Association of Hepatosplenic Schistosomiasis with HLA-DQB1*0201


Major histocompatibility class II alleles of 351 persons living in an area endemic for Schistosoma mansoni in northeastern Brazil were characterized at three loci (DRB1, DQA1, and DQB1). Contingency analyses were used to compare allele frequencies with high egg excretion, proliferative response to schistosome soluble egg antigens (SEA), and occurrence of severe, biopsy-confirmed hepatosplenic disease. There were no associations of HLA-DR or DQ with egg excretion. Patients positive for DRB1*01, DQA1*0101, or DQB1*0501 were less likely to respond to SEA than was the overall study population. However, using stringent Bonferroni correction (multiplying P values by the number of alleles tested; P × 35), none of these associations with SEA responsiveness remained significant. Hepatosplenic disease was less likely in patients positive for DRB1*11 and was more likely in patients positive for DRB1*07 or DQB1*0201. However, only the DQB1*0201 association remained significant (odds ratio = 3.72; P < .005) following Bonferroni correction.

Infection with Schistosoma mansoni causes severe morbidity and mortality in several regions of the developing world. Adult female worms living in the mesenteric venules can release up to 300 eggs per day, many of which exit the body to continue the parasite's life cycle. However, some eggs become lodged in the presinusoidal capillaries of the liver and induce CD4 T cell–dependent granuloma formation. In most persons, the infection is relatively benign. Patients with this asymptomatic or “intestinal” form of the disease only experience occasional gastrointestinal discomfort. In contrast, some persons develop a severe, “hepatosplenic,” form of schistosomiasis. Hepatosplenic disease is characterized by liver fibrosis, spleen congestion, portal hypertension, ascites accumulation, collateral circulation, esophageal varices, and eventual death, barring medical intervention.

The factors that cause some persons to develop the perportal fibrosis associated with hepatosplenic disease are poorly understood. Some studies have implicated the level of infection [1, 2], while others suggest that the intensity of the immune response to the lodged eggs (or failure to regulate this response) may be responsible [3, 4]. In either case, CD4 T cells may be centrally involved, either by producing the cytokines that regulate antibody isotype levels associated with resistance to infection [5, 6] or by generating the increased responses to egg antigens of patients who are in the process of developing hepatosplenic disease [3, 4]. In addition, the cytokines produced by CD4 T cells are of critical importance in granuloma formation, modulation, and egg-induced hepatic fibrosis [7, 8]. Because CD4 T cell responses are dependent on antigen presentation in the context of the class II major histocompatibility complex (MHC), patients’ MHC class II phenotypes may exert an influence on susceptibility to infection, regulation of immune responsiveness to egg antigens, and/or progression to hepatosplenic disease. This study was designed as a cross-sectional survey to examine possible correlations of the HLA class II alleles DRB1, DQA1, and DQB1 with the intensity and clinical form (intestinal or hepatosplenic) of infection and response to egg antigens in Brazilian patients with S. mansoni infections.

Materials and Methods

Study population. For this study, 301 patients with the intestinal form of schistosomiasis and 50 with severe hepatosplenic disease were analyzed. Patients with the intestinal form were residents of the schistosomiasis-endemic villages of Itaquara and Itririqui in the state of Bahia, Brazil. Infection was diagnosed and fecal egg count was estimated by use of the modified Kato-Katz method. A geometric mean number of eggs per gram of stool (EPG) was calculated for 3 stool samples for each patient. Patients with severe hepatosplenomegaly came from various villages in Bahia (including 4 patients from Itaquara and Itririqui) to undergo splenectomy and portal shunt surgery at the Hospital Roberto Santos in Salvador. During surgery, diagnostic wedge biopsies were obtained from
these patients for pathologic analysis and confirmation that the hepatosplenomegalyies were due to schistosomiasis [1]. There were granulomas surrounding schistosome eggs and fibrosis in biopsy samples from all surgical patients. Seri from these patients were also tested for antibodies to hepatitis B and C. Although the method of enrollment into the study differed for intestinal and hepatosplenic patients (community vs. hospital), there were no apparent racial, socioeconomic, or living condition differences between the 2 groups. All patients were offered treatment for schistosomiasis.

Assay for proliferative response. Peripheral blood mononuclear cells (PBMC) were isolated from venous blood and plated in 96-well plates at 250,000 cells/well in RPMI containing 5% normal human serum, 3% penicillin-streptomycin, and 1% L-glutamine (GIBCO BRL, Grand Island, NY). Cells were stimulated with soluble schistosome egg antigens (SEA) at a final concentration of 5 μg/mL for 5 days at 37°C in a CO2-enriched environment. Tritiated thymidine (0.5 μCi, 5 Ci/mmol; Amersham, Arlington Heights, IL) was added for the final 8 h of culture. Incorporation of tritiated thymidine was recorded as counts per minute (cpm). A patient was considered to have a positive proliferative response if the stimulation index (experimental value divided by control value) was ≥2.0 and the experimental minus the control cpm was ≥2000.

HLA typing. DNA was isolated from PBMC or spleen cells (hepatosplenic patients), DRB1, DQA1, and DQB1 DNA of the human MHC were amplified by use of the polymerase chain reaction using sets of primers and conditions specified by the 11th human MHC class II alleles are shown in figure 1A. Patients positive for DRB1*01 (OR = 0.416, P = .034), DQA1*0101 (OR = 0.379, P = .005), or DQB1*0501 (OR = 0.486, P = .037) were all less likely to respond to SEA (ORs <1) than was the overall study population. Although none of these P values remained significant following the stringent Bonferroni correction (i.e., after multiplication by 35), the fact that these alleles are all in strong linkage disequilibrium suggests that these observations are not the result of chance. These data are compatible with studies showing that the HLA-DQw1 molecule is associated with nonresponsiveness to Schistosoma japonicum antigens [10].

Allelic associations with hepatosplenic disease. Associations of the form of clinical disease and MHC class II alleles are shown in figure 1B. As with egg excretion was nonparametric Spearman’s rank correlation analysis (P < .02). The value did not maintain significance following Bonferroni correction. Similarly, patients positive for DRB1*07 had an initial positive association with hepatosplenic disease (OR = 2.416, P = .024) that did not maintain significance after correction. However, patients positive for DQB1*0201 had a strong association with hepatosplenic disease (OR = 3.724, P = .00014) that maintained significance (P = .005) following Bonferroni correction. Because some studies have associated hepatosplenic disease with hepatitis infection [12], patients with hepatosplenic disease were tested for hepatitis B and C. Only 7 of the 50 hepatosplenic patients had antibody against hepatitis, and they were represented by both HLA-DQB1*0201-positive and -negative persons. Removal of these subjects from analysis did not affect the significance of the association.

Correlation between ORs for hepatosplenic disease and proliferative responsiveness. As shown in figure 2A, ORs for hepatosplenic disease were significantly correlated with ORs for SEA responsiveness (Pearson’s correlation coefficient, r = .565; P = .001). A correlation analysis weighted for the percentage of subjects positive for a given allele (to ensure that the curve was not unduly influenced by alleles representative of few people) was also significant (r = .636, P < .001), as was nonparametric Spearman’s rank correlation analysis (P < .02). A similar analysis comparing ORs for hepatosplenic disease with ORs for high egg excretion (figure 2B) showed no relationship (unweighted r = .261, P = .172; weighted r = .132, P = .495; Spearman’s P > .05).

Discussion

For schistosomiasis patients >15 years old, these data indicate that the presence of HLA-DQB1*0201 in the pa-
Figure 1. Associations of major histocompatibility complex class II alleles with clinical aspects of schistosomiasis. A, Odds ratios (ORs) and 95% confidence intervals (CIs) for each allele in relation to responsiveness to schistosome soluble egg antigens (experimental - control, >2000 cpm; E/C, >2.0). B, ORs and CIs for each allele in relation to likelihood for severe hepatosplenic disease. CIs that do not transverse 1 are in bold.

The patient’s MHC is associated with an increased risk for that individual to develop severe hepatosplenic disease. Previous studies have demonstrated HLA class I associations with severe disease in *S. mansoni* [4, 13], *S. japonicum* [14], and *Schistosoma haematobium* [15]; however, to our knowledge, the current study is the first to demonstrate a correlation between severe hepatosplenic disease caused by *S. mansoni* and an MHC class II allele. A study of an Egyptian population [4] demonstrated a negative association of DR2 with fibrosis in *S. mansoni*. Although the OR for DR2 with respect to hepatosplenic disease was also <1 in our study (i.e., subjects with hepatosplenic disease were less likely to be DR2+ than was the overall population), this finding was not significant.
ever, a 1988 survey of this region indicated a schistosomiasis prevalence of 87%, suggesting that these data for persons with schistosomiasis are very likely a good representation of the overall population. Because the population was not sampled at random, the possibility that certain unknown confounders or selection biases influenced the observations described cannot be ruled out. Nevertheless, it would be very unlikely that an association as strong as the one observed here was obtained simply by chance.

In addition to the specific finding that HLA-DQBl *0201 is strongly associated with hepatosplenic disease, information from this study may also help elucidate the general factors contributing to hepatosplenomegaly. Development of hepatosplenic schistosomiasis, like most HLA-associated diseases, most likely involves both genetic and environmental factors. Obviously, without an infection, no matter what genetic background an individual has, he or she will not develop hepatosplenic schistosomiasis. Several studies have indicated that intense infection increases the incidence of severe disease [1, 2], while others suggest that the strength of an individual’s immune response (or failure to regulate that response) can similarly lead to hepatosplenic disease [3, 4]. Therefore, it might be expected that alleles positively or negatively associated with hepatosplenomegaly may show similar positive and negative associations with intensity of infection or proliferative responses to egg antigens.

Analysis at any one allele did not show a clear relationship between hepatosplenic pathology and intensity of infection (as estimated by egg excretion in subjects <19 years old) or strength of anti-SEA response. For example, DQBl*0201 did not demonstrate a statistically significant correlation with either high egg excretion or PBMC responsiveness to SEA. However, the ORs for hepatosplenic disease at a given allele showed a significant correlation with the ORs for SEA responsiveness. Persons with alleles that confer a greater risk for hepatosplenic disease tend to have stronger responses to SEA. In contrast, the ORs for hepatosplenic disease and high egg excretion do not correlate. These observations strengthen previous suggestions that hepatosplenic disease is related to more intense, perhaps less regulated, T cell responses to SEA [3, 4] and suggest that the mechanisms linking intensity of infection with hepatosplenic disease [1, 2] are not MHC class II-dependent.

Further investigation of the role of HLA in schistosomiasis is clearly warranted. Information from additional geographic areas with larger populations is needed to determine how universal these findings may be. Because Bonferroni correction is very conservative and may lead to type II statistical errors (rejection of a significant finding), it may also be useful to reexamine the other alleles that demonstrated significance prior to Bonferroni correction. In addition, further studies with HLA-DQBl*0201 could reveal important information regarding T cell response regulatory mechanisms and what roles these mechanisms may play in development of hepatosplenic schistosomiasis.

Figure 2. Correlation of odds ratios (ORs) for hepatosplenic disease and schistosome soluble egg antigen (SEA) responsiveness (A) and hepatosplenic disease and high egg excretion (>800 eggs/g of stool) (B).

Although there was a very strong association between the presence of the HLA-DQBl*0201 and the development of hepatosplenic disease, this study does not provide enough evidence that the association is causal in nature. The fact that there is no previous knowledge of the most common HLA haplotypes in this region of Brazil does not allow us to rule out the possibility that the association described is due to an overrepresentation of this allele in the population at large.
Acknowledgments

We thank Claudio Roberto dos Santos, Clea Moreira Noqueira, Argemiro Francisco de Carvalho Pereira, and Jackson Cerqueira for their assistance in the field site; the Bahian branch of the Brazilian National Health Service (FNS) for its cooperation; Edgar Milford and Ivan Yunis for helpful discussions; Daniel G. Colley and Patrick J. Lammie for critical reading of the manuscript; and Allen W. Hightower for advice on statistical analyses.

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