HLA-DP CONTROL OF HUMAN SCHISTOSOMA HAEMATOBIUM INFECTION

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Abstract. The DPA1 and DPB1 alleles of the major histocompatibility complex (MHC) class II were determined in 110 patients and 120 healthy controls of a Gabonese population from an area endemic for *Schistosoma haematobium* infection. The MHC-DP alleles of the variable second exons and their human leukocyte antigen (HLA) epitopes were correlated with egg excretion, interleukin-4 and interferon-γ patterns, and bladder abnormalities, as detected by ultrasonography. Met-histidine at position 11 of the DPα molecule (Met-11) and DPA1*0301 were associated with schistosomiasis when compared with controls (phenotypic gene frequencies = 0.791 versus 0.583 and 0.555 versus 0.375, respectively). Met-11 homozygosity occurred more often in patients, whereas healthy controls were more frequently homozygous for an alanine at position 11 (Ala-11). The combination of the DPB1-epitope DEAV (positions 84–87 of the DPβ molecule) and Met-11 positive DPA1 alleles was more frequent in patients than in controls (0.573 versus 0.316). Two years after antischistosomal treatment, the rate of reinfection as examined in 55 of the 110 former patients was higher in DPA1*0301-positive individuals than in those not possessing this allele (*P* < 0.001). Ala-11 positive individuals showed less frequently ultrasonographic signs of bladder pathology than Ala-11 negative individuals (*P* < 0.05). Our results suggest a role of MHC-DP elements in the manifestation of disease in *S. haematobium* infection.

Schistosomiasis is a widespread disease in many tropical countries with substantial morbidity and mortality and enormous socioeconomic significance. *Schistosoma haematobium* infection leads to pathologic changes due to cellular infiltrates formed by lymphocytes, eosinophils, and macrophages, and a delayed-type hypersensitivity granulomatous inflammation around eggs deposited in the urinary tract. The severity of these pathologic changes correlates with the intensity of infection. More than 10 million *S. haematobium* eggs in the bladder wall have been observed in autopsies. Chronic damage to the urinary tract can result in obstructive uropathy, and extensive granuloma formation is often associated with progressive fibrosis and calcification. There is also some evidence that *S. haematobium* infection can cause squamous cancer of the bladder. The diagnostic method for the classification of bladder wall abnormalities is ultrasonography.

*Schistosoma haematobium* infection is treated efficiently with praziquantel. However, reinfection after specific therapy is frequent, depending on several factors that may influence the probability and the manifestation of reinfection.

There is evidence for a major gene in human susceptibility and resistance to infection by the intestinal blood fluke *S. mansoni*, and a locus controlling the intensity of that distinct infection has recently been mapped to chromosome 5q31-q33. Factors of the major histocompatibility complex (MHC) have also been shown to correlate with various manifestations of schistosomiasis, mostly in studies on *S. mansoni* and *S. japonicum* infections. Among the human leukocyte antigens (HLA), alleles found to be associated with hepatosplenomegaly in *S. mansoni* infection were HLA-A1 and -B5. In a recent study, an association of hepatosplenic schistosomiasis with HLA-DQB1*0201 was reported. Infection-associated colonic polyposis correlated positively with HLA-B3 and -B8, and asymptomatic infection was found to be associated with HLA-CW2 in one study. In *S. japonicum* infection, the HLA haplotype Dw19-DRw13-DQw1 was shown to be associated with strong proliferative T cell responses and with post schistosomal liver cirrhosis, and low proliferative responses were observed in individuals carrying the haplotype Dw12-DR2-DQw1. In contrast, HLA-association studies on *S. haematobium* infection are scanty. Negative associations with disease were found with HLA-A9 and HLA-A24, while HLA-B16 and -CW2 correlated positively with *S. haematobium*-associated bladder cancer and HLA-B7 with severity of bladder inflammation. The purpose of this study was to define HLA-DP elements in *S. haematobium* infection and their involvement in susceptibility to infection, bladder pathology, and the rate of reinfection after treatment.

**PATIENTS, MATERIALS, AND METHODS**

**Patients and controls.** One hundred ten individuals infected with *S. haematobium* infections (patient group = 36 adults and 74 schoolchildren) from the vicinity of Lambaréné, Gabon, and 120 healthy blood donors were recruited. Patients and controls were members of several tribes (Fang, Kele, Myene, Punu, Sira, and Tsogo). Informed consent was obtained after ethical approval of the study by the Internation Foundation of the Albert Schweitzer Hospital in Lambaréné. All individuals were long-term residents of an area endemic for *S. haematobium* and had undergone clinical and laboratory examination. After diagnosis of schistosomiasis, all patients were treated with praziquantel. Fifty-five of the 110 patients were re-examined two years after that specific antiparasitic treatment. The patient group has been described in detail previously. The 120 controls were healthy blood donors and residents of the same area as the patients. They had no evidence of parasitic infections.

**Clinical, parasitologic, and immunologic examination.** Viable eggs in urine were stained with trypan blue and counted to measure the intensity of infection. Ultrasonog-
Phenotypic frequencies of DPA1 alleles in the study population

<table>
<thead>
<tr>
<th>Study group</th>
<th>Bladder pathology</th>
<th>Reinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPA1*0103</td>
<td>Schistosomiasis</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>(n = 110)</td>
<td>(n = 120)</td>
</tr>
<tr>
<td></td>
<td>(n = 60)</td>
<td>(n = 49)</td>
</tr>
<tr>
<td></td>
<td>Not detectable</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>(n = 60)</td>
<td>(n = 34)</td>
</tr>
<tr>
<td>*0103</td>
<td>0.518‡</td>
<td>0.692‡</td>
</tr>
<tr>
<td>*02011</td>
<td>0.309</td>
<td>0.317</td>
</tr>
<tr>
<td>*02022</td>
<td>0.300</td>
<td>0.233</td>
</tr>
<tr>
<td>*0301</td>
<td>0.555‖</td>
<td>0.379‖</td>
</tr>
<tr>
<td>*0401</td>
<td>0.064</td>
<td>0.075</td>
</tr>
<tr>
<td>≤ 5 observations</td>
<td>0.045</td>
<td>0.042</td>
</tr>
</tbody>
</table>

† Alleles observed five times or less are summarized. ND = not detected. OR = odds ratio.
‡ Significantly higher frequency of DPA1*0301 in controls than in patients (P < 0.05, OR = 2.1).
§ Significantly higher frequency of DPA1*0301 in patients with detectable bladder pathology (P < 0.05, OR = 5.1).
‖ Significantly higher frequency of DPA1*0301 in patients than in controls (P < 0.01, OR = 2.1).
# Significantly higher frequency of DPA1*0301 in reinfected individuals (P < 0.001, OR = 7.7).

Hypothesis of the bladder was performed and individuals were classified as belonging to one of two groups (bladder abnormalities detectable/not detectable). Interleukin-4 (IL-4) and interferon-γ (IFN-γ) production by lymphocytes after stimulation with schistosomal antigen was measured in vitro as well as lymphocyte proliferation after stimulation with adult worm antigen or soluble egg antigen. Details on the clinical, parasitologic, and immunologic investigations have been previously reported.15,16

**HLA typing.** Genomic DNA was isolated from urea-preserved (4 M) peripheral blood following standard procedures. The variable second exons of the MHC class II loci DPA1 and DPB1 were amplified in vitro (35 cycles of the polymerase chain reaction [PCR]) using the oligonucleotide primers DPAampA (GCGGACCATGTTGTCACCTAT), DPA1I2.3 (CTTCCAGTGGCCTCCGCTCAT), and DPB1I2 (GAGAGTGGCGCCTCCGCTCAT). For the detection of hybridization patterns, the PCR products were labeled with digoxigenin-11-dideoxy-uridine 5' triphosphate (DIG-11-ddUTP; LKB, Uppsala, Sweden). For the detection of hybridization patterns, the PCR products giving ambiguous signals underwent DNA sequencing for definitive assignment of the alleles.

**Statistical analysis.** Phenotypic and haplotypic frequencies of DP alleles and of DPα- and DPβ-epitopes were calculated. Contingency analyses were performed to test for heterogeneity of DP factors among the groups and subgroups of the study population. Candidate elements for associations with S. haematobium infection or disease-specific conditions were identified by chi-square tests, and P values were estimated. For factors with expected values smaller than five, Fisher’s exact test was applied. Odds ratios (ORs) were assigned according to the method of Haldane.20

### RESULTS

**Frequencies of DP elements.** One hundred ten patients infected with *S. haematobium* were compared with 120 controls with respect to their MHC-DP alleles and epitopes. The phenotypic frequencies of the DP elements observed in our study are listed in Tables 1, 2, and 3. DPA1*0103 was the most frequent DPA1 allele in the control group (phenotypic frequency = 0.692; Table 1). Among patients, DPA1*0301 (0.555) and DPA1*0103 (0.518) occurred in comparably high frequencies. The prevailing DPB1 allele in both patients and controls was DPB1*0402 (patients = 0.518, controls = 0.426). As expected from the allele distribution, the haplotypic combination DPA1*0301-DPB1*0402 was observed in the highest frequency in both groups (patients = 0.436, controls = 0.317; Table 4). Other haplotypic combinations found in the study group are given in Table 4.

**Comparison patients versus controls.** The distribution of DP factor frequencies was compared between schistosomiasis patients and the control group. As shown in Table 2, an association of a methionine at position 11 of the DPα-
chain (Met-11) was found in patients ($P < 0.001; OR = 2.7$). Inversely, the only alternative amino acid at that position, alanine (Ala-11), was more frequent among controls (0.867 versus 0.755; $P < 0.05; OR = 2.1$). Accordingly, Met-11 homozygosity was higher among patients (19.1%) than among controls (10.0%) and Ala-11 homozygotes were more frequent in controls (41.7%) than in patients (20.9%) ($P < 0.002$) (Figure 1).

Metionine at position 11 is encoded by the alleles DPA1*0201, *0301. Consistent with this, the prevalence of DPA1*0301 was significantly higher in patients than in controls (0.555 versus 0.375; $P < 0.01; OR = 2.1$) (Table 1). Conversely, DPA1*0103 was more frequent in controls (0.692 versus 0.518; $P < 0.05; OR = 2.1$). The DPB1 epitope DEAV (aspartic acid, glutamic acid, alanine, valine; positions 84-87) encoded by the DPB1 second exon in combination with the DPA1-epitope Met-11 occurred significantly more frequently among patients (0.573 versus 0.317; $P = 0.0002; OR = 3.7$).

**Bladder pathology.** Ultrasonography of the bladder was performed in 109 patients. In 49 individuals, pathologic changes were detectable and in 60 individuals, abnormalities were not detected. Detectable bladder pathology was less frequent in Ala-11-positive than in Ala-11-negative schistosomiasis patients (48.2% versus 76.9%; $P < 0.02, OR = 0.2$). Abnormalities of the urinary tract were found only in one patient positive for DPB1*1801 compared with 10 subjects negative for DPB1*1801 ($P < 0.005, OR = 14.3$; Table 3).

**Reinfection.** Two years after specific antiparasitic chemotherapy the rate of reinfection was examined in 55 of the former 110 patients. As diagnosed by *S. haematobium* egg excretion, 34 individuals were reinfected. The rate of reinfection was higher among DPA1*0301-positive individuals (76.3%) than among those not possessing this allele (29.4%; $P < 0.001; OR = 7.7$). This applies also to the reinfection rate in DPB1*0402 positive individuals (79.3% versus 42.3%; $P < 0.005; OR = 5.2$). Reinfection was also more common among carriers of the haplotypic combination DPA1*0301-DPB1*0402 (77.7% versus 46.4%) ($P < 0.05; OR = 4.0$) than among individuals not carrying this particular DP combination.

No associations between MHC-DP elements and the intensity of infection (number of excreted eggs in urine) were found and no correlations were observed with IL-4 and IFN-γ production after stimulation of lymphocytes with schistosomiasis patients ($P < 0.005, OR = 14.3$; Table 3).

**Table 4**

<table>
<thead>
<tr>
<th>DPA1</th>
<th>DPB1</th>
<th>Schistosomiasis (n = 110)</th>
<th>Control (n = 120)</th>
<th>Detectable (n = 60)</th>
<th>Not detectable (n = 49)</th>
<th>Yes (n = 34)</th>
<th>No (n = 21)</th>
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<tbody>
<tr>
<td>*0103</td>
<td>*0101</td>
<td>0.191</td>
<td>0.200</td>
<td>0.183</td>
<td>0.204</td>
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<td>0.200</td>
<td>0.224</td>
<td>0.118</td>
<td>0.286</td>
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<td>0.117</td>
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<td>*1801</td>
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<td>0.204§</td>
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<td>0.095</td>
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<td>*02011</td>
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<td>*0101</td>
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<td>0.206</td>
<td>0.095</td>
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<td>0.450</td>
<td>0.429</td>
<td>0.618¶</td>
<td>0.286¶</td>
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</table>

† All combinations of DPA1 alleles from Table 1 and DPB1 alleles from Table 3 observed more than 10 times were included. ND = not detected; OR = odds ratio.
‡ Significantly higher frequency of DPA1*0103-DPB1*1801 in patients with absent bladder pathology ($P < 0.005, OR = 14.3$).
§ Significantly higher frequency of DPA1*0202-DPB1*0101 in patients with detectable bladder pathology ($P < 0.01, OR = 4.0$).
¶ Significantly higher frequency of DPA1*0301-DPB1*0402 in reinfecced individuals ($P < 0.05, OR = 4.0$).
With the DP\textsubscript{a} acids at DP\textsubscript{a} position 11 (Met-11 and Ala-11) is given for patients and controls. Ala-11/Ala-11, homozygosity (alanine); Ala-11/Met-11, heterozygosity (alanine, methionine); Met-11/Met-11, homozygosity (methionine). Three row by two column test: $\chi^2 = 0.126, P < 0.002$.

![Graph showing the percentage of patients and controls with homozygosity and heterozygosity at DP\textsubscript{a} position 11](image)

**Figure 1.** Rate of homozygosity and heterozygosity of amino acids at DP\textsubscript{a}-position 11 (Met-11 and Ala-11) is given for patients and controls. Ala-11/Ala-11, homozygosity (alanine); Ala-11/Met-11, heterozygosity (alanine, methionine); Met-11/Met-11, homozygosity (methionine). Three row by two column test: $\chi^2 = 0.126, P < 0.002$.

There was no correlation between HLA-DP factors and the intensity of infection. This supports findings of others who did not find associations between HLA-DR and -DQ epitope DEAV with the DP\textsubscript{a} epitope Met-11 was strongly associated with infection. As expected, an inverse association was found with alanine at position 11 of the DP\textsubscript{a} chain (Ala-11). This epitope is associated with relative protection from infection. There was no correlation between HLA-DP factors and the intensity of infection. This supports findings of others who did not find associations between HLA-DR and -DQ elements and egg excretion in *S. mansoni* infection.

We found that 1) susceptibility and resistance to *S. haematobium* infection, 2) bladder abnormalities as detected by ultrasonography, and 3) reinfection rates are associated with the MHC loci coding for the HLA class II DP molecule.

Susceptibility to disease was significantly associated with the allele DPA1*0301 and with a methionine at position 11 of the DP\textsubscript{a} chain (also present in the DPA1 alleles *02021 and *02022). The combination of the DP\textsubscript{b} epitope DEAV with the DP\textsubscript{a} epitope Met-11 was strongly associated with infection. As expected, an inverse association was found with alanine at position 11 of the DP\textsubscript{a} chain (Ala-11). This epitope is associated with relative protection from infection.

During HLA DP typing one novel DPA1 (DPA1*0105) and one DPB1 allele (DPB1*6601) were identified. The sequences of these novel variants are given elsewhere.

**DISCUSSION**

We found that 1) susceptibility and resistance to *S. haematobium* infection, 2) bladder abnormalities as detected by ultrasonography, and 3) reinfection rates are associated with the MHC loci coding for the HLA class II DP molecule.

Susceptibility to disease was significantly associated with the allele DPA1*0301 and with a methionine at position 11 of the DP\textsubscript{a} chain (also present in the DPA1 alleles *02021 and *02022). The combination of the DP\textsubscript{b} epitope DEAV with the DP\textsubscript{a} epitope Met-11 was strongly associated with infection. As expected, an inverse association was found with alanine at position 11 of the DP\textsubscript{a} chain (Ala-11). This epitope is associated with relative protection from infection.

There was no correlation between HLA-DP factors and the intensity of infection. This supports findings of others who did not find associations between HLA-DR and -DQ elements and egg excretion in *S. mansoni* infection.

DP\textsubscript{a} Ala-11 was correlated with a lower degree of detectable bladder abnormalities, a clinical manifestation of chronic disease. Ala-11 is frequent in healthy controls, relatively frequent in patients without bladder pathology, and rare in individuals with bladder abnormalities. This observation indicates a remarkable role of that particular position and points towards a genetically determined immunologic spectrum of the immune response against *S. haematobium* infection.

The observation of the DPA1*0301 association with disease is supported by the fact that a higher rate of *S. haematobium* reinfection was observed with DPA1*0301-positive reconvalescent individuals after treatment than in DPA1*0301-negative individuals. This applies also to those individuals bearing the haplotypic combination DPA1*0301-DPB1*0402. Both alleles contribute in comparable extents to the association.

It has previously been shown that the HLA molecule DP(\alpha*0301,\beta*0402) is associated with a distinct clinical manifestation of another helminth infection, namely with localized onchocerciasis. Interestingly, the presence or absence of DP\textsubscript{a}-Met-11 correlates with susceptibility and protection (putative immunity) in *Onchocerca volvulus* infection.

Genetic predisposition of relative resistance to both a nematode and a trematode infection exhibited by the same amino acid of the DP\textsubscript{a} molecule and identical alleles is surprising. We have no causal explanation for the finding that in both cases a disease with high IgE and eosinophil levels correlates positively with the alleles DPA1*0301 and DPB1*0402 and, in particular, with the position 11 of the DP\textsubscript{a} molecule. In schistosomiasis, low levels of reinfection are associated with high blood levels of activated eosinophils and an adequate IgE response. It may be assumed that in schistosomiasis haematobium, protective HLA elements regulate the immune response in terms of higher levels of protective IgE antibodies and eosinophilia. This would be consistent with a T helper cell 2 (Th2)-type response. Although it has been shown that HLA molecules may direct immune responses towards either Th1- or Th2-type patterns of reactivity, in our study no associations between HLA-DP factors and IL-4 or IFN-\gamma production rates were observed.

Presently, there is no evidence in schistosomiasis or onchocerciasis that the associated HLA-DP elements lead to protection via specifically presented peptides, which trigger the course of infection. It is not clear whether both infectious organisms (*S. haematobium* and *O. volvulus*) exhibit identical or resembling antigenic peptides to be presented by the same HLA molecule. When adapting the crystallographic structure of the DR molecule to DP one becomes aware that position 11 of the DP\textsubscript{a}-chain as well as the DP\textsubscript{b} epitope at positions 84-87 may essentially be involved in antigen binding. Both epitopes are important structural components in shaping the cleft for antigen binding.

Several other parallels of HLA associations between onchocerciasis and schistosomiasis are worth mentioning. The alleles DRB1*01, DQA1*0101, and DQB1*0501 were associated with the generalized manifestation and immunosuppression in *O. volvulus* infection (DRB1*01 not significant). The same alleles, which all are in strong linkage disequilibrium and constitute a particular haplotype, were also associated with weak proliferative lymphocyte responses after stimulation with soluble egg antigen induced in *S. mansoni* infection. DQB1*0201, which is associated with generalized disease in onchocerciasis, is also associated with hepatosplenic *S. mansoni* infection.

The significance of MHC associations with infectious diseases is the subject of continuous controversial discussion. It needs to be considered that other polymorphic genes, being in linkage with associated DP alleles, could be responsible for the associations observed in our study. Attention must be paid to evading mechanisms of schistosomes and it...
has to be remembered that schistosomes do incorporate macromolecules of the host. However, when one simplistically looks at HLA-dependent antigen presentation as an early step in generating specific immune responses and when one relates later events such as the type of T cell responsiveness and pathology to MHC-polymorphism, the puzzle becomes in part resolvable.

Although other genetic factors might also be responsible, HLA-DP elements appear to be involved in the genetic determination of susceptibility to S. haematobium infection and the associated pathology of the urinary tract.

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