Amino Acid Residue 67 (Isoleucine) of HLA-DRB Is Associated with POHS

Jenny V. Ongkosuwito,1 Marcel G. J. Tilanus,2 Allegonda Van der Lelij,3 Mary J. van Schooneveld,3 Martine J. Jager,1 Erik H. Rozemuller,2 Marc D. de Smet,1 and Maria S. A. Suttorp-Schulten5

PURPOSE. To investigate whether presumed ocular histoplasmosis syndrome (POHS) in The Netherlands is associated with HLA-DR2 and HLA-B7, as previously shown in the United States.

METHODS. Twenty-four Dutch patients with POHS were included in this study. DNA isolated from peripheral blood leukocytes was typed for HLA by a sequence-based method. Associations were statistically determined. The frequencies of HLA alleles in bone marrow of donors listed on the European donor registry was used to represent the distribution in the normal population. Patients were included in the study only when no cells were present in the vitreous at any time and when fundus photographs fit the diagnosis made according to the following criteria: presence of peripapillary atrophy, presence of punched out chorioretinal lesions (histospots), and presence of a submacular scar. After the fundus photographs were judged, the patients were divided into two groups. Group 1 contained patients who met all three diagnostic criteria (complete POHS), and group 2 contained patients who met one or two of the criteria (incomplete POHS).

RESULTS. Group 1 consisted of 14 patients and group 2 of 10 patients. An association between POHS and HLA-DR2 and -B7 was present, compared with the normal Dutch control subjects. Although significant, the association between the frequency of HLA-DR2 and -B7 of all patients with POHS was less striking than the findings in patients with POHS in the United States. The association with DR2 in patients with incomplete POHS (group 2) was significantly different from that in the group with complete POHS (group 1). According to the defined criteria the association of POHS with HLA-B7 and -DR2 was confined to the incomplete POHS group and was not found in the complete POHS group. Furthermore, analysis of DR at the amino acid level, rather than at the allele level (DR2) showed that amino acid 67 of the DRB1 alleles had the most significant HLA association with POHS, independent of the two groups.

CONCLUSIONS. POHS in Dutch patients was associated with HLA-B7 and -DR2, but more striking was the presence of isoleucine at position 67 of the HLA-DR molecule. (Invest Ophthalmol Vis Sci. 2002;43:1725-1729)

In uveitis, a constellation of syndromes affecting the pigment epithelium and the inner choroid are known as white-dot syndromes. Among these, POHS is a well-characterized clinical entity. It typically consists of white, atrophic, sharply circumscribed scars (0.2–0.3 disc diameter) scattered throughout the fundus (histoplasmosis spots, or histospots), disciform macular scars, peripapillary scarring, and the absence of active vitreous inflammation. POHS usually occurs in young individuals. When subretinal neovascularization develops in the macular region, it results in permanent loss of vision. Poor vision is present in 50% of affected individuals. Although the characteristics are often used as criteria to diagnose this ocular syndrome, not all patients fulfill all criteria.

In the United States, POHS is predominantly, but not exclusively, seen in the Midwest, where the fungus Histoplasma capsulatum is endemic. Based on epidemiologic studies in the United States, a correlation between the clinical appearance characteristic of POHS and the presence of this fungus has been established—hence, the name POHS. Previous studies concerning this ocular syndrome in the Netherlands has shown no etiological association between Histoplasma capsulatum and POHS in the Netherlands. Therefore, this infectious agent is unlikely to be responsible for the clinical manifestations observed in European patients with POHS.

An association of POHS in the United States with HLA-DR2 (of which DR15 is a subgroup) and HLA-B7 has been reported with a frequency of 81% and 77%, respectively. This association with both HLA-DR2 and -B7 is explained by the strong linkage disequilibrium between these two genes. Other HLA-B alleles that are in linkage disequilibrium with DR2, such as B7–DR2, have been associated with certain autoimmune diseases, such as ankylosing spondylitis.

With the advent of more refined techniques for HLA typing, it has been possible to link certain disease entities to specific amino acid substitutions at defined positions within the alleles rather than to the individual alleles as a whole.

The use of DNA sequencing techniques has facilitated the detailed mapping of binding domains within the HLA class II molecules. For HLA-DRB alleles, amino acid residues located at position, 47, 67, 70, 71, and 86 are essential for peptide binding (antigen) and subsequently for T-cell recognition. The purpose of the present study was to determine the HLA association in Dutch patients with POHS and to investigate whether specific loci within the HLA-DRB allele were particularly associated with this syndrome.

PATIENTS AND METHODS

Patients with diagnosed POHS were selected from the Ophthalmology department of the University Medical Centre Utrecht and of the Aca-
In short, for each HLA locus, specific amplifications were performed by polymerase chain reaction (PCR). Both primers were elongated with templates for the universal M13 forward and reverse sequencing primers for direct sequencing (ABI 373; PE Biosystems, Foster City, CA). Once a sequence had been obtained, sequence data were analyzed with sequencing analysis software (PE Biosystems). Heterozygous positions were assigned with the Hetero program and allele assignments were made with the Allele program. Both are software programs developed for sequence-based typing of HLA genes (PE Biosystems).²⁶,²⁷

Statistical analysis was performed as described by Sveigaard et al.²⁸

For the association of DR types, the Fisher exact test for two-by-two tables was performed. The Bonferroni correction of probability was applied as a correction for the comparisons made (α = 9). For frequency of B7, no other comparisons were performed except with the presence of B7 in the control group. As the control frequency, we used the frequency from the Dutch bone marrow donor registry, as reported by Schipper et al.²⁹

RESULTS

Twenty-four patients (18 women and 6 men) with POHS were typed for HLA class I (HLA-A and -B) and class II (HLA-DRB, -DQB, and -DPB; Table 1). In patients 2.3 and 2.5, possible new HLA alleles were identified. Further analysis and cloning is necessary to confirm these findings.

Patients as a Whole

In our group of patients with POHS HLA-DR15 (a subgroup of HLA-DR2) was found at a frequency of 0.25, significantly higher than in the control group (frequency, 0.137; P < 0.001). The previously observed association between POHS and HLA-B7 was also confirmed, with a frequency of 0.273 (control frequency, 0.001). No alleles of the other, low-frequency DR2 subgroup, DR16, were identified (Table 2).

The frequency of other alleles present in the patients was not significantly different between patients and the Dutch
control (e.g., the frequency of HLA-DR13 was 0.18 in patients with POHS and 0.15 in the Dutch control; $\chi^2 0.73, P = 0.3$). No association was found with the HLA-A, DQB, and DPB alleles. For clarity in Table 2, only statistically significant results were presented.

Because in our group of patients with POHS the strongest association was found for HLA-DR15 (subgroup of DR2), we analyzed for the presence of specific amino acids in peptide binding (positions 47, 67, 70, 71, and 86) in the DRB alleles. The frequencies were compared with those known frequencies in a large cohort of the Dutch population (Table 3), estimated from the allele frequencies and assuming the highest frequency of the individual allele types, because no high resolution was available from the control panel. This overestimates the frequency, because some alleles do not have that amino acid. The amino acid isoleucine at position 67 was significantly more frequently detected in patients with POHS than in the Dutch control ($P = 0.0003$). It was present in almost all patients with POHS. This consequently resulted in a significantly lower presence of phenylalanine at position 67. Some significant differences were noted in positions 70 (arginine was significantly more often present) and 71 (alanine and glutamic acid were significantly more often present), but these were present in very few patients and therefore were not taken into consideration. Analysis of positions 47 and 86 did not reveal any significant differences. Only the results of the analysis of position 47 and 67 are stated in Table 3.

### Subgroups

As mentioned earlier, to assess the possible role of HLA-DR15 and/or isoleucine at position 67 in the clinical picture, patients were divided among patients into two groups: group 1 (complete POHS), 14 patients; and group 2 (incomplete POHS), 10 patients. All patients in group 1 had all three clinical characteristics; in group 2, nine patients had two characteristics and one patient had one.

A significant association of DR15 with group 2 (incomplete POHS) was found ($P < 0.0001$), whereas this association with group 1 (complete POHS) was not significant ($P = 0.9862$). Accordingly, as a result of the linkage disequilibrium between HLA-B7 and HLA-DR2, HLA-B7 was also significantly associated with group 2 ($P < 0.0001$), whereas group 1 showed no significant association ($P = 0.3398$).

For isoleucine at position 67 the calculated frequencies were statistically significant in group 1 ($P = 0.0002$) and group 2 ($P = 0.0006$) when analyzed individually. No significant difference between the groups and their association with isoleucine at position 67 was seen (Table 3).

Evaluation of individual symptoms (the presence of peripapillary atrophy, histospots, or submucular neovascularization) in groups 1 and 2 did not show an association with HLA-B7-DR15 or isoleucine at position 67.

### Discussion

In the present study, we showed, as was observed in US patients, an association between presumed ocular histoplasmosis syndrome and HLA-DR2 and -B7. This association in our study sample was much stronger for HLA-DR15 (a subtype of DR2), and more specifically for the presence of an isoleucine at position 67 of the HLA-DRB1 locus.

Initially, POHS was thought to result from a systemic or ocular infection with *H. capsulatum*, but the presence of the same clinical entity in Europe and other areas nonendemic for histoplasmosis argues for a multifactorial origin. Studies in the United States have shown an association between HLA-DR2 and -B7.5,20 However, a known linkage disequilibrium exists between these two genes.19 The present study shows a stronger association with HLA-DR15 and is probably not only relevant to the European population, but also to the US one. When our patients were divided into two groups (complete and incomplete POHS), according to the presence or absence of all clinical criteria defining the syndrome, the HLA-DR15 association (and in particular HLA-DRB1*15011) was obvious in the incomplete POHS group ($P < 0.0001$). Such an analysis has not been previously performed, in part because patients have not

### Table 2. Frequency of HLA-DR15 and HLA-B7 Alleles of Patients with POHS Compared with Dutch Control Subjects

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Whole Group (n = 24; 48 alleles)</th>
<th>Group 1 (n = 14; 28 alleles)</th>
<th>Group 2 (n = 10; 20 alleles)</th>
<th>Dutch Control Frequency (n = 2440)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Frequency</td>
<td>$P$</td>
<td>n</td>
</tr>
<tr>
<td>DR15</td>
<td>11</td>
<td>0.250</td>
<td>&lt;0.0001</td>
<td>4</td>
</tr>
<tr>
<td>*15011</td>
<td>10</td>
<td>0.229</td>
<td>&lt;0.0001</td>
<td>3</td>
</tr>
<tr>
<td>*15021</td>
<td>1</td>
<td>0.200</td>
<td>NS</td>
<td>1</td>
</tr>
<tr>
<td>B7*07021</td>
<td>12</td>
<td>0.273</td>
<td>&lt;0.0001</td>
<td>4</td>
</tr>
</tbody>
</table>

* Schipper et al.19
been so strictly classified according to the presence or absence of all criteria defining the entity known as POHS. The number of patients is limited, making speculation difficult. However, one can envisage that HLA class II is associated with only a portion of the criteria defining the syndrome, explaining why it shows a stronger relationship to the incomplete type. To obtain a complete typing, either external factors or possibly the action of a separate gene or genes is necessary. Indeed, the presence of chorioretinal atrophy in the peripapillary and/or at extrafocal sites does not necessarily imply a linkage with the process leading to macular scarring or the development of a subfoveal neovascular membrane.

The present study, by making use of high resolution HLA typing was able to identify a critical amino acid at the peptide-binding locus within the HLA-DRB1 allele. This has not been reported previously in the US population. We specifically found that this syndrome is associated with isoleucine at position 67 of HLA-DRB1. Isoleucine at position 67 was found in almost all patients with POHS, regardless of their HLA-DR2 typing. Other peptide binding sites of the DRB1 allele, which showed statistically significant associations with specific amino acids were positions 70 and 71. However, at these two positions, too few patients showed an association to make it clinically relevant. A study of a larger sample might allow a complete delineation of the peptide binding motif in this population and identify the source of antigenic response. Alternatively, it might identify the importance of protein encoded just outside the HLA locus in the inflammatory process.

In a recent study, Kobayashi et al. demonstrated that T cells from patients with Vogt-Koyanagi-Harada (VKH) disease recognize tyrosinase-derived peptides. This article appeared shortly after VKH disease was shown to be associated with HLA-DRB1*0405. Tyrosinase was suggested as a possible target of the known binding motif of HLA-DRB1*0405. Their results suggest that tyrosinase, a normal self protein may be involved in the pathophysiology of VKH. Disease susceptibility associated with specific class II genes can be the result of linkage disequilibrium with proteins located just outside the HLA locus. In a recent study of patients with multiple sclerosis, in which direct sequencing of exon 2 of the HLA-DRB1 allele was used, disease susceptibility was associated with the presence of specific amino acids at positions 11, 13, and 71. These were all related to HLA-DR2, and no allele-independent residues relevant to antigen binding conferred disease susceptibility, possibly by increasing proinflammatory cytokine secretion or HLA class II expression. Similarly in Behcet disease a recent study has suggested that disease susceptibility relates to HLA-B51 is more closely reflected by its association with major histocompatibility complex class I chain-related genes (MICA). This gene is located near HLA-B and is associated with HLA-B through linkage disequilibrium.

Our present study was too limited to determine which process is predominant. Isoleucine at position 67 appeared to be independent of the specific HLA-DR2, possibly indicating that it is related to a specific binding motif rather than linked by linkage disequilibrium to an unknown protein. Too few data are available on the other two positions that were identified. In essence, a larger study is needed in which patients with complete and incomplete POHS are studied with advanced genotyping techniques. Such a study would help to answer the question of whether allelic association to DRB1*1501 is based on antigen presentation through a specific peptide determinant or is the result of immune enhancement from a protein located adjacent to the HLA site. If it is related to a specific amino acid-binding motif, the peptides capable of activating the immune process can then be identified. This, in turn, will allow the identification of the putative organism(s) capable of causing the clinical syndrome known as POHS.

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References

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