Role of the CCL2/MCP-1 -2518A>G gene polymorphism in HLA-B27 associated uveitis

Beate Julia Wegscheider,1 Martin Weger,1 Wilfried Renner,2 Ursula Posch,3 Silvia Ulrich,2 Josef Hermann,4 Navid Ardjomand,1 Eva-Maria Haller-Schober,1 Yosuf El-Shabrawi1

Departments of 1 Ophthalmology, 3 Transfusion Medicine and Blood Serology, and 4 Internal Medicine, and the 2 Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, Graz, Austria

Purpose: Acute anterior uveitis (AAU) is the most common form of uveitis. Up to 50% of patients with AAU are HLA-B27 positive. Since HLA B27 itself plays only a minor role in the overall genetic background, other genetic variants are likely to contribute to the susceptibility to AAU. The chemokine (C-C motif) ligand 2 (CCL2) gene, coding for monocyte chemoattractant protein-1 (MCP-1), a chemotactic cytokine, is involved in the induction of uveitis. A CCL2 gene polymorphism, which is characterized by an A>G substitution at nucleotide -2518 in the promoter region of CCL2 has been previously shown to affect MCP-1 synthesis. The purpose of the present study was to investigate a hypothesized association between this genetic variant and the presence of HLA-B27 associated AAU.

Methods: The study group comprised 114 patients with HLA-B27 associated AAU. One hundred and eleven healthy HLA-B27 positive individuals served as the HLA-B27 positive control group, whereas 81 healthy HLA-B27 negative individuals served as a HLA-B27 negative control group. Genotyping for the CCL2 -2518A>G polymorphism was performed by polymerase chain reaction.

Results: Carriers of a CCL2 -2518G allele were found significantly more often in patients with HLA-B27 associated AAU than among HLA-B27 positive controls (49.2% and 31.5%, respectively; odds ratio 2.1; 95% confidence interval 1.2-3.6; p=0.007).

Conclusions: Our data suggest that the CCL2 -2518A>G polymorphism may play a role in HLA-B27 associated acute anterior uveitis.

The most common form of uveitis is acute anterior uveitis (AAU) [1,2]. AAU is a well defined entity and its clinical features are typical. It is usually abrupt in onset, with significant circumlimal hyperemia. Patients suffer from pain, photophobia, and epiphora [3]. Unilateral attacks with mildly or severely decreased vision in the affected eye are common, but a “flip-flop” to the contralateral eye may occur [3]. Up to 50% of patients with AAU show a positive HLA-B27 haplotype [1,2]. HLA-B27 associated uveitis is closely linked to well defined diseases such as ankylosing spondylitis (AS, a prototype of spondylarthropathies), reactive arthritis (ReA), psoriatic arthritis (PsA), or inflammatory bowel disease (IBD) [3].

Genetic factors appear to account for the majority of the susceptibility to HLA-B27 associated AAU, although HLA-B27 itself accounts for only a minority of the overall genetic background of AAU [4,5]. In a Caucasian population approximately 7-8% of the general population are found to be HLA-B27 positive [6]. The estimated cumulative lifetime risk of uveitis in HLA-B27 positive individuals, however, is only 2% [7]. Thus, recent studies focused on other genetic variants that may also contribute to the development of HLA-B27 associated AAU [8-10]. Indeed, single nucleotide polymorphisms (SNPs) within the promotor region of the tumor necrosis factor alpha (TNFα) gene have been most recently suggested to play a role in AS [11] and uveitis [10].

Monocyte chemoattractant protein-1 (MCP-1) is a chemotactic cytokine, belonging to the CC family of chemokines. It has been detected in various cells including monocytes, lymphocytes and endothelial cells [12]. MCP-1 is also known to attract monocytes, neutrophils [13], memory T-cells and natural killer cells to a site of inflammation [12,14]. In numerous animal models MCP-1 was shown to play a part in autoimmune response [14-16]. It is thought to participate in leukocyte infiltration and protein leakage in AAU and in the induction of uveitis itself [14-16].

Furthermore, MCP-1 can easily be detected in the aqueous humor of patients with active AAU, while the cytokine is not present in the aqueous humor of healthy controls and patients with inactive AAU [17].

Stimuli for the production and secretion of MCP-1 are proinflammatory cytokines such as IL-1β, TNF-α [18], or infectious agents like Trichinella spiralis [19], Chlamydia pneumoniae [20], or Salmonella species [21].

Recently Rovin et al. [22] identified a functional A>G gene polymorphism in the MCP-1 gene (chemokine [C-C motif] ligand 2 [CCL2]) distal regulatory region at position -2518 relatively to the transcription start site (based on the published sequence in GenBank accession number D26087; CCL2 -2518A>G [SNP number rs1024611]). After interleukin-1β
stimulation peripheral mononuclear cells (PBMC) from individuals heterozygous or homozygous for the CCL2 -2518G allele showed a significantly higher MCP-1 synthesis than cells from individuals carrying the -2518AA genotype [22]. An association between the CCL2 -2518A>G polymorphism and various pathologic conditions such as Crohn’s disease [23], cutaneous vasculitis, and arthritis in patients suffering from systemic lupus erythematosus [24,25], and more severe hepatic inflammation and fibrosis in patients with hepatitis C has been reported [26]. Most recently, Tucci et al. [27] observed a strong association between the aforementioned gene polymorphism and lupus nephritis.

To the best of our knowledge, the role of the CCL2 -2518A>G polymorphism has not yet been studied in patients with HLA-B27 associated AAU. The purpose of the present study was therefore to investigate a hypothesized association between this genetic variant and the presence of the HLAB27 associated AAU.

METHODS

Study design and population: The study was designed as a retrospective case-control study. It was conducted according to the tenets of the Declaration of Helsinki and approved by the local Ethics Committee. All participants gave written, informed consent prior to enrolment.

The study group comprised of 114 patients with HLA-B27 associated AAU who were seen at the Department of Ophthalmology between January, 1999 and July, 2004. The following data were obtained from all patients: gender, age at presentation, age at onset of uveitis, follow-up time, clinical diagnosis of systemic disease association, number and duration of recurrent inflammatory attacks or chronic disease, and the prevalence of severe ocular complications. Ocular complications were defined as significant cataract (greater than or equal to 2+ opacity), and secondary glaucoma, vitreous inflammation (greater than or equal to 2+ cells), clinically significant macular edema as seen in optic coherence tomography or fluorescein angiography. HLA-B27 positive patients with Fuchs’ heterochromic iridocyclitis, sarcoidosis, or any history of malignancy were not eligible for enrolment. All patients and control subjects were Caucasian from the same area in Central Europe.

Laboratory methods: Genomic DNA was isolated from peripheral blood lymphocytes by standard methods (QiAmp DNA Blood Mini Kit; QIAGEN, Hilden, Germany) and stored at -20 °C. HLA-B27 status was determined using sequence specific primer application (Olerup SSPTM HLA-B*27; GenoVision AB, Saltsjöbaden, Sweden). Genotyping for the CCL2 -2518A>G polymorphism was performed as previously described [29].

Statistics: SPSS for Windows (release 10.0.5; SPSS Inc., Chicago, IL) was used for statistical analysis. Categorical variables were compared using the χ² test. Odds ratios (OR) and 95% confidence intervals (95% CI) were determined by logistic regression analysis. A p<0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics of patients and controls are shown in Table 1, while clinical features of patients are presented in Table 2. In 83 (74.8%) out of 114 patients a systemic manifestation was found; 51 (44.3%) patients suffered from AS, 18 (15.7%) from undifferentiated spondylarthropathy, eleven (9.6%) from PsA, five (4.3%) from ReA, and one (0.9%) each from IBD and JIA.

| Table 1. Baseline characteristics of patients and controls |
|-------------|-----------------|-----------------|-----------------|
| HLA-B27    | Controls        |                 |
| associated AAU patients (n=114) | negative (n=88) | positive (n=111) |
| Male (%)   | 60 (52.6)       | 57 (64.8)       | 59 (53.2)       |
| Age ± SD   | 44.89 ± 14.29   | 37.68 ± 12.34   | 32.39 ± 7.34    |

This table shows the baseline characteristics of patients and the two control groups.

Table 2. Clinical features of patients

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age of onset ± SD (years)</td>
<td>35.1 ± 13.14</td>
</tr>
<tr>
<td>Mean number of flares ± SD</td>
<td>7.92 ± 7.62</td>
</tr>
<tr>
<td>Mean duration of flares ± SD (weeks)</td>
<td>4.96 ± 2.68</td>
</tr>
<tr>
<td>Mean duration between flares ± SD (months)</td>
<td>22.97 ± 18.7</td>
</tr>
<tr>
<td>Early onset (&lt;40 years)</td>
<td>72 (63.2)</td>
</tr>
<tr>
<td>Late onset (&gt;40 years)</td>
<td>42 (36.8)</td>
</tr>
<tr>
<td>One eye affected</td>
<td>64 (56.2)</td>
</tr>
<tr>
<td>Both eyes alternating</td>
<td>42 (36.8)</td>
</tr>
<tr>
<td>Both eyes concomitant</td>
<td>8 (7.0)</td>
</tr>
<tr>
<td>One attack of inflammation</td>
<td>14 (12.3)</td>
</tr>
<tr>
<td>Recurrent attacks of inflammation</td>
<td>89 (78.1)</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>11 (9.6)</td>
</tr>
<tr>
<td>Secondary cataract</td>
<td>17 (14.9)</td>
</tr>
<tr>
<td>Secondary glaucoma</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>Posterior segment inflammation</td>
<td>30 (26.4)</td>
</tr>
<tr>
<td>Macular edema</td>
<td>18 (15.8)</td>
</tr>
</tbody>
</table>

This table presents the patients’ clinical features and their opthalmic complications.
Genotypes were successfully determined in all participants and did not deviate from the distribution predicted by the Hardy-Weinberg equilibrium. Table 3 shows the genotype distribution of the CCL2 -2518A>G polymorphism in patients with HLA-B27 associated AAU and the two control groups. Carriers of a CCL2 -2518G allele were found significantly more often in HLA-B27 positive patients than in HLA-B27 positive controls (OR=2.1, 95% CI 1.2-3.6; p=0.007). Genotype distribution of the CCL2 -2518A>G polymorphism did not significantly differ between patients and healthy HLA-B27 negative control subjects (p=0.97).

No significant association between CCL2 -2518A>G genotypes and number or duration of attacks, chronic disease, and occurrence of any systemic manifestation was observed.

**DISCUSSION**

In the present study we found a significant increase in the prevalence of the CCL2 -2518AG and GG genotypes in HLA-B27 associated AAU patients compared to healthy HLA-B27 positive controls.

Given the appropriate stimulus, cells from individuals homozygous or heterozygous for the CCL2 -2518G allele produce significantly higher levels of CCL2 than cells from individuals with the AA genotype [22,30].

Since this increase in the prevalence of the CCL2 -2518G allele among AAU patients was not seen when compared to the HLA-B27 negative control individuals, in whom we found a G-allele frequency comparable to other central European control populations [24,29-32], it might be postulated that an increase in CCL2 predisposes only HLA-B27 positive individuals to the development of an AAU. This might be explained by a coherence in function related to the HLA-B27 haplotype. A genetic linkage in the inheritance of HLA-B27 and CCL2 is rather unlikely as the gene for the histocompatibility complex class I molecule HLA-B27 is located on chromosome 6 (6p21.3) and the CCL2 gene is located on chromosome 17 (17q11.2-q21.1). Thus a possible explanation is more likely to be found focusing on functional properties related to the HLA-B27 haplotype.

The precise mechanism by which HLA-B27 contributes to the susceptibility of uveitis is still incompletely understood. It is evident, however, that HLA-B27 by itself is responsible for only a minor predisposition to AAU [4,5]. Along with genetic conditions, other factors [9,10], especially infectious agents, as for example Salmonella, _Yersinia enterocolitica_, _Bartonella henselae_, Campylobacter species, or _Chlamydia pneumoniae_ have been suggested to contribute to AAU [33-37]. Recently, Saarin et al. [21] demonstrated that intestinal epithelial cells carrying HLA-B27 are invaded by higher numbers of _Salmonella enteritidis_ than HLA-B27 negative control cells. _S. enteritidis_ has also been shown to survive better in HLA B27 transfected human monocytes and mouse fibroblasts than in HLA B27 negative cells in vitro [38,39].

It could possibly be argued that in HLA-B27 positive individuals, the uptake of a certain infectious agent is facilitated or its elimination impaired. The infectious agent might then serve as a persistent stimulus for MCP-1 production, with carriers of the CCL2 -2518AG and GG genotypes synthesizing higher concentrations of MCP-1. This could lead to an upregulated inflammatory response and hence induce AAU in HLA-B27 positive individuals.

The finding that the CCL2 -2518G>A polymorphism might play a role only under the same stimulatory conditions, such as the HLA-B27 haplotype, is in concordance with the findings of Gonzalez-Escribano et al. [24]. They found the CCL2 -2518G>A polymorphism to contribute to rheumatoid arthritis only if patients were lacking HLA shared epitopes.

Chen et al. [40] investigated two different CCL2 SNPs, one of them being the CCL2 -2518G>A polymorphism. The group reported one haplotype to be overrepresented in male patients suffering from Behcets’s disease. In our cohort of patients with HLA-B27 associated AAU the CCL2 -2518G>A polymorphism was not associated with either gender.

This study shares the same limitations as any other retrospective study. Genetic factors, however, unlike many other biologic parameters, do not change during lifetime. Hence, our findings strongly suggest a contributory role of the CCL2 -2518A>G polymorphism in HLA-B27 associated uveitis. Further research will be needed to support this hypothesis and elucidate more precisely the underlying pathologic mechanisms.

**ACKNOWLEDGEMENTS**

The authors kindly thank Mrs. Manuela Fischl, Mrs. Evelyn Elshatti, and Mrs. Helga Spitzenberger for their excellent technical assistance.

**REFERENCES**

3. Suhler EB, Martin TM, Rosenbaum JT. HLA-B27-associated uvei-


33. Hamre RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats representing HLA-B27 and human beta 2m: an animal model of HLA-


