ORIGINAL PAPERS

The association of the IL-1β-31 polymorphism and the development of neuroinfections

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ABSTRACT

Introduction. Inflammation of the meninges can have various clinical courses, from mild, self-limiting in some viral neuroinfections to severe, sometimes ending in death. The pro-inflammatory cascade and defects in the inhibitors of the inflammatory response play an important prognostic role. Single nucleotide polymorphisms (SNPs) of the genes encoding cytokines, influence the severity of the inflammatory response.
Aim. The aim of this study was to evaluate the effect of selected polymorphisms of proinflammatory cytokines IL-1β, TNF-α and IL-8 on the development of neuroinfections.
Material and Methods. We evaluated the laboratory results of 30 patients treated for bacterial and viral meningitis and compared those to 30 healthy volunteers. The following 4 variants were analyzed for occurrence of genetic polymorphism in patients with meningitis versus the control group: IL-1β 3953, IL-1β -31, TNF-α -308, and IL-8 781. Then, we assessed the association between these genetic polymorphisms and the inflammatory response during the course of meningitis.
Results and Conclusions. We observed that polymorphism of the IL-1β-31 significantly differs between patients and healthy subjects, the IL-1β -31AA polymorphism existed only in healthy individuals (p < 0.001). The WBC count was dependent on the TNF-α -308 polymorphism with a statistically significant difference (p = 0.021) occurring among persons with variants AA and AG. In conclusion the study showed that the presence of the AA genotype of IL-1β-31polymorphism may have a protective effect on the development of meningitis. This polymorphism was not observed in any patient with meningitis.

Keywords: polymorphisms of genes, neuroinfection, IL-1β, TNF-α, IL-8.

Introduction

The pathophysiology of meningitis is a complex process which consists of factors dependent on the micro-organisms (eg. endotoxins, adhesive ability, the presence of the areola), and host factors [1, 2]. Proinflammatory cytokines, activated by bacterial or viral antigens, play a very important role in causing damage to the blood-brain barrier, and the result is the penetration of bacteria into the subarachnoid space and the development of the classic symptoms of the disease [3, 4]. Various courses of disease from mild to very severe in the case of the same causative pathogens have been observed, indicating that the proinflammatory pathway and defects in inhibitors of the inflammatory pathway play a very important predictive role. Single nucleotide polymorphisms (SNPs), genes that encode cytokines, influence the severity of the inflammatory response. Identifying these will allow an accurate assessment of the patient's prognosis. Classification of meningitis depends on the type of etiologic

agent, there are two main groups: bacterial meningitis (BM) and viral meningitis (VM) [5, 6]. BM, due to the type of pathogens that cause inflammation are divided into purulent and non-purulent [5, 7]. VM are usually mild, with a self-limited course and rarely cause neurological sequelae, and have a low mortality rate [6, 8, 9]. However, there are cases of VM described with quite dramatic courses [5,8–10].

Aim

The aim of our study was to evaluate the influence of interleukin-1β (IL-1β), tumor necrosis factor (TNF-α) and interleukin 8 (IL-8) gene polymorphisms on the development and intensity of inflammation markers during the course of neurologic infections in patients treated in the Department of Infectious and Tropical Diseases, University Hospital in Krakow, Poland.

Material and Methods

We evaluated the results of 30 patients, 12 men and 18 women treated for BM and VM in the Department of Infectious and Tropical Diseases at the University Hospital in Krakow [mean age: 39.9 years]. The control group consisted of 30 healthy volunteers, 19 men and 11 women [mean age 44.6]. The study included 11 patients with BM and 19 patients with VM. Exclusion criteria included: other acute and chronic inflammatory states of immunosuppression and immunosuppressive therapy. In the test group, we analyzed blood morphology, the concentration of C reactive protein (CRP) in the blood, and tested the cerebrospinal fluid (CSF) for the number of cells, and concentrations of glucose and protein using standard methods. Patients and control subjects were evaluated for polymorphisms of IL-1β + 3953AG (rs1143634), -31AG (rs 1143627), TNF-α -308AG (rs1800629) and IL-8 + 781AG (rs2227306). For this purpose, DNA was isolated from blood using a DNA Qiamp DNA Mini Kit in accordance with manufacturer recommendations (Qiagen, Germany). After quantitative and qualitative assessments, the DNA samples were normalized to a concentration of 9 ng/ul. Genotyping was performed using a TaqMan SNP Genotyping Kit (Life Technologies, USA) and CFX384 Touch Real Time PCR Detection System (Bio-Rad, USA).

Statistical methods

We used nonparametric tests for statistical analysis. For comparison of the two groups, we used the Mann-Whitney test. Interdependence between select-ed parameters was determined by the Pearson correlation coefficient. Effects of polymorphisms on the level of the selected parameters were analyzed using ANOVA-Kruskal-Wallis. We also used the Chi^2 test of independence. A P value <0.05 was considered statistically significant. Calculations were performed using Statistica 10 (StatSoft® Inc., U.S.).

The study was Conducted in Accordance with the Declaration of Helsinki (1975) and approved by the Jagiellonian University Ethics Committee. All participants of the study signed an informed consent form.

Results

In the test and control groups, there was no statistically significant difference in gender (p = 0.07) or age (p = 0.21). The 19 patients with VM had an average CRP concentration of 7.40 mg/l (1.00–37.10 mg/l) whereas the group of 11 patients with BM the average CRP concentration was 74.02 mg/l (34.00–135.62 mg/l); the difference between the groups was statistically significant (p = 0.005). The WBC count (x10'3 /ml) was 7.83 (5.43–10.00) in the VM and 16.54 (6.37–22.12) in the BM, with a statistically significant (<0.001) difference. CSF analysis of the VM group showed 83.00 cells/ml (37.00–272.00), 0.72 g/L of protein (0.43–0.83) and 2.79 mmol/L of glucose (2.49–3.20). The BM group had an average of 398 cells/ml (37.00–1150.00), 1.45 g/L of protein (1.00–2.89), and 1.51 mmol/L of glucose (1.10–2.05). Statistically significant differences were observed between the two groups in the blood glucose (p = 0.001) and protein (p = 0.003).

There was a statistically significant difference in the distribution of AA genotype of the IL-1β -31 polymorphism (p < 0.001) between the test and control groups. The IL-1β -31AA genotype was present in the healthy group but not in the test group. In addition, the GG polymorphism was more frequently observed in the test group than in the control group. In terms of the other assessed polymorphisms, IL-1β 3953, TNF-α -308, IL-8 781, there was no statistically significant difference in the distribution of these polymorphisms between the test and control groups (**Table 1**).

We assessed for the association between the examined gene polymorphisms and inflammatory parameters of blood and CSF. We observed a relationship between the number of WBC and the TNF-α -308 polymorphism, wherein a statistically significant difference existed between the AA and AG (p = 0.02). In terms of the other assessed polymorphisms, IL-1β 3953, TNF-α -308, and IL-8 781, there was no statisti-

Table 1. Genotype frequencies of IL-1β, TNF-α and IL-8 genes in patients with meningitis and controls

SNP	Test group		Control group		Chi2 test
	n	GF (%)	n	GF (%)	p
IL-1β +3953					
GG	20	66.67	18	60	
AG	10	33.33	11	36.67	p = 0.56
AA	0	0.00	1	3.33	
IL-1β -31					
GG	18	60.00	3	10.00	
AG	12	40.00	16	53.33	p < 0.001
AA	0	0.00	11	36.67	
TNF-α -308					
GG	19	63.33	20	66.67	
AG	9	30.00	9	30.00	p = 0.84
AA	2	6.67	1	3.33	
IL-8 +781					
GG	3	10.00	7	23.33	
AG	8	26.67	7	23.33	p = 0.20
AA	16	53.33	16	53.33	

GF, genotype frequencies; IL-1β, interleukin 1β; IL-8, interleukin 8; SNP, single nucleotide polymorphism, TNF-α, tumor necrosis factor α

cally significant relationship between them and WBC, CRP, TNF-α IL-1β in CSF, or inflammatory parameters of CSF: white cells, protein, and glucose.

Discussion

Our study for the first time evaluated the IL-8 + 781 polymorphism in infection, and polymorphisms of the IL-1β: -31 and +3953 in neurological infections. A limitation of our study is that due to the small sample size, the selected gene polymorphisms of IL-1β, TNF-α and IL-8 were analyzed together in both types of meningitis.

We have shown that the IL-1β -31AA genotype demonstrates a statistically significant (p <0.001) difference between the test and control groups. Patients in the test group did not have the IL-1β -31AA genotype, while this genotype was present in the control group. It is possible that this genotype protects against the development of meningitis. This issue requires further research on a larger group of patients. In addition, more patients in the test group were found to have the GG genotype.

Thus far, the incidence of the IL-1β -31 polymorphism in neurologic infections has not been evaluated. The effects of IL-1β polymorphisms have been demonstrated in sepsis. Wen et al. observed that a polymorphism at position 1470 GG, AG and 51 31GA increases the risk of severe sepsis in patients after trauma [11]. Polymorphisms at positions -31 (G/A) and -511 (A/G) are also associated with more severe infections of

Plasmodium falciparum [12]. Liu et al., demonstrated a correlation between the presence of a G allele at position 31, and susceptibility to infection with influenza virus AH1N1pdm09 [13]. Allele 511G, the GG genotype, and haplotype 511G/3953G can be considered one of the factors responsible for susceptibility to the development of visceral leishmaniasis, as opposed to the A allele or AA genotype at position 511 and haplotype 511A/3953, which can be considered as factors promoting immunity against the disease [14]. Polymorphisms at position 3953 (G/A) was also observed in chronic hepatitis caused by HCV genotype 4, where the presence of the A allele was associated with poorer clinical response and more severe fibrosis [15]. Sa-Ngasang et al., showed that carriers of IL1β -31G have a higher risk of developing shock during Dengue fever (Dengue Shock Syndrome), which suggests a connection with production of interleukins in the pathogenesis of the disease [16].

The most widely investigated polymorphism of the gene encoding TNF-α is a polymorphism in the promoter region at position 308. Depending on the purine presence, two TNF-α alleles may be present: guanine at position -308 is associated with TNF-α 1 and adenine at this position is associated with TNF-α 2. Allele TNF-α 2 is less frequent, and it is associated with higher production of TNF-α as compared to TNF-α 1 [17]. However, the occurrence of a polymorphism at position -863 (G/A) is associated with a lower expression of the gene and lower levels of this cytokine [18]. Research on

the effects of the presence of TNF-α polymorphisms on the course neurologic infections are few. Titmarsh et al., evaluated the polymorphism of TNF-α -308 in groups of patients with VM, specifically invasive meningococcal disease (IMD) compared to a control group. They showed significant differences between the groups, with the genotype GG of TNF-α -308 polymorphism associated with a lower production of TNF-α which correlated with a higher risk of developing IMD [19]. Pujikhari et al. showed that people with -308 alleles and -863G allele were more likely to develop severe Japanese encephalitis [20]. Fontes et al., in comparing the distribution of TNF-α -308 genotypes demonstrated that the TNF-α -308 is present more often in BM patients than in healthy people [21].

Polymorphisms in the region of -308 have been demonstrated in other acute infections, bacterial, viral, or parasitic. Thus far a correlation between the occurrence of allele TNF-α 2 and more severe malaria has been shown. McGuire et al. observed that children who are homozygous for TNF-α 2/2 were at seven times greater risk of developing the cerebral form of malaria or death [22]. Similarly, Cabrera et al. observed a correlation between the presence of the allele of TNF-α 2 and susceptibility to mucocutaneous leishmaniasis infection [23]. With respect to the role of TNF-α in the development of septic shock, polymorphisms of the gene coding for TNF-α have been demonstrated to have an effect on the course of sepsis. Song et al. have demonstrated the relationship between the presence of the TNF-α 2 allele and the risk of severe sepsis, but no correlation was observed with the occurrence of this polymorphism and death [24]. Teuffel et al., came to similar conclusions in their meta-analysis [25]. Polymorphisms in the region of 308 were also demonstrated in the course of viral infections. The presence of the TNF-α 2 allele, and thus increased production of TNF-α, was associated with an increased risk of haemorrhagic dengue fever when re-developing the disease [26]. In the course of infection with influenza virus AH1N1pdm09 the presence of -308G allele was associated with more severe disease [27].

In terms of the analyzed polymorphisms of TNF-α -308 in our study there was no statistically significant difference in the distribution between the test and control groups. The WBC count was dependent on the TNF-α-308 polymorphism, but statistical significance was only reached between AA and AG. TNF -308 polymorphisms did not affect other analyzed parameters: CRP in the peripheral blood and CSF studies: TNF-α, IL-1β, cell count, protein, or glucose.

Studies on the effect of SNP IL-8 in the course of infection are few, and primarily surround infections of the gastrointestinal tract. Jiang et al. demonstrated in 2 separate studies that the AA genotype at the -251 position of the IL-8 is a significant risk factor for primary CDI [28, 29]. Jiang et al. further demonstrated the influence of the same polymorphism on the development of the enteroaggregative forms of Escherichia coli (EAEC) [30]. In terms of neurologic infection, Titmarsh et al., compared the prevalence of polymorphisms of IL-8 -251 in groups of patients with VM, IMD, and healthy controls. Significant difference between groups was noted; for the IL-8 -251 polymorphism, IL-8 -251AA was associated with a higher risk of VM [19].

In our study we chose the IL-8 SNP +781, which has not been analyzed in the progression of any infection. No statistically significant difference in IL-8 +781 polymorphisms were noted between the test and control groups. Polymorphisms of IL-8 +781 had no impact on peripheral blood WBC, CRP, TNF-α, IL-1β or CSF studies, specifically: cell count, protein and glucose.

As a conclusion, we observed that the IL-1β -31AA genotype may play a protective role in the course of neurologic infection – it was not observed in patients who presented with this disease. The WBC count in peripheral blood correlated with the TNF-α -308 polymorphism, and there was a statistically significant difference between the AA and AG subsets.

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