Abstract

BACKGROUND: HLA molecules play a central role in the immune response. HLA class II are involved in the selection of the T-cell repertoire in the thymus, and in presentation of antigenic peptides to antigen reactive CD4-positive T cells. The HLA class II act as restriction determinants in the presentation of antigens to T lymphocytes and their expression on the cell surface is necessary for triggering the immune responses. Regulated transcription of HLA class II genes is a complex system involving *cis*-acting sequence elements and *trans*-acting protein factors. It has been reported that allelic polymorphism exists in the regulatory regions of HLA class II DQA1, DQB1 and DRB1 genes. Most of the polymorphisms appear to be conserved within a haplotype. The hierarchy of sequence homology which exists among the structural genes is not paralleled among their promoter sequences. It is of interest that the most striking discrepancy was found for the DR4 linked alleles, DQB1*0301 and DQB1*0302, which were described as frequent risk factors for a variety of autoimmune diseases. The structural genes of these two alleles are the most closely related, but their regulatory sequences are the most heterogeneous among DQB1 variants. These sequence differences correspond to functional variation: The promoter strength of the DQB1*0301 allele is three- to four- fold greater than the comparable one of DQB1*0302. Another cause of the functional differences between HLA class II alleles might be a different level of methylation of this region.

AIMS: We aimed to determine the correlation between methylation of CpG dinucleotides in HLA-DQA1 gene promoter and their genotype.

METHODS: 89 healthy donors, age 25-45 years, were included to the pilot study. The genotyping of HLA-DRB1, HLA-DQB1 and HLA-DQA1 was performed using PCR with sequence specific primers. The genomic DNA was converted by bisulfite treatment and the target segment in the promoter of HLA-DQA1 gene was amplified using nested PCR. The PCR product was cloned into *Escherichia coli* (XL-1 Blue). Successful transformants were selected on medium with ampicillin, IPTG and X-Gal. Succesful transformation was confirmed by colony PCR. Then sequencing of individual clones was performed.

RESULTS: We found differential methylation of some CpG sites in the region studied: at site -639, QAP 4.1 allele was methylated to greater extent than QAP 1.1 and 1.3 alleles; at site -540 QAP 3.1 allele was methylated less than QAP 1.1 a 1.3 alleles; at site -374 QAP 1.3 allele was methylated less than any other allele; at site -139 allele QAP 1.2 was methylated less than QAP 1.4 allele. We observed difference in total count of methylated CpG sites in QAP 1.1 allele compared to QAP 1.4 and 4.1 alleles.

CONSLUSION: We found correlation between genotype and epigenotype of promoter alleles of HLA-DQA1 gene. Differences in average methylation of alleles, and also differences in methylation state of individual CpG sites were observed. We also described new polymorphic sites in this region.

Keywords: DNA methylation, polymorphism, promoter, HLA-DQA1