INVESTIGATION OF HLA ASSOCIATION AND EPITOPE SPECIFICITY OF ANTI-ADAMTS13 ANTIBODIES

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Introduction: TTP (thrombotic thrombocytopenic purpura) belongs to the group of thrombotic microangiopathies. The primary form of the disease is characterized by a severe deficiency of the von Willebrand factor-cleaving protease ADAMTS13. In most cases the deficiency is caused by inhibitory autoantibodies, evolvement of which is associated with HLA alleles. These antibodies target conformational and linear epitopes of ADAMTS13.

Aims of our experiments were to confirm the HLA association of the development of anti-ADAMTS13 antibodies in the Hungarian population and to investigate the epitope specificity of anti-ADAMTS13 antibodies of acute TTP patients.

Patients and methods: HLA-DR typing was performed in 64 TTP patients (mean age 41 years, 49 females) and 204 healthy Caucasian controls (mean age 46 years, 117 females).
The binding of antibodies to linear epitopes of the ADAMTS13 enzyme was determined by the pin-ELISA method using 15-amino-acid-long overlapping peptide sequences of the protease. Serum- or citrate-anticoagulated plasma-samples of 15 acute TTP-patients (mean age 37 years, 12 females) were measured with this assay. Samples of 10 healthy individuals (mean age 36 years, 8 females) were used as controls.

Results: There was a significant increase in the frequency of the HLA-DR11 (54,7% vs. 28,9%) and HLA-DR15 (29,7% vs. 14,2%) phenotypes in TTP patients as compared with controls, while the HLA-DR4 (7,8% vs. 20,6%) and HLA-DR7 (12,5% vs. 27,0%) phenotypes were present at a lower frequency in the patient group.
The antibodies showed significant binding to 42 of the 94 ADAMTS13-peptides. We found significant differences in the binding to acute versus control samples in the case of 8 of these peptide epitopes. Antibodies of acute TTP patients bound to the 558Arg-Thr572 and 1163Gln-Arg1177 sequences with higher affinity, while the 568Arg-Ser582, the 573Phe-Asn587, the 578Pro-Phe592, the 583Val-Val597, the 618Pro-Leu632 and the 668Leu-Pro682 peptides showed stronger antibody-binding in the case of control samples.

Discussion: We confirmed the HLA association in the development of TTP with HLA-DR11 and HLA-DR15 increasing the susceptibility to the disease and HLA-DR4 and HLA-DR7 being protective. Our results are in accordance with those of previous studies, although in our investigation not only the associations with the phenotypes HLA-DR11 and HLA-4 proved to be significant, but also those with HLA-DR15 and HLA-DR7.

We have furthermore identified linear peptide-epitopes of the ADAMTS13 protease which are recognized with different affinities by autoantibodies of acute patients and of healthy controls. These epitopes may form the base of a new method facilitating the diagnosis of TTP.

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Poster/Clinical