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# SPONDYLOARTHRITIS – ASSOCIATED HLA-B PHENOTYPES IN NORTHEASTERN ROMANIA POPULATION

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Abstract

Aim: The aim of our survey was to identify spondyloarthritis – associated to HLA -B phenotypes in the population from north-eastern part of Romania. Genetic (HLA or non-HLA genes) and infectious factors are involved in determining spondyloarthritis susceptibility and severity. The most frequent association is with HLA-B27 antigen, but the extent of association of this antigen with spondyloarthritis depends on the ethnical and geographical context of the population investigated.

Patients and method: HLA-B antigens were determined using the CDC-NIH (complement- dependent- cytotoxicity- National Institute of Health) assay. We compared the frequency of HLA-B antigens expression in the study group, consisting of 40 spondyloarthritis patients (35 with ankylosing spondilitis) and the control group, including 100 healthy people.

Results and conclusions: HLA-B27, HLA-B7 and HLA-B35 antigens were expressed more frequently in patients with spondyloarthritis compared to controls. The difference was statistically significant for HLA-B27 and HLA-B7 antigens ($p = 0.009$ and $p = 0.041$). Although HLA-B35 was present in 31.4% of the patients it did not involve a significant risk for developing ankylosing spondilitis compared to controls ($p = 0.445$).

Key words: spondiloarthritis, HLA and non HLA genes, HLA-B27 antigen, susceptibility.

Rezumat

Scop: Scopul studiului a fost evaluarea fenotipurilor HLA-B asociate cu spondiloartrite in populatia din NE Romaniei. Factori genetici (gene HLA sau non-HLA) si infecţioşi sunt implicaţi în determinismul susceptibilităţii şi severităţii spondiloartritelor. Cea mai frecventă asociere este cu antigenul HLA-B27, dar ponderea asocierii acestei fenotip la spondiloartrite este dependentă de contextul etnic şi geografic al populaţiei analizate.

Material şi metodă: Antigenele HLA-B au fost determinate prin tehnica CDC-NIH (complement-dependent-cytotoxicity - National Institute of Health) la 40 de pacienţi cu spondiloartrite (dintre care 35 cu spondilită anchilozantă) şi 100 de martori sănătoşi şi s-a comparat frecvenţa expresiei antigenelor HLA-B în cele două loturi studiate.

Rezultate si concluzii: Antigenele HLA-B27, HLA-B7 si HLA-B35 au fost exprimate cu frecvenţă mai mare la pacienţii cu spondiloartrite comparativ cu lotul martor.

Diferențe semnificative statistic au inregistrat antigenele HLA-B27 si HLA-B7 (p=0,009 si p=0,041). HLA-B35, deşi prezent la 31,4% dintre pacienti, nu a prezentat un risc semnificativ de dezvoltare a spondilitei anchilozante față de lotul martor (p=0,445).
Cuvinte cheie: spodiloartrite, gene HLA şi non HLA, antigen HLA-B27, susceptibilitate.

## INTRODUCTION

Spondyloarthritis include a heterogeneous group of chronic inflammatory diseases characterized by vertebral and peripheral involvement of the musculoskeletal system, ossifying lesions of the locomotor system, high frequency of enthesopathies, often associated with mucocutaneous, intestinal and ocular involvement, in the absence of the rheumatoid factor (1, 2). Spondyloarthritis include ankylosing spondylitis, reactive arthritis, psoriatic arthritis, enteropathic arthritis, juvenile spondyloarthritis, undifferentiated spondyloarthropathies.

The most important disease in the group is ankylosing spondylitis, defined as a chronic inflammatory disease, mainly affecting spinal and sacroiliac joints, that progressively evolves towards ankylosis. It mainly affects men aged 20-30. About 5% of patients present a family history of the disease (3). During progression the disease there are often associated psoriasis, reactive arthritis, chronic enteropathies, anterior uveitis. The inflammation is localized at the synovium, subchondral bone, capsular fibrous tissue, ligamentous insertions, muscle sheaths, affecting sacroiliac, posterior inter-vertebral, costo-vertebral, inter-vertebral, hips, shoulders and peripheral joints.

Pathological lesions reveal a chronic inflammation characterized initially by hypervascularization and an inflammatory infiltrate with lymphocytes, plasma cells, macrophages, followed by fibrosis.

Genetic and infectious factors are linked with spondyloarthritis susceptibility. Some distinct MHC alleles, especially HLA-B27, are frequently associated with these diseases. More than 90% of the European patients with ankylosing spondylitis are HLA-B27 – positive, compared to 6-10% incidence of the antigen in normal population (4, 5, 6).

HLA-B27 expression was also reported in 70% of the patients with reactive arthritis and inflammatory bowel disease-associated spondyloarthritis , in 50% of acute anterior uveitis patients and in 25% of the patients with psoriatic peripheral arthritis (2). HLA-B27 implication in spondylo-arthritis pathogeny was not confirmed in other geographic areas (7).

Recent data suggest that genetic susceptibility in spondylo-arthritis extends beyond the HLA-

B27 allelic variants and MHC - complex genes (2, 7, 8, 9, 10, 11). The aim of our study was to evaluate spondyloarthritis – associated HLA-B phenotypes in Northeastern Romanians.

MATERIALS AND METHODS:

The patients group included 40 persons, 15 women and 35 men, aged between 15 and 56, diagnosed with ankylosing spondylitis (35 patients), arthropatic psoriasis (3 patients), acute anterior uveitis (2 patients). The diagnosis of spondyloarthritis was based on clinical and radiological criteria.

The control group included 100 unrelated persons, 59 women and 41 men, aged between 20 and 60, with similar socio-economical and ethnical background to the study group. Controls are employees of the Immunology and Genetics Laboratory, Oncology Clinic of the "Saint Spiridon" Hospital and potential donors for renal transplantation.

HLA typing. HLA-B antigens were determined using the microlymphocytotoxicity assay CDC-NIH (complement- dependent - cytotoxicity- National Institute of Health), as previously described (12). The Terasaki plates preparation and HLA antisera distribution was done in our laboratory. Human HLA antisera with known specificities were a kind gift from Professor Marc De Bruyere and of Lutgarde Berckmans, from the Immuno-hematology Laboratory, "St. Luc" Hospital in Brussels. The 58 HLA antisera used allow the identification of all types and subtypes of HLA-B molecules described to date in the HLA nomenclator. A quantity of 1 µl from a 5000 lymphocytes/µl suspension was distributed in each well of the Terasaki plate. Lymphocytes were incubated with antisera for 30 minutes, at room temperature, and then 5-6 µl of rabbit complement were added to each well. After 60 minutes of incubation the reaction was visualized with eosin, at an inverted microscope with phase contrast. Cells were lysed in the presence of complement and corresponding antibodies. The designation of a certain HLA phenotype was made when a positive specific reaction of that phenotype was detected in at least two wells of the Terasaki plate.

Statistical analysis. Data were processed by calculating the percentages. Odd ratio (OR) corresponding to a 95% confidence interval (CI) was calculated for the frequently – expressed HLA phenotypes (13). OR indicates the susceptibility of a person with a disease - associated HLA phenotype to develop a certain disease, compared to a person that does not have that HLA phenotype.

Data were analysed using Epi Info 6 program and considered statistically significant when $p< 0.05$.

RESULTS:

HLA-B phenotypes frequently identified in the spondyloarthritis patients included in our study group were HLA-B27, -B7 and –B35. The patients with psoriatic arthropathy, acute anterior uveitis and 45.7% of the patients with ankylosing spondylitis expressed HLA-B27, while this phenotype characterized only 21% of controls (OR=3.17; p=0.009) (Table1).

Table 1. HLA-B phenotypes frequently expressed in ankylosing spondylitis patients compared to controls.

| HLA –B antigens | Antigens frequency (%) | | Results | |
|---|---|---|---|---|
| | Patients n=35 | Controls n =100 | Controls vs patients OR (CI 95%) | p |
| HLA-B27 | 45.7 | 21 | 3.17 (1.29-7.81) | 0.009 |
| HLA-7 | 22.8 | 8 | 3.41 (1.04-11.23) | 0.041 |
| HLA-B35 | 31.4 | 23 | 1.53 (0.60-3.89) | 0.445 |

HLA-B7 (22.8% in patients versus 8% in controls, OR=3.41, p=0.041) and HLA-B35 antigens (31.4% in patients compared to 23% in controls, OR=1.53, p=0.445) were as well expressed with higher frequency in patients with ankylosing spondylitis compared to controls. Despite its presence in 31.4% of the patients, HLA-B35 molecule did not reach the statistically significant threshold (p=0.445). In the study group a positive association with ankylosing spondilitis, with statistically significant differences, was noticed only for HLA-B27 and HLA-B7 (p=0.009 and p=0.041, respectively).

DISCUSSIONS

Genetic (HLA and non-HLA genes) and infectious factors are involved in inducing susceptibility and severity in spondylarthritis (10, 11). The most frequent association is related to HLA-B27 antigen. The extent of the association of this phenotype with spondylarthritis

depends on the ethnical and geographical context of the studied population. European and Asian populations associate HLA-B27 antigen with 80-95% of ankylosing spondylitis (4, 6, 14, 15, 16), while in Africans ankylosing spondylitis susceptibility does not imply HLA-B27 phenotype (7).

More than 25 HLA-B27 alleles have been identified, those associated with inducing disease susceptibility being HLA-B*2705, HLA-B*2704, HLA-B*2702 and HLA-B*2707; HLA-B*2706 and HLA-B*2709 confer spondylarthritis resistance (11, 17, 18, 19). The details of HLA-B27 – dependant mechanism of susceptibility to spondylarthritis have not been completely described, but there have been described several ways that might be operational in the intervention of this molecule to spondyloarthritis pathogenesis:

1. The actual model of HLA-B27 pathogenic implication is dominated by its conformational characteristics. α chains form homodimers with disulfide links between cys 67 from the α1 domains. This allows an atypical membrane expression, independent of β2-microglobulin association, resulting from aberrant folding (20, 21). HLA-B27 homodimers were identified on cells from spondyloarthritis patients. These atypical molecules are functional and present peptides to T or NK cells (2). HLA-B27 homodimers resulted from aberrant folding accumulate in the endoplasmic reticulum and activate, through chaperons, cellular stress responses with proinflammatory cytokine production (22). 2. The theory of HLA-B27 implication in spondyloarthritis relies on the ability of these molecules to present to CD8+ T cells, and possibly to NK cells, certain specific "arthritogenic" peptides (self, HLA-B27-derived or bacterial), present only in joints and/or spondyloarthritis-affected organs. The theory of HLA-B27 - restriction of cytotoxic T cells from the inflammatory synovial fluid is sustained by the experimental blocking effect of anti-HLA-B27 antibodies, in cytotoxicity assays using cartilage –derived peptides (collagen IV-derived nanomer) (23). Molecular mimetism seems to be the mechanism of such anti-self responses. The elements of this mimetism are several sets of exogenous peptides, either bacterial or peptide fragments from self molecules (HLA-B27-derived, peptidoglycans). In each set, the association of the peptides with the HLA-B27 binding cleft creates the same TCR-binding surface (17, 21, 24). Peptides in every such set can be presented in different

ways in the binding grooves of the risk-associated-HLA-B27 alleles compared to those of the alleles that are not spondyloarthritis-associated (24). Although arthritogenic peptides have not been identified, the ability to present them, depending on the aminoacid residues forming the groove, is important in inducing spondyloarthritis susceptibility. Thus, HLA-B*1403 presents similar sets of peptides as HLA-B*2705 molecule (11). By consequence, these alleles are considered to be important for susceptibility to spondyloarthritis in HLA-B27 - negative persons. There are some reports on other HLA class I molecules with high risk for ankylosing spondilitis development in HLA-B27 negative persons: HLA-B60, –B61 and molecules of B7 cross-reactive group (10, 18). By contrast, the difference between aminoacid residues in position 116 in HLA-B*2709 and HLA-B*2705 (His vs. Asp) renders the first molecule protective and the second susceptible in spondyloarthritis (19).

Due to the contribution of the exposed surfaces of the HLA groove to TCR paratopes binding, it is possible that other HLA-B27 cross-reactive alleles might be involved in stimulating reactive T cells in ankylosing spondylitis.

In conclusion, the details of HLA-B allele determinism in spondyloarthritis are incompletely clarified.

In our geographical area, northeastern Romania, characterized by a relatively diverse ethnicity, the contribution of various HLA alleles in determining spondyloarthritis susceptibility was not studied. The present work addresses this issue using a serological approach for HLA-B phenotype identification with a panel of antisera against all HLA-B molecules described to date in HLA nomenclator.

The study group included 40 persons: 35 patients with ankylosing spondylitis, 3 with arthropatic psoriasis, 2 with acute anterior uveitis. HLA-B27 phenotype characterized the patient group, with a highly significant relative risk ($p=0.009$) (35). None of the patients was HLA-B27 homozygous. HLA-B7 phenotype, reported to be spondyloarthritis-associated in HLA-B27-negative persons, is characteristic for autochthonous population (18). The OR calculated for this phenotype registered statistically significant differences compared to controls ($p=0.041$). It is not surprising that HLA-B7 phenotype is disease-associated since HLA-B27 and HLA-B7 belong to the same cross-reactive group. The both molecules have similar aminoacid residues in the HLA cleft, allowing binding of arthritogenic peptides associated with ankylosing spondylitis. HLA-B7- HLA-B27

haplotype characterized a single patient with ankylosing spondylitis. The presence of both HLA predisposing phenotypes in developing ankylosing spondylitis seems to be related to disease severity and treatment resistance. HLA-B35 phenotype present with high frequency among patients did not present significant differences when compared to controls (p=0.445).

CONCLUSIONS:

HLA-B27 and HLA-B7 phenotypes are frequently expressed in spondyloarthritis patients from northeastern Romania, with statistically significant relative risks for these phenotypes (p=0.009 and p=0.041, respectively).

Although present in 31.4% of the patients, HLA-B35 did not present a statistically significant risk of developing ankylosing spondylitis compared to controls (p=0.445).

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