Analysis of the 8.1 ancestral haplotype and the *PAI-1* 4G/5G promoter polymorphism in pneumonia-related sepsis

Ph.D. Thesis

István Aladzsity

Semmelweis University
Molecular Medicine PhD School

Project Leader:	Dr. Prohászka Zoltán
Opponents:	Dr. Bobek Ilona Dr. Erdélyi Dániel
Head of the exam:	Dr. Ligeti Erzsébet
Participants of the exam:	Dr. Tóth Sára, Dr. Prechl József

Budapest
2012

1. Introduction

In the Caucasian population the most frequent haplotype of the major histocompatibility complex (MHC) region is the 8.1 ancestral haplotype (AH8.1). This haplotype is associated with numerous immunopathological differences (high titer of antibodies, autoantibodies and circulating immunocomplexes, increased TNF-α production) certain autoimmune diseases and colorectal carcinoma. The immunopathological alterations described in AH8.1 carriers may also influence the effectiveness of host-defenses against various microorganisms. Recently Laki J et. al. reported delaying effect of AH8.1 on bacterial colonization, observed in patients with cystic fibrosis (CF), may be relevant for other infectious diseases, such as sepsis.

The plasminogen activator inhibitor-1 (PAI-1) is a key element in the inhibition of fibrinolysis and activated protein C. Several studies demonstrated worse outcomes in patients hospitalised due to acute lung injury (ALI) and severe pneumonia who had increased levels of PAI-1. The most studied polymorphism of the *PAI-1* gene is the 4G/5G insertion/deletion polymorphism in the promoter region. The 4G/5G polymorphism has been associated with increased susceptibility to community-acquired pneumonia, and increased mortality in hospitalized patients with meningococcal and trauma sepsis.

2. Aims of the study

- Is there any association between the frequency of 8.1 ancestral haplotype and the poor outcome of pneumonia sepsis? If there is, is it the effect of AH8.1 or the effect of five other polymorphisms which required to identify the AH8.1?

- Does the chronic obstructive pulmonary disease (COPD) as a fellow illness influence the outcome of the pneumonia sepsis and the effect of the AH8.1 in sepsis?

- Is there any association between the carriages of AH8.1 and the studied clinical parameters?

- Is there any association between the carriages of the 5G allele and the 5G/5G genotype of *PAI-1* 4G/5G polymorphism and the lower risk for worse outcome in pneumonia induced sepsis?

- If there is, how this genetic factor influences the found effects of AH8.1 in pneumonia related sepsis?

3. Methods

3.1. The studied group

We enrolled 207 patients with pneumonia-related sepsis who were treated in the Department of Anesthesia and Intensive Therapy Clinic, Semmelweis University.

3.2. Genotyping

Genomic DNA was extracted from white blood cells using the method of Miller et al. Genotyping of *LTA* 252A/G (rs909253), *HSP70-2* 1267A/G (rs1061581) and *PAI-1* 4G/5G (rs1799768) polymorphisms were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the *TNF-α* -308G>A (rs1800629), *AGER* -429T>C (rs1800625) single nucleotide polymorphisms (SNPs) were genotyped by TaqMan® probe based allele discrimination. Copy numbers of the *C4A* and *C4B* genes were determined by the method of Szilagyi et al.

3.3. Serological

HLA-B, HLA-DQB1 and HLA-DRB1 alleles were detected by HLA-B, HLA-DBR1 and HLA-DQB1 kits (Olerup SSP TM DQ kit, Olerup SSP AB, Saltsjöbaden, Sweden) using sequence specific primer (SSP-) PCR method.

3.4. AH8.1

Simultaneous carriage of C4A*Q0, *TNF* -308A, *AGER* -429C, *HSP70-2* 1267G and *LTA* 252G alleles was assumed as the carrier state of AH8.1, which was confirmed by HLA determination (patients who had at least one of the HLA-B8, HLA-DR3 and HLA-DQ2 alleles were considered AH8.1 carriers).

4. Results

We successfully genotyped the *AGER* -429T/C, *HSP70-2* 1267A/G, *TNF-α* -308G/A and *LTA* 252A/G polymorphisms and determined the number of *C4A* genes. All genotype distributions were in Hardy-Weinberg equilibrium. Based on the carriage of rare variants and C4A*Q0, thirty-two hypothetical 8.1 haplotype carriers were identified. After the determination of HLA alleles in this group of patients, twenty-five individuals were found to carry B8, DR3 and DQ2 alleles in addition, and considered heterozygous for AH8.1 (12.1%) – there were no homozygotes.

Comparing the allele frequencies of *AGER -429*, *HSP70-2* 1267, *TNF-α* -308, and *LTA* 252 polymorphisms and the *C4* gene copy number frequencies of the studied population and that of other collections of Caucasian healthy individuals did not reveal any difference. The AH8.1 frequency determined from the assigned polymorphisms and HLA typing was similar to the frequencies reported previously in Hungarians (5-10%).

Our findings indicate that in the COPD-free cohort of patients with severe, pneumonia-related sepsis, the carriers of the 8.1 ancestral haplotype have a significantly lower risk for septic shock, compared to non-carriers. After the multivariate logistic regression analysis, which contained among others COPD, this haplotype assumed an independent protective role against septic shock for the whole study cohort as well. Moreover, this adjusted effect was much stronger in COPD-free patients. In addition in the group of COPD-free patients the carriers of the AH8.1 had raised total white blood cell count, higher blood pressures and better mechanical ventilation parameters which are good prognostic markers in sepsis.

Comparing the distribution of the studied genetic factors between the groups with severe sepsis and septic shock, the genotype frequencies of *AGER* -429, *HSP70-2* 1267, *TNF-α* -308 and *LTA* 252 polymorphisms, as well as the incidence of C4A*Q0 were not different between the two severity grades. No correlation could be shown between the genotype frequencies of the SNPs and the survival, except for the *LTA* 252 polymorphism. However, none of the studied variations was associated with mortality in the adjusted models.

The PAI-1 4G/5G polymorphism was successfully genotyped in 207 patients with pneumonia–related sepsis. Genotype frequencies were in Hardy-Weinberg equilibrium. Comparing the allele frequencies of *PAI-1* 4G/5G polymorphism of the studied population

and that of other collections of Caucasian healthy individuals, no difference could be found.

Our results indicate that among patients hospitalized with severe sepsis due to pneumonia carriers of the *PAI-1* 5G/5G genotype have lower risk for septic shock. This effect was also seen in the group of COPD-free patients. The multivariate logistic regression analysis also supported that carriers of the 5G/5G genotype have lower prevalence of septic shock. In addition patients bearing the 5G/5G genotype had lower disseminated intravascular coagulation (DIC) score at admission, lower blood pressures and better mechanical ventilation parameters.

Analysing the interactions of AH8.1 and *PAI-1* 5G/5G genotype we found the follows. The AH8.1 showed the same protective effect in the COPD free patients when the carriers of 5G/5G genotype were exclude from the studied group. The patients who carried the AH8.1 and the *PAI-1* 5G/5G genetic factors together had not significantly lower odds ratio than who's carried the AH8.1 or *PAI-1* 5G/5G separately. Furthermore, when these two parameters were introduced simultaneously into the logistic regression models of septic shock of COPD free cohort only the AH8.1 showed independent protective role in the.

5. Discussion

Our findings demonstrate that the 8.1 ancestral haplotype has a protective role against septic shock in pneumonia-related sepsis, particularly in COPD-free patients. This observation indicating that the influence of this genetic trait may manifest only under certain conditions. The observed protective role was consistent with previous observation showing that the carriers of AH8.1 are relatively protected against bacterial infection in cystic fibrosis. The genetically encoded alteration of immune response in AH8.1 carriers may be a disadvantage in other conditions (such as tumours and autoimmune diseases), but may be efficient against infections. This relative protection might have been an advantage for positive selection during evolution that can explain the accumulation of AH8.1 in the Caucasian population.

The abundance of contradictory data on SNP analysis in sepsis emphasizes the importance of preferring haplotype analysis to the determination of individual SNPs as modifier factors. Haplotypes reflect the sequence of a whole gene (or genes) including coding and non-coding regions therefore they correspond more directly to the unit of biological function. In addition, it allows mapping of unknown risk or protective variants as well; therefore, haplotype-based analysis may enable susceptibility gene identification in complex diseases such as sepsis more effectively than individual SNPs.

Moreover our results demonstrate that among patients hospitalized with severe sepsis due to pneumonia carriers of the *PAI-1* 5G/5G genotype have lower risk for septic shock. This observation supports previous studies reporting that the activation of coagulation and the inhibition of fibrinolysis are important in the pathogenesis of sepsis and severe lung disease and support the notion that genetic factors may predispose to outcome in severe sepsis.

Analysing the interactions of AH8.1 and 5G/5G genotype we indicated the follows. The found protective effect of AH8.1 against the septic shock does not due to the protection of the 5G/5G genotype. Analysing the two genetic factors together an additive association was not found. In the group of COPD free patients the AH8.1 showed stronger protection than *PAI-1* 5G/5G.

6. Publication

6.1. Publications required for the thesis

Aladzsity I, Madách K, Szilágyi Á, Gál J, Pénzes I, Prohászka Z, Fust G, *Analysis of the 8.1 ancestral MHC haplotype in severe, pneumonia-related sepsis.* Clin Immunol. 2011 Jun;139(3):282-9

Madách K, **Aladzsity I**, Szilágyi A, Fust G, Gál J, Pénzes I, Prohászka Z, *4G/5G polymorphism of PAI-1 gene is associated with multiple organ dysfunction and septic shock in pneumonia induced severe sepsis: prospective, observational, genetic study.* Crit Care. 2010 Apr 29;14(2):R79

6.2. Others

Aladzsity I, Tóth ML, Sigmond T, Szabó E, Bicsák B, Barna J, Regos A, Orosz L, Kovács AL, Vellai T, *Autophagy genes unc-51 and bec-1 are required for normal cell size in Caenorhabditis elegan.; Genetics*. 2007 Sep; 177 (1): 655-60.

Aladzsity I, Kovács M, Semsei A, Falus A, Szilágyi A, Karádi I, Varga G, Füst G, Várkonyi J, *Comparative analysis of IL6 promoter and receptor polymorphisms in myelodysplasia and multiple myeloma.* Leuk Res. 2009 Nov; 33 (11): 1570-3. Epub 2009 Apr 29.