR cycles consisted of 50 sec at 94°C (denaturation step), 50 sec at 55°C (primers annealing) and 45 sec at 72°C (DNA synthesis). Then PCR-product was hybridized with the panel of sequence-specific membrane-bound oligonucleotide probes as described by Saiki et al.¹⁷ Visualization of the results of hybridization by coloured enzymatic reaction allowed to identify HLA-DRB genotype. Respective serological specificities of DR antigen were estimated according to the WHO Nomenclature.³ Standard chi-square test was used for statistical analysis of the data.

Results

The distribution of DRB alleles and corresponding HLA-DR specificities in AA patients and healthy persons is shown in *Table 1*. The frequency of one of the DR2 subtypes, namely DR15, was almost twice higher in patients compared to control group (23.0% vs. 13.3%, $p < 0.05$). However, the incidence of the remaining subvariant of DR2 specificity, known as DR16 antigen, did not follow the same tendency. This controversy resulted in the lack of statistically significant variations for the overall DR2 serotype (DR15+DR16 splits) occurrence between AA and controls (24.4% vs. 17.5%, $p < 0.2$). The incidence of other HLADR specificities in both groups was rather similar, except for border-line DR4 frequency increase in AA individuals (21.6% vs. 14.1%, $p < 0.1$).

Discussion

The discovery of the links between HLA genotype peculiarities and AA susceptibility has attracted a great deal of attention due to possible practical benefits. In general, such studies may allow to develop clinical tests for the determination of individual predisposition to AA, and, thus to optimize the disease prevention, diagnosis and treatment. In the present study we actually failed to confirm a published correlation^{8,14} between DR2 serotype and aplastic anemia, but our observations suggest key role of the one of DR2 subtypes, namely DR15 split, in AA susceptibility.

The development of preventive actions for AA on the basis of sole HLA-DR typing is unlikely, since on the one

hand the occurrence of DR15 specificity in the general population is as high as compared to the incidence of the disease, and on the other hand, many AA patients do not contain predisposing HLA-DRB alleles in their genotype. However, clinical applications of the published data^{8,11-13,15} regarding the association between treatment efficiency and HLA pattern look more probable. Unfortunately, therapeutic response has not yet been considered in our study, since only small number of patients received specific therapy.

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