Association of *KIR, HLA* and antigen-presenting machinery genes with susceptibility to and outcome of atopic dermatitis

SUMMARY

Atopic dermatitis (AD) is one of the most common dermatoses. About one fifth of the world's population suffer from AD, but the prevalence of the disease varies in different parts of the world. The pathogenesis of this disease is still unclear, although recent studies emphasize the importance of genetic predisposition in the development of AD. The increase in the incidence rate of atopic dermatitis in developed countries, which coincided with the increase in industrialization, suggests the involvement of environmental factors in the development of atopic dermatitis. Despite the quite well-known immune mechanisms of AD, the role of NK cells in the development of this disease is poorly understood. The most important receptors that inhibit or stimulate the activity of these cells are KIR molecules, the ligands of which are HLA class I molecules. KIR molecules are also present on the surface of a subpopulation of T lymphocytes and regulate their activity. The presence of HLA class I molecules on the cell surface and the effective presentation of antigens in the form of HLA class I peptides depends on the activity of proteins belonging to antigen presenting machinery (APM). The polymorphisms of APM genes: *LMP2, LMP7, TAP1, TAP2, ERAP1* and *ERAP2* may have impact on the activity of their protein products and in this way they may affect the effectiveness of antigen presentation.

The aim of my thesis was to investigate the association between *KIR* genes and their HLA ligands and polymorphic variants of APM genes: *LMP2, LMP7, TAP1, TAP2, ERAP1* and *ERAP2* with susceptibility to atopic dermatitis and the clinical course of the disease.

KIR genes are the major factor influencing phenotype variability and NK cells function. The analysis of the association between activatory and inhibitory KIR receptors and their ligands with the risk of developing AD showed a protective effect only for the *KIR2DS1* gene. There was no difference in the frequency of HLA-C epitopes (C1 and C2) and HLA-B Bw4 and HLA-A Bw4 epitopes between the examined groups. Moreover, the association of the *KIR2DS1* gene with lower risk of AD was independent of the presence or absence of C2 epitope which is a ligand for the product of this gene. Most of the known ligands for KIR receptors are HLA-C molecules. The analysis of their polymorphisms showed that people who do not have the *HLA-C*05* allele have more than two times higher risk of developing AD than people who have this allele.

The repertoire of peptides bound by a given HLA class I molecule depends not only on the peptide binding preferences of a given molecule but also on the APM activity. Thirteen polymorphisms of selected genes coding for proteins in the antigen presenting machinery were analyzed: *LMP2, LMP7, TAP1, TAP2, ERAP1, ERAP2*. It has been shown that among the studied polymorphisms, only *rs26618* in the *ERAP1* gene was associated with the risk of developing AD. Individuals who were carriers of the *C* allele (*C/C* and *C/T*) compared to *T/T* homozygotes had a higher risk of developing atopic dermatitis. In addition, it has been shown that subjects without the *KIR2DS1* gene and being the *C/T* heterozygotes had 1.5 times higher risk of developing the disease than individuals possessing homozygous *T/T* genotype. Moreover, individuals with the *C/C* homozygous genotype, had over 2 times higher risk of developing the disease. The association of SNP *rs26618* with the risk of developing AD only in KIR2DS1-negative subject is difficult to explain, because its biological function has not been described, although it encodes an isoleucin to methionin change in position 276 of the ERAP1 protein. In an effort to understand the functional relevance of the ERAP1 aminopeptidase *rs26618* (Ile276Met) polymorphism, we tested the activity of two variants of aminopeptidase (with Ile267 and Met267) against a N-terminally extended precursor LIVDRPVTLV of the HLA-C*05:01 epitope IVDRPVTLV. Both ERAP1 variants were able to efficiently generate the epitope, although the isoleucine allotype at position 276 was able to generate this about 50% faster. Both variants were quite inefficient in further trimming of the mature epitope to shorter peptides. Their kinetics were up to 100-fold slower compared to epitope generation, but with very similar performance rates for both variants. No association between other tested polymorphic variants of genes encoding proteins belonging to AMP and susceptibility to AD was demonstrated. On the other hand, the analysis of the influence of the *rs2248374 ERAP2* polymorphism which affects the expression of the ERAP2 protein on the *rs26618 ERAP1* polymorphism associated with the risk of AD, showed a synergistic effect only for the *rs2248374*A/A - ERAP1 rs26618*C/C* genotype. Individuals with this genotype had over three times higher risk of developing AD than those with the other genotypes.

The association between the studied genes and selected clinical data of patients was also analyzed. The influence of some polymorphic variants in the *KIR, HLA, LMP2, TAP1, ERAP1* genes on the age of diagnosis, the severity of the disease measured by the SCORAD coefficient and the association with comorbidities such as asthma or allergic rhinitis was observed.

The obtained results showed that genetic variants of investigated here genes may be considered as potential risk factors of atopic dermatitis. The studies are innovative since it is the first report on the contribution of the *KIR* and *HLA-C* genes and variants of *ERAP (1* and *2)* genes to the risk of AD. However, these results should be confirmed on larger European and other populations. In fact, in ethnically diverse populations from different geographic regions, the additional factors favoring the development of AD may exist. In addition, my result is the first evidence of biological function of *rs26618*C>T* (Ile276Met) polymorphism.