

Carriage of *DRB1*13* Is Associated with Increased Posttreatment IgE Levels against *Schistosoma mansoni* Antigens and Lower Long-Term Reinfection Levels¹

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Praziquantel treatment for *Schistosoma mansoni* infection enhances Th2 responsiveness against parasite Ags, but also increases the variance in Ab isotype levels. This effect may arise partly from genetic heterogeneity. In this study, associations between HLA polymorphisms at three loci (*HLA-DQB1*, *HLA-DQAI*, and *HLA-DRB1*) and posttreatment Ig responses to *S. mansoni* Ags were assessed in 199 individuals aged 7–50 years from Uganda. Blood samples were assayed for IgG1, IgG4, and IgE levels against soluble worm Ag (SWA), soluble egg Ag, tegument Ag, and a recombinant tegumental Ag (rSm 22.6) 7 wk after treatment. Multivariate ANOVA analysis initially revealed associations between carriage of *DRB1*13* and increased levels of IgG1, IgG4, and IgE against SWA, tegument Ag, and rSM22.6. Subsequent analysis of covariance, which controlled for correlations between isotype levels and also included pretreatment IL-4, IL-5, and IL-13 responsiveness against SWA as covariates, revealed an independent association only between *DRB1*13* and a factor score summarizing IgE levels to worm-derived Ags, which was strongest in adults. A post hoc age- and sex-stratified analysis revealed lower reinfection intensities at 1 year, 22 mo, and 6 years after the first round of treatment among carriers of *DRB1*13*. These results indicate that genetic background has a prominent influence on the posttreatment Th2 immune response to *S. mansoni* Ags, as well as a downstream association with long-term reinfection levels. *The Journal of Immunology*, 2006, 176: 7112–7118.

A common observation during parasitological surveys, in areas of endemic infection, is that *Schistosoma mansoni* infection levels, as measured by egg counts in fecal samples, are considerably lower in adults compared with children (1). Conversely, IgE levels in response to *S. mansoni* worm Ags increase with age before treatment (2) and are increased further when measured several weeks after treatment (3, 4). These increases in IgE levels after treatment are concomitant with increases in Th2 cytokine responsiveness to worm Ags (5) and have been correlated with resistance to reinfection measured several years after treatment (3).

IgE production is limited to B cells that have undergone a tightly regulated process of isotype class switching, whereby production of IgE is preceded by IgG1 and occurs alongside production of IgG4 (6). Although the molecular mechanisms of IgE regulation

have been partly characterized, especially in relation to atopic disease, the causal pathway between parasite death, increased IgE levels, and reinfection status in relation to schistosomiasis remains undefined. Underpinning IgE levels, and those of other isotype responses, is likely to be the genetic background of the host. Total IgE has been known for many years to be under genetic control, with heritability estimates of ~50% (7). The best known linkage for control of a quantitative immune trait is the linkage of total IgE to the chromosome 5q31 gene cluster, encoding predominantly Th2 cytokines such as IL-4 and IL-13, first described by Marsh et al. (8), and also implicated in control of intensity of *S. mansoni* infection (9). This linkage has been confirmed in a number of populations (10). In addition, total IgE serum levels have been associated previously with carriage of the HLA class II allele, *DRB1*0701* (11).

The HLA class II region is also a candidate region for genes involved in the restriction of Ag-specific humoral responses to parasite Ags, including those of *S. mansoni*. A number of studies have therefore sought evidence of associations between HLA genes and control of infection or disease. Early studies concerned HLA control of proliferative responses to *Schistosoma japonicum* Ags (12, 13). These were accompanied by an observed increase in *HLA-B40* in high responders to schistosomal Ags and hepatosplenic patients (14). More recent studies on *S. japonicum* were devoted to HLA class II associations and advanced disease/fibrosis (15–18), or to the combined influence of HLA class II and the chromosome 5 gene cluster (12). Elsewhere, *HLA-DQB1*0201* has been positively associated with hepatosplenic disease in Brazilian patients >15 years old (19). A study specifically examining the role of *HLA-DP* observed that *HLA-DPA1*0301* was

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Received for publication November 23, 2005. Accepted for publication March 15, 2006.

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¹ This project was funded by the Wellcome Trust, the Medical Research Council, and the Commission of the European Community's Science and Technology for Development Programme (INCO-DC Contract IC18 CT97-0237 and INCO-DEV Contract ICA4-CT-1999-1003).

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associated with *Schistosoma hematobium* infection, and a high reinfection rate posttreatment (20).

In the context of *S. mansoni* immunoepidemiology, alleles within the HLA region may be a marker (through linkage disequilibrium) with an overall increase in T cell-dependent response against *S. mansoni* Ags. Alternatively, individual alleles may yield HLA proteins that specifically present peptides derived from *S. mansoni* Ags to T cells and initiate a class-switching pathway leading to production of IgE. Because IgG1 and IgG4 are involved in the class-switching pathway, and their levels are often correlated with those of IgE (2, 21), it will be important to consider all these three isotypes in any analysis.

The aim of the present study was to examine HLA class II associations between alleles of *HLA-DQB1*, *HLA-DQA1*, and *HLA-DRB1* loci, and posttreatment IgE, IgG1, or IgG4 Ab isotype levels against recombinant Ag and soluble extracts of adult worm and egg Ags of *S. mansoni*. Any alleles significantly associated with an Ab response were then assessed as markers of reinfection intensity over a time period that included several rounds of treatment separated by up to 4 years. This cohort study took place in a focus of endemic *S. mansoni* infection in Uganda, characterized by very high infection levels before the first treatment due to community-wide occupational or domestic exposure.

Materials and Methods

Study design and population

The present study was conducted in Booma village, situated on the Ugandan shore of Lake Albert, where the entire population is exposed through either domestic or occupational contacts with lake water. The study area and population are described in detail elsewhere (22, 23). Briefly, the study cohort consisted of 199 individuals aged 7–50 years, selected on the basis of residency in the village of at least 10 years or since birth if under the age of 10. Before enrollment in the study, informed consent was obtained from adults, and assent was obtained from parents or guardians of the children. Ethical permission for the study was obtained from the ethical review committee of the Ugandan Ministry of Health, and the study was conducted in line with their guidelines on informed consent.

Parasitology and treatment

Before treatment, three stool samples were collected on consecutive days from each member of the cohort. From each sample, two slide readings were made using the Kato Katz method for detection of helminth eggs (24). After collection of the final sample, each member of the cohort and the other members of the village were treated with praziquantel (40 mg/kg). Two weeks later, the entire community was retreated at the same dose. Follow-up stool samples were collected a year, 22 mo, and 6 years after the first treatment to estimate reinfection levels. The village population, including the cohort, was treated again 2 years after the first round of treatment. Thus, the longest period of exposure after treatment was 4 years.

Genotyping

Genotyping of the HLA class II candidate markers, *HLA-DQB1*, *HLA-DQA1*, and *HLA-DRB1*, was conducted through PCR, then allele detection by digoxigenin-labeled sequence-specific oligos. The PCR conditions are previously described (25), and the specificity and sequence of the individual sequence-specific oligo probes are detailed in Lundin et al. (26), Ronningen et al. (27), and Kimura and Sasazuki (28).

Ab levels and cytokine responsiveness

Ab isotype levels (IgG1, IgG4, IgE) were measured by ELISA 7 wk after the first praziquantel treatment, using the same method as described previously (2). Ags against which isotype levels were measured were: the soluble fraction of homogenized adult worms (soluble worm Ag (SWA)),³ soluble egg Ag (SEA), the outer tegumental layer of adult worms (tegument Ag (TEG)), and the recombinant, tegument-derived Ag rSm 22.6,

prepared as described previously (29). Pretreatment IL-4, IL-5, and IL-13 responsiveness to SWA was measured by capture ELISA in venous blood samples, as described previously (29).

Analysis

All analyses were conducted in SPSS version 13. Each isotype response was first age standardized using the same procedures previously described (2). The overall association between the carriage of alleles at each of the *HLA-DQB1*, *-DQA1*, and *-DRB1* loci and each isotype response to *S. mansoni*-derived Ags was then assessed using the age-standardized variables. All alleles with a frequency of >10% were tested for significant associations in a multivariate ANOVA that compared isotype levels with carriage of a particular allele.

A factor analysis was conducted on the age-standardized IgG1, IgG4, and IgE responses to SWA, TEG, SEA, and rSm22.6, to identify highly correlated variables and reduce the amount of covariates and dependent variables in subsequent analysis. Variables that had similarly high coefficients in a particular factor were substituted in further analysis with the corresponding factor score variable. Alleles significantly ($p < 0.01$) and positively associated with a particular isotype in the ANOVA analysis were then entered into a univariate analysis of covariance (ANCOVA) in which the relationship between each response and the allele of interest was reassessed while controlling for the covariance effects of the other isotype responses and in vitro, whole blood Th2 responsiveness (IL-4, IL-5, and IL-13) against the same Ags. The ANCOVA analysis was stratified into two age classes (7–15 and 16+ years) to allow for potential age modification of cytokine-Ab associations.

Peptide prediction

The published amino acid sequence of any Ag significantly associated with a *DRB* allele was entered into a web-based program called ProPred (30). This program calculates peptide-binding scores for particular amino acid sequence/allele combinations and presents those sequences that have the highest peptide scores (within the 2 percentile). The score for each sequence is given as a percentage of the maximum obtainable score for a particular allelotype.

Post hoc analysis of egg count data

Reinfection egg counts (eggs/g of feces) were estimated at 1 year, 22 mo, and 6 years after the first praziquantel treatment (4 years after the last treatment). Due to low reinfection intensities and an overdispersed distribution at each time point, analysis was restricted to a nonparametric analysis. The Moses Extreme Reactions Test, as implemented in SPSS, offers group trimming (5% of the outliers are trimmed from each end of the distribution of each group), thereby reducing the probability of type II errors. We tested whether or not the tails of the egg count distributions were significantly longer in either carriers or noncarriers, thus indicating whether or not reinfection intensities were restricted in one group or the other, for each allele exhibiting a significant association in the above analysis. The potentially modifying effects of age and sex were controlled for independently by stratifying by sex or age group.

Results

HLA-DQB1

The *HLA-DQB1* locus was typed for 176 individuals in the post-treatment cohort. The following alleles were present at >10% frequency in the cohort (percentage of frequency in brackets): *DQB1*0201* (45.2%), *DQB1*0301* (20.5%), *DQB1*0501* (20.9%), *DQB1*0602* (14.8%), and *DQB1*0604* (14.2%). In the multivariate analysis, no allele at this locus was significantly associated with any isotype response and no further analysis was conducted.

HLA-DQA1

The *HLA-DQA1* locus was typed for 177 individuals in the post-treatment cohort. The following alleles were present at greater than 10% frequency in the cohort: *DQA1*0101* (29.4%), *DQA1*0102* (49.2%), *DQA1*0103* (12.4%), *DQA1*0201* (24.5%), *DQA1*0401* (21.5%), and *DQA1*0501* (20.3%). There were no significant associations observed between carriage of any of these

³ Abbreviations used in this paper: SWA, soluble worm Ag; ANCOVA, analysis of covariance; SEA, soluble egg Ag; SIT, specific allergen immunotherapy; TEG, tegument Ag.

alleles and any isotype response, and no further analysis was conducted.

HLA-DRB1

A total of 147 individuals donated blood samples 7 wk after treatment that were assayed for IgE responses and that were typed at the *HLA-DRB1* locus. The following alleles had a frequency of >10% among this posttreatment cohort: *DRB1*1* (18.5%), *DRB1*2* (17.7%), *DRB1*3* (27.0%), *DRB1*7* (14.9%), *DRB1*11* (32.4%), *DRB1*12* (14.3%), *DRB1*13* (17.3%), and *DRB1*14* (36.7%).

ANOVA

In the multivariate ANOVA that compared carriage of each *DRB1* allele with each isotype response, the carriage of *DRB1*13* was associated with significantly higher IgE responses to SWA ($F = 18.18$, $p < 0.001$), SEA ($F = 6.29$, $p = 0.013$), TEG ($F = 16.6$, $p < 0.001$), and rSm22.6 ($F = 29.93$, $p < 0.001$). Fig. 1 illustrates the magnitude of the association between *DRB1*13* carriage and IgE vs rSm22.6 responses across age groups. Positive associations were also observed between IgG4 responses to SWA ($F = 11.67$, $p = 0.001$), TEG ($F = 6.9$, $p = 0.009$), and rSm22.6 ($F = 7.40$, $p = 0.007$). An association with higher IgG1 responses to rSm22.6 was also observed, but this did not reach statistical significance ($F = 3.53$, $p = 0.062$). The only other positive association observed was between carriage of *DRB1*11* and the IgG1 response to SEA ($F = 4.65$, $p = 0.033$). This was not considered a strong enough association for further analysis. Subsequent analysis therefore focused on *DRB1*13*.

Factor analysis

IgG1 levels against SWA, TEG, and rSm22.6 were all highly correlated, and therefore combined into a single factor score variable representing the anti-adult worm IgG1 response using the method described in a previous analysis of Th2 responsiveness (29). The age-standardized IgG1 SEA response was used separately in subsequent analysis as it was only partially correlated with the anti-adult worm responses.

IgG4 levels against SEA, TEG, and SWA were all correlated within the same factor. A single factor score variable was therefore used to represent the overall IgG4 response.

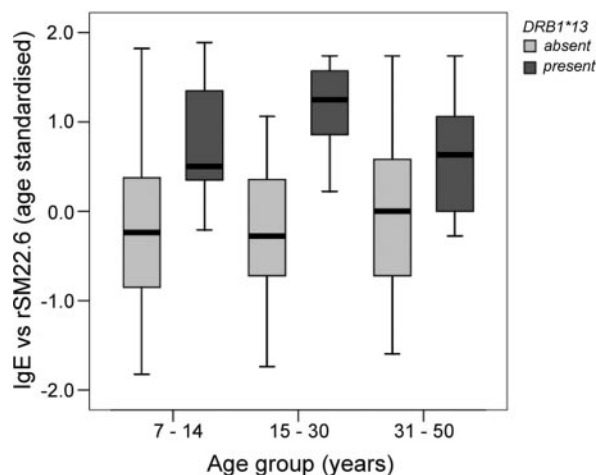


FIGURE 1. Box and whisker plot of the age-standardized IgE response against the rSm22.6 Ag, in three age groups, stratified in each age group by the carriage status of *DRB1*13* (absent or present). Horizontal lines within each box represent the median value. The lower bound of the box represents the 25th percentile, and the upper bound represents the 75th percentile. The whiskers indicate the range of values, excluding outliers.

IgE levels against SWA, TEG, and rSm22.6 were all highly correlated, and therefore combined into a single factor variable representing the anti-adult worm IgE response. The age-standardized IgE level against SEA was used separately in further analysis due to the relatively low correlation between this isotype level and the anti-adult worm isotypes.

ANCOVA

As not all individuals were assayed for every measure of cytokine responsiveness, every isotype level, or typed for carriage of *DRB1*13*, the full ANCOVA analysis was run on a reduced dataset, comprising 122 individuals. Eight children and 11 adults carried the *DRB1*13* allele in this subset.

Factor score variables representing IgG1, IgG4, and IgE levels were individually tested for their association with *DRB1*13*, while the other variables were added as covariates alongside the standardized values of IL-4, IL-5, and IL-13 SWA. After age stratification, and with all covariates included, there was no longer a significant association between the carriage of *DRB1*13* and any factor score variable. A stepwise backward elimination procedure first removed the IL-5 vs SWA as a covariate. This yielded a borderline significant result in adults for the association between carriage of *DRB1*13* and IgE levels against adult worm Ag ($F = 4.06$, $p = 0.048$). Elimination of the other cytokine covariates, while keeping the other Ab isotypes, increased the sample size to 139 and also increased the strength of the association between carriage of *DRB1*13* and IgE in adults ($F = 10.0$, $p = 0.002$). No other association between Ab isotype level and carriage of *DRB1*13* was maintained during this analysis.

Peptide prediction

The amino acid sequence of Sm22.6 was analyzed using ProPred for potential binding sites against 37 *DRB* allelotypes. Each analysis yielded a potential sequence of between 9 and 13 residues. The highest score with respect to *DRB1*13* was obtained for a 13-mer sequence predicted to bind with the HLA protein encoded by *DRB1*1321* (Fig. 2). Peptide-binding scores for other *DRB1* alleles were observed both within and beyond those obtained for *DRB1*13* allelotypes (data not shown).

Post hoc analysis of egg counts

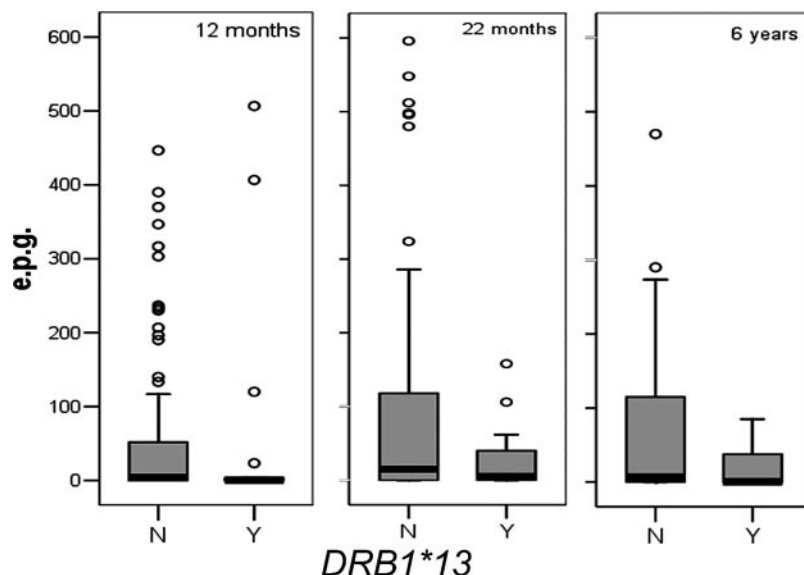
The carriage of *DRB1*13* was tested for an association with reinfection intensity (Fig. 3) using the Moses Extreme Reactions Test. Reinfection egg counts were highest in children at each time point, and always higher in males. The potentially modifying effects of age and sex were therefore tested by stratifying the data by either

	30-----40-----50-----	
DRB1_1301:	KQELIKYCQKYRLDMKLDPWIARF	33.0%
DRB1_1302:	KQELIKYCQKYRLDMKLDPWIARF	37.5%
DRB1_1304:	KQELIKYCQKYRLDMKLDPWIARF	38.9%*
DRB1_1305:	KQELIKYCQKYRLDMKLDPWIARF	42.5%
DRB1_1307:	KQELIKYCQKYRLDMKLDPWIARF	19.1%
DRB1_1311:	KQELIKYCQKYRLDMKLDPWIARF	37.4%
DRB1_1321:	KQELIKYCQKYRLDMKLDPWIARF	59.6%
DRB1_1322:	KQELIKYCQKYRLDMKLDPWIARF	28.6%
DRB1_1323:	KQELIKYCQKYRLDMKLDPWIARF	21.4%
DRB1_1327:	KQELIKYCQKYRLDMKLDPWIARF	33.6%
DRB1_1328:	KQELIKYCQKYRLDMKLDPWIARF	33.6%

*sequence LKVSEIRRE starting at position 72 scored 46.7%

FIGURE 2. Highest scoring amino acid sequences from rSm22.6 predicted to bind with each allelotype of *DRB1*13*. Peptide anchors are shown in bold; other amino acids in the binding sequence are underlined. The percentage value at the end of each row is the peptide score as a percentage of the highest possible score for that allelotype.

FIGURE 3. Box and whisker plots illustrating the association between carriage of *DRB1*13* (absent, N or present, Y) and reinfection egg counts measured 1 year, 22 mo, and 6 years after the first praziquantel treatment. The horizontal lines within each box represent the median. The lower and upper bounds represent the 25th and 75th percentiles of the egg count distribution. Whiskers indicate the range of nonoutlying data. Individual outliers below 600 eggs/g are also shown.



sex or age group. The intervals for each age group were updated according to time elapsed since first treatment. At 1 year posttreatment, 118 individuals with known *DRB1*13* status contributed fecal samples. The Moses test was significant in both males ($p < 0.001$) and females ($p < 0.001$). After stratifying by age, the test was significant ($p < 0.001$) in each age group. At 22 mo posttreatment, 122 individuals with known *DRB1*13* status contributed stool samples. The Moses test was significant ($p < 0.001$) in both males and females, and in each age group (9–16 years, $p = 0.019$; 17–32 years, $p < 0.001$; 33+ years, $p < 0.001$). At 6 years posttreatment, 78 individuals contributed stool samples. The Moses test was significant in both males ($p = 0.006$) and females ($p < 0.001$). The test was also significant ($p < 0.001$) in each age group.

Discussion

In seeking to understand the mechanisms behind observed variation and changes in immune responses to parasitic infections, it is important to consider a number of potential influences. As well as behavioral and environmental factors, there may be a heritable component that is of importance. It is therefore plausible to hypothesize that polymorphisms at certain loci may be influential in restricting the host response to challenge with parasite Ag as well as any downstream consequences of that restriction. Reasons for interest in this field are clear, and include assessing the burden of disease in relation to transmission intensity, predicting the benefits of treatment, and understanding the genetic restriction of vaccine candidates. This report is the first attempt to identify genetic restriction of the humoral response to challenge with schistosome Ags in a human population undergoing exposure to infection after treatment.

Early immunoepidemiological studies of *S. mansoni* focused on establishing whether acquired immunity contributes to the typical decline of egg counts in older individuals, and a lack of reinfection in adults. These were conducted through a series of reinfection studies that also measured changes in Ab isotype responses (31). A tegumental 22.6 K_d Ag was identified as a target for these responses (3), and the IgE response to this Ag in particular has been implicated in protection from reinfection (21). More recent studies on the cohort from Booma have focused on pre- and posttreatment levels of cellular Th1 and Th2 responsiveness to parasite Ags (5, 32, 33). Short-term responses have also been measured in a cohort

from a neighboring village (34). There, an increased level of circulating IL-5 1 day after treatment was associated with a lower worm-specific IgE response and an increased level of reinfection 1 year later. The present analysis, a direct extension of these reinfection studies, indicates for the first time the importance of genetic factors in driving these IgE responses and reinfection levels.

In the initial screening undertaken with a multivariate ANOVA, carriage of *DRB1*13* was associated with many of the Ig responses to adult worm Ags, but most strongly with IgE levels against the rSm22.6 peptide. No other allelotype was associated with any other isotype response, which restricted further analysis to associations involving *DRB1*13*. The questions asked during subsequent analysis focused on whether there was a unique association between carriage of *DRB1*13* and IgE levels against *S. mansoni*-derived Ags, or whether the carriage of this allele was a marker for a qualitatively different response to antigenic challenge.

Peptide-binding analysis based on the sequence of Sm22.6 and multiple *DRB1* allelotypes revealed that many *DRB1* alleles are potentially capable of presenting peptides from this Ag. The probability values obtained when assessing the potential of *DRB1*13* allelotypes to bind with Sm22.6 peptides were within the range of values obtained with other *DRB1* alleles, suggesting that Sm22.6 is likely to be a promiscuous Ag, rather than being uniquely associated with *DRB1*13*. This observation was consistent with the hypothesis that *DRB1*13* is a qualitative marker, rather than coding for an HLA protein that uniquely binds to, and presents, Sm22.6 peptides in the classical manner.

That hypothesis was further strengthened during the ANCOVA analysis, which introduced variables measuring pretreatment Th2 responsiveness as potential covariates of the IgE response. Pretreatment IL-5 responsiveness to worm Ag in particular was an influential covariate. When controlling for this cytokine, the association between *DRB1*13* and IgE levels was abrogated in children and reduced in adults. Further analysis will focus in detail on the association between pretreatment cytokine responsiveness and Ab levels in this cohort (K. Walter, A. Fulford, R. McBeath, S. Joseph, F. Jones, M. Kariuki, J. Mwatha, G. Kimani, N. Kabatereine, B. Vennervald, J. Ouma, and D. Dunne, manuscript in preparation), but this preliminary analysis nonetheless further points toward carriage of *DRB1*13* as an important marker of an overall enhanced ability to mount a Th2 response to *S. mansoni* Ags, rather than an

allele coding for protein that presents peptides derived from *S. mansoni* Ags.

Notably, the apparent association between *DRB1*13* and the IgE response to SEA was initially weak, and then disappeared during the ANCOVA analysis. It is possible that the initial correlation was due to partial correlations between IgE responses to Ags present in both egg and adult worms. Subsequent analysis will also therefore focus on the other parasite Ags related to rSm22.6 that are shared between life stages (C. Fitzsimmons, R. McBeath, S. Joseph, F. Jones, K. Walter, K. Hoffmann, M. Kariuki, J. Mwatha, G. Kimani, N. Kabatereine, B. Vennerwald, J. Ouma, and D. Dunne, manuscript in preparation). The apparent contribution of *DRB1*13* to variation in the IgE response was also reduced during the ANCOVA because of correlations between IgE and IgG4 levels. IgG4 is one of several isotypes that have been observed under experimental conditions to prevent eosinophil-mediated killing of schistosomula (35), and it has been proposed that the schistosomes induce the production of this blocking Ab as part of their defense mechanism against the host immune system (36, 37). After treatment, the influence of the parasite is diminished, suggesting that the correlation between IgE and IgG4 is host mediated.

Observations from immunotherapy studies offer further insight. An increase in IgG levels is often seen in patients on specific allergen immunotherapy (SIT), with IgG4 as the dominant isotype (reviewed by Ref. 38). This increase in the allergen-specific IgG4 response after SIT may be accompanied by an improvement in clinical status, and sera from SIT patients block histamine release from basophils (39). Such observations have led to the hypothesis that the blocking effect of IgG stems from the patient's attempt to prevent IgE-mediated hypersensitivity to Ag (reviewed elsewhere (40)). Experimental evidence suggests a mechanism for default coproduction of IgG4 and IgE, because B cells can be induced to produce both IgE and IgG4 by IL-4, particularly when cultured with RANTES or MIP 1- α (41). Thus, the observed correlations between IgG4 and IgE responses in the present and previous studies may be a result of both parasite and host signals aiming to reduce the IgE response against parasite Ags.

The current analysis did not include haplotype analysis, due to the relatively small sample size and a lack of material available for typing all individuals at each HLA locus. This does not diminish the value of the observed associations involving *DRB1*13*, but does leave open the question of which other alleles may be influential. In other published studies that included a haplotype analysis, the effects of *DRB1*13* on IgE responses have been associated with carriage of either *LTA*₊₃₆₈*1 (42) or *DRB1*07* (43). Elsewhere, linkage disequilibrium has been reported between the *DRB1*13* allele and *TNF*₊₆₉₁*G^{del} in the class III region (44). Such linkages can make the functional implications of single-allele associations hard to unravel, but insights may be gained from comprehensive analyses of existing data, such as the peptide-binding and ANCOVA analyses.

We did not observe any significant association between the carriage of *DRB1*13* and pretreatment IgE, IgG1, or IgG4 responses to any of the parasite Ags, although there was a trend for increased responses in carriers of this allele (data not shown). This was not unexpected, as treatment with praziquantel in an area of ongoing exposure appears to increase the visibility of immunogenic peptides to the immune system after the death and disintegration of the worms.

IgE levels against adult worm Ags have been associated previously with resistance to reinfection by schistosome worms. It was therefore plausible for us to ascertain whether or not carriage of *DRB1*13* was not only a marker for enhanced ability to mount a Th2 response, but also a marker for the downstream effects of a

more effective response. In this context, we examined the association between carriage of *DRB1*13* and reinfection levels measured up to 6 years after initial praziquantel treatment, and up to 4 years since the last round of treatment of the cohort. At each time point, and after several rounds of treatment, the effect of carrying *DRB1*13* was still evident. Both children and adults carrying this allele were reinfected at lower levels than their counterparts without the allele, at each follow-up.

We did not observe any overall association between IgE responses at 7 wk posttreatment and reinfection status. Reasons for this may stem from the low levels of reinfection that were observed. We are currently investigating ecological and demographic factors that may have played a role in low reinfection levels in this cohort. In particular, we have anecdotal information that certain population subgroups had a reduced level of water contact after the initial intervention. This may have created a scenario whereby lower IgE levels were observed in people no longer exposed. Nonetheless, we observed a consistent association between the carriage of *DRB1*13* and reinfection status at 1 year, 22 mo, and 6 year posttreatment follow-ups. This observation can be compared with previous work on the genetic control of *S. mansoni* infections, in which a major gene (SM1) was identified as restricting infection intensity (45). Localization studies placed SM1 in the q31–33 region of chromosome 5 (9), but through linkage analysis identified a minor candidate region on chromosome 6 (p21–q21), ~40 cM from the HLA region (46). The authors of that study considered the distance to be too great to implicate alleles within the HLA, but did not examine the influence of genetic background after treatment, thus leaving open the question of HLA gene associations with reinfection.

Although the probability of being infected was not significantly different between carriers and noncarriers at either survey, we observed that the probability of being relatively heavily infected was significantly lower among individuals carrying the allele. The consistency of this result over several years suggests that the HLA region does contain influential markers of infection intensity. In particular, carriage of *DRB1*13* appears to be an important marker for the downstream effect of more effective immune responses against *S. mansoni*. These results highlight the need to consider genetic background when investigating the effects of any intervention that is designed to improve the effectiveness of immune responses.

The strong association between carriage of *DRB1*13* and improved prognosis observed in the present study is consistent with the broader literature, in which carriage of this allele has been linked to both increased responsiveness and/or improved prognosis for a range of other pathogens. Reports include increased responsiveness to vaccination with hepatitis B surface Ag (47), increased rate of clearance of hepatitis B virus during acute infection (48), lower risk of chronic infection with hepatitis C (49), lower grade of fibrosis associated with hepatitis C (50), suppression of HIV-1 viremia (51), and faster regression of cervical neoplasias associated with human papilloma virus (52). By extension, it is reasonable to suggest that carriage of *DRB1*13* may contribute significantly to the state of individuals living in areas in which infectious diseases are still the main threat to health.

In conclusion, we have demonstrated that carriage of *DRB1*13* was a significant marker for the enhancement of Ab responses, particularly IgE responses, to adult worm-derived Ags of *S. mansoni* after praziquantel treatment. This association most likely stems from linkage with genes involved in a wider Th2 response, rather than a classical Ag presentation, class-switching pathway.

Carriage of this allele also was a significant marker of lower re-infection intensities up to 4 years after praziquantel treatment despite low reinfection levels. These observations provide strong evidence that genetic factors are important in restricting or promoting the Th2 response to specific schistosome Ags after treatment and confirm that carriage of the *DRB1*13* allele is an important marker of the quality of the immune response against a range of pathogens.

Acknowledgments

We acknowledge the valuable cooperation of Booma community, and thank the fieldworkers for their work during this project. The late Jovanice Kemijumbi is also remembered for her substantial contribution to the fieldwork.

Disclosures

The authors have no financial conflict of interest.

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