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Absence of linkage between MHC and a gene involved in susceptibility to human schistosomiasis

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Abstract

Six hundred million people are at risk of infection by Schistosoma mansoni. MHC haplotypes have been reported to segregate with susceptibility to schistosomiasis in murine models. In humans, a major gene related to susceptibility/resistance to infection by S. mansoni (SM1) and displaying the mean fecal egg count as phenotype was detected by segregation analysis. This gene displayed a codominant mode of inheritance with an estimated frequency of 0.20-0.25 for the deleterious allele and accounted for more than 50% of the variance of infection levels. To determine if the SM1 gene segregates with the human MHC chromosomal region, we performed a linkage study by the lod score method. We typed for HLA-A, B, C, DR and DQ antigens in 11 informative families from an endemic area for schistosomiasis in Bahia, Brazil, by the microlymphocytotoxicity technique. HLA-DR typing by the polymerase chain reaction with sequence-specific primers (PCR-SSP) and HLA-DQ were confirmed by PCR-sequence-specific oligonucleotide probes (PCR-SSOP). The lod scores for the different θ values obtained clearly indicate that there is no physical linkage between HLA and SM1 genes. Thus, susceptibility or resistance to schistosomiasis, as defined by mean fecal egg count, is not primarily dependent on the host's HLA profile. However, if the HLA molecule plays an important role in specific immune responses to S. mansoni, this may involve the development of the different clinical aspects of the disease such as granuloma formation and development of hepatosplenomegaly.

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• HLA
• Linkage study
• Human schistosomiasis

Introduction

Schistosomiasis is a parasitic disease that has widely spread throughout the world. Six hundred million people are at risk of infection by schistosomes. It is estimated that two hundred million are currently infected and around twenty thousand deaths occur each year (1). Humans become infected in rivers or ponds populated by infected snails that release schistosome larvae during the hottest hours of the day. After entering their human host by digesting the skin, the larvae migrate to the mesenteric veins where they mature into adult worms within 4 to 5 weeks. Severe liver disease that develops in 5 to 10% of

infected subjects is the consequence of the rejection triggered by S. mansoni eggs in the liver. This reaction may lead to a heightened formation of scars that cause high portal blood hypertension and consequently esophageal varices and ascites. Most subjects with advanced schistosomiasis will die of the disease (2). Severe schistosomiasis is associated with high infection levels and several studies have attempted to identify which factors account for these heavy infections observed in parts of a population from an endemic area.

Preliminary epidemiological data indicate that some people are prone to rapid and severe reinfection after being cured (3). In addition, higher infection levels are clustered within certain families. On the other hand, hepatosplenomegaly, a severe clinical feature of S. mansoni infection, is not found in Brazilian black people in spite of similar high infection levels (4). These and other studies suggest the presence of a genetic influence on the resistance to infection by Schistosoma. Correa-Oliveira et al. (5) described a gene called Rsm-1 involved in IgM production in mice vaccinated with irradiated cercariae. This gene is not localized on chromosome 17, site of the H-2. However, the presence of $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes has been correlated to protection against infection after vaccination with irradiated cercariae when compared with haplotypes $H\text{-}2^k$ and $H\text{-}2^a$ (6). The H-2 complex, in addition to having an undefined genetic background, also affects the number of eggs in the stool.

Few genetic studies have been carried out on human resistance to parasites. In 1991, our group reported that subjects with high infection levels are less resistant than the rest of the population of endemic areas to renewed infection, and this resistance level was shown to be under the control of a major gene (7).

In family studies, segregation analysis was performed to determine the mode of inheritance of this trait, which was based on the mean fecal egg count in 20 pedigrees from an endemic area in Brazil. Corrected data were grouped into four categories according to level of exposure to water, and the results showed a mixture of three normal distribution curves indicating a codominant mode of inheritance for this gene. To test this hypothesis two different models were used in the segregation analysis, i.e., the unified mixed model and the regressive model. Both also take into account other genetic, environmental, and behavioral factors involved in the onset and development of the disease. The hypothesis of dominance, recessivity or absence of gene transmission was rejected. However, the hypothesis of codominance for the SM1 gene was accepted with $P<10^{-9}$. An estimated frequency of the susceptibility allele (A) of 0.20 to 0.25 was calculated, and according to the Hardy-Weinberg law we would expect to find 5% susceptible individuals (AA), 60% heterozygotes (Aa) and 35% would be considered resistant (aa). This major gene accounted for more than 50% of the variance of infection levels after the effects of environmental factors and age were taken into account.

Several association studies have correlated HLA with hepatosplenomegaly in human schistosomiasis. In Egyptian children, HLA-A1 and B5 were associated with hepatosplenomegaly with a relative risk of 55.6 when both alleles were present (8). In Philippine patients, presence of HLA-B40 was also correlated to hepatosplenomegaly. In addition, a suppressor effect associated with the presence of the HLA-DQ molecule has been suggested by Ohta et al. (9), when response to a soluble egg antigen is tested. In a recent study, Secor et al. (10) have shown that there is no association between HLA class II alleles and egg excretion in a Brazilian population, but that an association between HLA DQB1*0201 and hepatosplenic disease is present. In addition, intensity of

infection as measured by fecal egg count has been shown to correlate with development of hepatosplenomegaly (11), suggesting that a gene within the Major Histocompatibility Complex (MHC) could be the candidate gene for SM1.

Family studies can be of great interest when linkage to a marker is being looked for. Linkage is usually tested using the lod score method. A lod score is the logarithm of the likelihood ratio of having observed a certain pedigree given various linkage distances compared to the same pedigree given no linkage. A linkage analysis will be further strengthened if the presence of the disease locus itself has already been suggested from other sources of evidence, as is the case for schistosomiasis (12,13).

Material and Methods

Samples

Peripheral blood mononuclear cells (PBMC) were obtained by a Ficoll-Hypaque gradient (d = 1.077) from 73 individuals (11 families) from the endemic area (Caatinga do Moura, BA, Brazil) who had been previously included in the study for the description of the SM1 gene (7).

HLA typing

HLA class I and class II typing was initially performed using the standard microlymphocytotoxicity test with HLA-specific antisera (14). We also typed for class II using molecular biology methods.

Serology typing

Class I and class II HLA typing was performed by complement-mediated cytotoxicity with 144 sera for class I HLA-A, B and C and 72 sera for class II HLA-DR and DQ from C-six Diagnostics Inc. (Mequon, WI).

DNA typing

DNA was extracted from frozen cells by a phenol/chloroform method. HLA-DR low resolution typing by the polymerase chain reaction with sequence-specific primers (PCR-SSP) was performed according to Olerup and Zetterquist (15), and DQA and B typing was performed by PCR followed by hybridization with sequence-specific oligonucleotide probes (SSOP) (16,17). We used generic primers for exon-2 amplification according to the 12th International Histocompatibility Workshop protocols. Ten percent of the PCR products were checked on 1.2% agarose gel electrophoresis. Three microliters of amplified product per blot was denatured. Replicate filters were prepared by loading the denatured sample onto a Hybridot Manifold apparatus (BRL, Gaithersburg, MD) and the membranes were dried. Each oligonucleotide probe was labeled with 40 µCi [γ^{32}P] ATP using T_4 polynucleotide kinase. Sequences of the 17 probes for DQA and 15 for DQB were based on published sequences. Blots were hybridized overnight with the probe at 54°C. Filters were washed twice in 2 x SSC/0.1% SDS at room temperature, and once in TMAC solution for 45 min at 59°C. We utilized each blot 2-3 times. To remove the probe, blots were washed 3 times in warmed (95°C) TE 10-10.

Linkage analysis

A lod score (Z) is the logarithm of the likelihood ratio of having observed a certain pedigree given various linkage distances compared to the same pedigree given no linkage. As linkage increases, the value of the recombination fraction (θ) approaches zero. A Z value that is the ratio between H_1 (hypothesis of no linkage $\theta = 0.5$) and H_0 (hypothesis for the presence of linkage $\theta<0.5$) is calculated. When the Z value obtained is more than 3 (odds of 1000 to 1) it is considered as significant evidence of linkage; a lod score

of -2 is taken as evidence against the existence of linkage between the gene and the marker (18). Lod score analysis requires information about some parameters of the locus under investigation. In the present case, these parameters were the frequency of the allele predisposing to high levels of infection, the genotype specific for the mean intensity of infection and the variance in infection intensity residual to the major gene effect. These parameters were obtained from the previous segregation analysis data (7). We used the Linkage package developed by J. Ott (18) (University of New York) to compute lod scores from pedigrees. Following the author's instructions we present the lod score values for scanning θ value between 0.00-0.40.

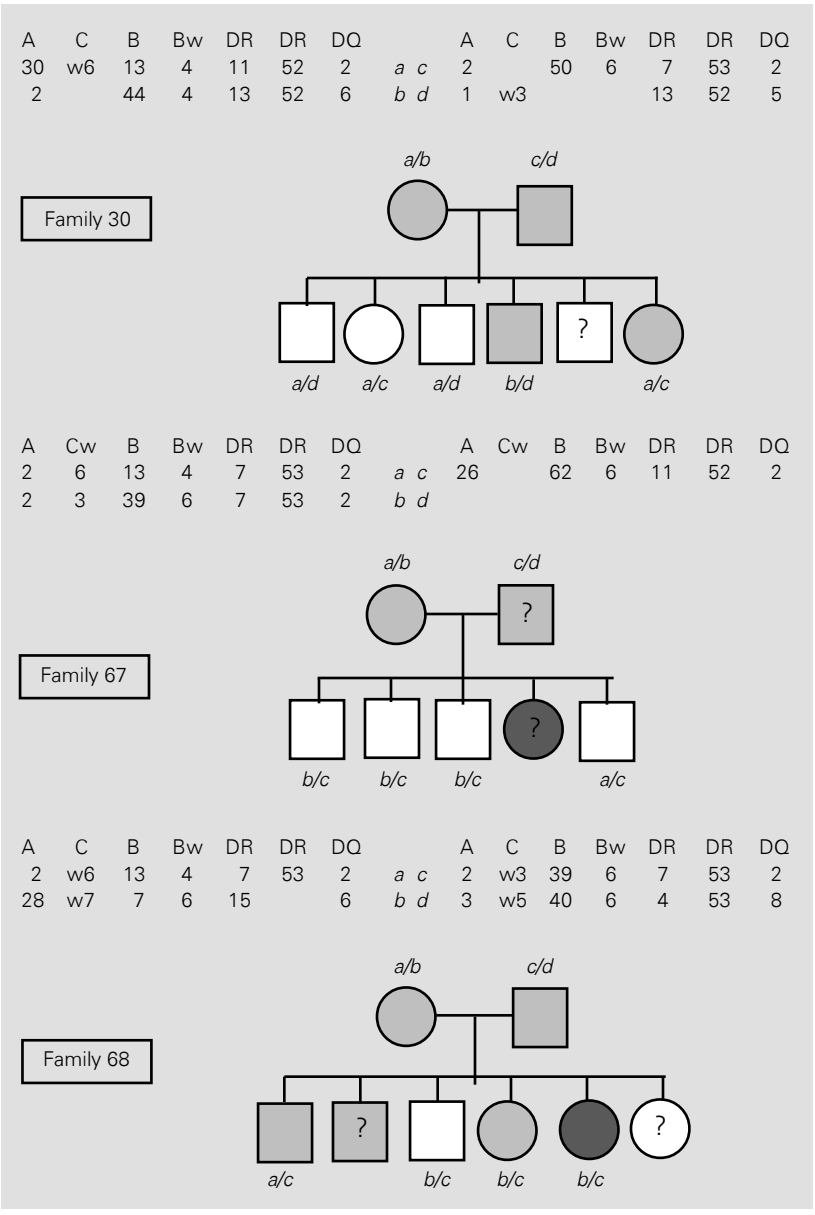

Figure 1 - Examples of individual pedigrees. ●, AA or homozygous susceptible individuals. ◐, Aa or heterozygous individuals. ○, aa or homozygous resistant individuals. ?, HLA typing not done.

Results

According to the results obtained by HLA-A, B, C, DR and DQ typing, we defined haplotypes using serologic notation (19). We used a, b, c or d to indicate the maternal and paternal HLA haplotypes and added the data to the pedigrees of the 11 individual informative families. Some examples are shown in Figure 1, where different patterns have been used to denote SM1 gene segregation. All families proved to be informative for this study since individual lod score values were found to be different from zero. The combined lod score obtained for all families is shown in Table 1a. The critical lod score value corresponding to $\theta = 0.0$ (co-segregating genes) was -3.56, providing strong evidence against linkage between the SM1 gene and the MHC. In addition, lod scores calculated with recombination fractions up to 0.4 were likewise non-significant, meaning that these loci are not even close to one another.

To determine if divergent results could be obtained with single families or family groups, we performed separate analyses using the following small pedigrees: a) family group 1: families 29, 35, 73, 76, 83 and 106, b) family group 2: families 45 and 117, c) family group 8: families 67 and 68, and d) family 30. The pedigree containing the 6 informative families in group 1 is shown in Figure 2. All lod scores obtained are shown in Table 1b. None of the pedigrees yielded a lod score close to the critical value (3.3) required for linkage, suggesting again that SM1 is not a gene of the HLA locus.

Discussion

Association of MHC genes with S. mansoni infection has been described in murine models comparing different H-2 backgrounds. On one hand, it has been shown that H-2 does not correlate with the number of adult worms, but some studies have shown that specific H-2 haplotypes are related to other traits such as antibody titers and maximum fecal egg count (20). Several studies on humans have shown that subjects carrying certain HLA haplotypes have a higher risk of developing severe liver disease when infected by S. mansoni. Since our group has obtained evidence for a major gene controlling infection levels in an endemic area that segregated in a codominant fashion, we tested whether this major gene could be part of the HLA complex.

For this purpose we analyzed families previously found to be informative for the segregation of the SM1 gene, applying the lod score method. A lod score of -3.56 was observed and indicated, with a P value greater than 0.001, that the SM1 is outside the MHC chromosomal region. In other words, the results clearly show that there is no physical linkage between SM1 and the MHC region.

Trying to locate the SM1, Marquet et al. (21) have recently employed a genome-wide study for the search. Using a two-point lod score analysis they have reported that the SM1 is located on chromosome 5q31-q33 close to the colony stimulating factor 1 receptor (CSF1R) with a maximum two-point lod score of +4.54. This region also carries genes coding for the granulocyte-macrophage colony-stimulating factor (CSF2), interleukins 4, 5, 3, 9, 13 and the immune regulatory factor IRP1. The present study confirms the well-known importance of the cytokine profile in human schistosomiasis (22), where resistance to infection correlates with a higher IL4/interferon γ ratio.

Although the MHC is not related to the SM1, could this system be implicated in other controlling points of the immune response to human schistosomiasis?

The HLA molecule itself is directly involved in the immune response, presenting peptides to the T cell receptor (TCR). This recognition is an essential step to trigger specific immune responses. A pivotal role for the HLA molecule can thus easily be envisaged, where these interactions will ultimately interfere with the course of the disease and the development of the different clinical aspects of the infection such as granuloma formation and development of hepatosplenomegaly. One major controlling point,

Table 1 - Lod scores considering different recombination fractions (θ) for linkage analysis between HLA antigens and the SM1 gene involved in susceptibility to infection by *Schistosoma mansoni*.

Family group 1 comprises families 29, 35, 73, 76, 83 and 106; family group 2 comprises families 45 and 117; family group 8 comprises families 67 and 68.

1a: combined lod score for all families

θ	0.00	0.01	0.05	0.10	0.20	0.30	0.40
	-3.56	-2.71	-1.30	-0.66	-0.11	-0.05	-0.04

1b: lod scores for isolated families or family groups

θ	0.00	0.01	0.05	0.1	0.2	0.3	0.4
Family group 1	-3.75	-2.91	-1.54	-0.91	-0.36	-0.12	-0.03
Family group 2	-0.21	-0.19	-0.14	-0.09	-0.03	-0.01	0.00
Family group 8	0.34	0.34	0.33	0.31	0.25	0.17	0.06
Family 30	0.06	0.06	0.05	0.04	0.02	0.01	0.00

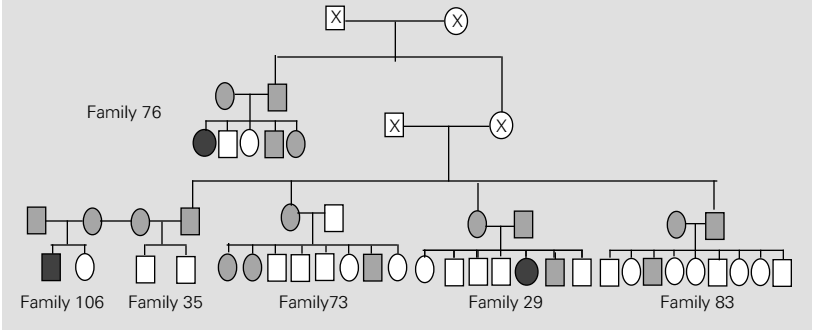

Figure 2 - Pedigree of 6 families analyzed in this study. ●, AA or homozygous susceptible individuals. ◍, Aa or heterozygous individuals. ○, aa or homozygous resistant individuals. ?, HLA typing not done.

defining level of resistance to the infection as measured by fecal egg count, would be accounted for by the SM1 gene that, together with age, corresponds to over 50% of the variance in infection levels. A second controlling point could be the inflammatory response to the egg leading to granuloma formation and the ensuing hepatosplenomegaly. The presentation of the relevant peptide by the HLA molecule itself may trigger the potent immune response of delayed hypersensitivity that is the hallmark of granuloma formation.

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