The 8.1 ancestral MHC haplotype: a double-edged sword

PhD thesis

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Introduction

The term "conserved extended haplotype" or as also known "ancestral haplotype" (AH) defines highly conserved haplotype—blocks of highly conserved genomic sequences—derived from a common remote ancestor. The main histocompatibility complex (MHC) or human leukocyte antigen (HLA) regions harbour genes—such as HLA-A, B, C, DQ, DR, heat shock proteins, complement components and cytokines like TNF-α and LT-α—that play important roles in the innate or acquired immune response. Variations, alleles of these genes make up conserved extended haplotypes in the MHC regions, extending through class I, class II and class III regions in few megabase. The so-called 8.1 AH consists of, among others, HLA-A1, HLA-B8, HLA-Cw7, TNF-α -308A, C2'C, Bf'S, C4A*Q0 (encoded in the so-called RCCX module), C4B*1 (encoded in the so-called RCCX module), HLA-DRB1*0301, HLA-DQA1*0501 és HLA-DQB1*0201 alleles. Individuals carrying the 8.1 AH have altered cytokine profile, increased TNF-α production, high titers of autoantibodies and circulating immune complexes. These features are thought to be beneficial in response to infections and are thought to have had positive selection during evolution, thereby resulting in accumulation in the Caucasian population. However, the 8.1AH may lead to the development of autoimmune diseases as a long-term side effect.
Aims

1. The RAGE gene is located in the close proximity of the MHC II region; previous preliminary results suggested a genetic linkage between RAGE -429C and TNF-α -308A alleles. Therefore our aim was to clarify whether RAGE -429 T>C polymorphism has a genetic linkage disequilibrium with TNF-α -308 G>A, HLA-DQβ1, HLA-DRβ1 polymorphisms and with an RCCX module that contribute to the 8.1AH.

2. Furthermore we checked if RAGE -429 polymorphism or the 8.1AH show association with type 1 diabetes mellitus or its complications.

3. Major cause of death in patients with cystic fibrosis (CF) is bacterial colonization. The wide phenotypic variation in CF patients suggests that genes other than the cystic fibrosis transmembrane conductance regulator (CFTR) gene modify the disease. Cystic fibrosis patients are constantly immunochallenged due to the disease causing genetic disorder. The 8.1AH is characterized by an altered immune response, high TNF-α and circulating immune complex levels that may be advantageous against infections. We hypothesized that the 8.1AH has a role in chronic infections (colonization) in CF.

4. Several data indicate that alleles encoded in the major histocompatibility complex (MHC) regions may contribute
to the development of colorectal cancer. Therefore we determined the role of a haplotype of alleles in the MHC III region that are known constituents of the 8.1AH in colorectal cancer.

**Methods**

*Detection of gene polymorphisms*

Genotyping of TNF-\(\alpha\) -308 G>A polymorphism was carried out by PCR-RFLP and SSP-PCR. RAGE -429 T>C, HSP70-2 +1267A>G and LTA 252 A>G polymorphisms were determined by PCR-RFLP. Detection of HLA-DQB1 alleles was carried out by SSP-PCR, HLA-DRB1 alleles were determined by SSP-PCR and SSO-PCR.

C4A and C4B polymorphisms in the RCCX module were determined by Petra Kiszel.

*Study populations*

TNF-\(\alpha\) -308, RAGE -429, HSP70-2 1267, C4A and C4B, HLA-DQB1 and HLA-DRB1 polymorphisms were analyzed in the DNA samples of eight informative families affected with type 1 diabetes mellitus, in 82 unrelated patients with type 1 diabetes mellitus, and in unrelated healthy individuals of three different Caucasian populations (Hungarian, Ohioian, Icelandic). TNF-\(\alpha\) -308, RAGE -429, HSP70-2 +1267A>G, HLA-DQB1 and HLA-DRB1 polymorphisms were detected in the DNA samples of
72 CF patients (39 homozygous and 33 heterozygous for ΔF508) and 139 healthy controls. Carriage of the 8.1AH haplotype was considered when RAGE -429C, HSP70-2 +1267 G, TNF-α -308A, HLA-DRB1*0301 and HLA-DQB*0201 were carried simultaneously.

TNF-α -308, RAGE -429, HSP70-2 +1267 and LTA +252 polymorphisms were detected in the DNA samples of 183 Hungarian patients with colorectal cancer (100 males, 83 females) and 141 age matched control subjects. Carriage of the 8.1AH haplotype was considered when RAGE -429C, HSP70-2 +1267 G, TNF-α -308A, and LTA +252G were carried simultaneously.

Results

We are the first to show the RAGE -429C and in another study LTA +252G and HSP70-2 +1267G alleles to be member alleles of the 8.1AH in population level. The detection of TNF-α -308A, RAGE -429C, LTA +252G and HSP70-2 +1267G alleles may simplify the detection of the 8.1AH.

In type 1 diabetics RAGE -429C allele showed strong association with high HbA1C levels (>10%) so with poor metabolic control (p=0.042).

In cystic fibrosis patients frequency of colonization was significantly (p=0.012) lower in the 8.1AH carriers; age, gender and ΔF508
genotype-adjusted odds ratio to be colonized of the carriers versus non-carriers was 0.112 (0.024–0.520). According to survival analysis, patients with 8.1AH had significantly (p<0.0001) longer colonization-free period compared with non-carriers. Our novel observations demonstrate that the 8.1AH is associated with delayed onset of colonization in CF, presumably by influencing defence mechanisms against infections, exerting a more efficient immune response against bacterial infections.

In the study of colorectal cancer patients, frequency of the 8.1AH was significantly (p=0.006) more frequent (19.1%) among patients than in the controls (7.8%). Age- and gender-adjusted ratio of the 8.1AH carriers vs. non-carriers to have colorectal cancer was 2.514. This risk was higher in <or=67 years old subjects (4.073) and in females (3.771). These findings-consistent with similar recent results with ovarian cancer-indicate that carriers of the 8.1AH, encoding for an altered immune response and known to be associated with alterations of several immune functions and autoimmune diseases have an increased risk for some cancer types.
Conclusions

These were the first studies to use TNF-α -308A, RAGE -429C, HSP70-2 1+267G and LTA +252G alleles for the relatively simpler and cheaper detection of the 8.1AH. This method is successfully applied by others in several studies in our laboratory.

Disease association studies focusing on single alleles lose the context and detection of the effect of the complex haplotype they belong to, therefore the importance of haplotype analysis rather than determination of single alleles as modifier factors in disease association studies is to be emphasized.

The genetically encoded alteration of the immune response of 8.1AH carriers, which is more efficient against infections –so advantageous in cystic fibrosis against colonization- may not be advantageous in terms of tumours. In two different disorders the 8.1AH showed different, once positive and once negative association. According to the mentioned hypothesis carriers of the 8.1AH could have a positive selection during evolution due to the alteration of the immune response that is believed to be more efficient against infections. This selection advantage may explain the accumulation of the 8.1AH in the Caucasian population. In the era of antibiotics when the host immune response does not play as important role in combating infections as earlier in evolution, the excessive immune response turns against the host itself, resulting in pathologic conditions.
Publications related to present work


Other publications


