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Human Immunoglobulin E Responses to a Recombinant 22.6-Kilodalton Antigen from *Schistosoma mansoni* Adult Worms Are Associated with Low Intensities of Reinfection after Treatment

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***Schistosoma mansoni*-infected individuals who have low intensities of reinfection following treatment produce immunoglobulin E (IgE) antibodies against a range of *S. mansoni* adult-worm antigens. One of the targets of the IgE response is an adult-worm sodium dodecyl sulfate-polyacrylamide gel electrophoresis band of 22 kDa (Sm22), which contains an antigen(s) located within the tegument and gut lining of adult worms and relatively late schistosomula life cycle stages only. A significant negative correlation between the level of anti-Sm22 IgE and the intensity of reinfection following treatment suggests that IgE responses against this antigen(s) are characteristic of individuals who are resistant to reinfection. To identify the antigen(s) in the Sm22 band that are associated with these IgE responses, we have cloned and characterized a recombinant 22-kDa protein (rSm22) that cross-reacts immunologically with Sm22. There was a high correlation between native and recombinant Sm22 isotype responses, indicating that the correct antigen had been cloned and that responses against rSm22 made up the majority of the responses against Sm22. By analyzing human isotype responses to rSm22 with human sera from a longitudinal treatment and reinfection study and correlating the anti-rSm22 isotype responses, retrospectively, with the intensity of reinfection following treatment for each individual, we observed a negative correlation between the IgE response to rSm22 and the intensity of reinfection. This relationship remained significant after allowing for age and other isotype responses to rSm22, in particular IgG4.**

Schistosomiasis is a debilitating parasitic disease that currently affects at least 200 million people throughout South America, Africa, and Asia (40). Although this disease can be treated chemotherapeutically, treatment is expensive and does not prevent reinfection (especially in children), which, as has been shown, can occur within 1 year following treatment for *Schistosoma mansoni* (19). The development of prophylactic immunization as an additional schistosomiasis control strategy was encouraged by the demonstration that low intensities of reinfection with *S. mansoni* following treatment in older individuals may be a result of an age-dependent acquired immune response rather than simply being due to decreased exposure to the parasite (7, 9, 15, 21, 30, 39).

Longitudinal treatment and reinfection studies have suggested the existence of human immunity to schistosomiasis. In a treatment-and-reinfection study in a community infected with *Schistosoma haematobium* in The Gambia, for example, Hagan et al. (15) showed that reinfection was significantly less likely to occur in those with high levels of schistosome-specific immunoglobulin E (IgE). Likewise, Rihet et al. (30) showed that antilarval IgE levels were, on average, six- to eightfold higher in the sera of the most resistant individuals in a study of *S. mansoni*-infected Brazilian subjects. Similarly, Dunne et al. (9) showed that IgE responses against *S. mansoni* adult worms, but not eggs, correlated negatively with the intensity of reinfection following treatment. This correlation remained significant even after accounting for age within the age group of 16 years and below. Collectively, these studies indicated that resistance to reinfection with schistosomiasis is associated with IgE antibody responses to the adult worm.

Dunne et al. (9) showed that the range of *S. mansoni* adult worm antigens recognized by human IgE was restricted in comparison with antigens recognized by IgG. An adult-worm band with an apparent molecular mass of 22 kDa (Sm22) in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was preeminent among the repertoire of antigens reactive with IgE. Qualitative Western blot (immunoblot) analysis showed that individuals with an IgE response against the 22-kDa band had significantly lower intensities of reinfection following treatment. Similarly, quantitative analysis with the 22-kDa band in an enzyme-linked immunosorbent assay (ELISA) revealed a significant negative correlation between the IgE response to this band and the intensity of reinfection following treatment (11a). These qualitative and quantitative associations, which were found with IgE reactivity only, were with a band isolated from SDS-PAGE gels by electroelution. A number of *S. mansoni* antigens with molecular masses in the 22-kDa region have been identified (2, 12, 18, 22, 25, 28, 33, 34, 37), and the 22-kDa band described here may have contained any mixture of them. The aim of this work, therefore, was to identify the antigen(s) within the 22-kDa band that, via its

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recognition by IgE, is associated with resistance to reinfection with schistosomiasis mansoni.

MATERIALS AND METHODS

Parasite and antigens. The life cycle of a Puerto Rican strain of *S. mansoni* was maintained in outbred TO mice (Tuck Ltd.) and the snail *Biomphalaria glabrata*. To prepare soluble adult-worm antigen (AW), adult worms recovered from mice were retrieved by perfusion of the hepatic portal system by the method of Smithers and Terry (36) and processed as described previously (9). Native Sm22 was prepared from 9 mg of AW by electroelution following SDS-PAGE (14% polyacrylamide) (23).

Cloning and purification of rSm22. Polyclonal, monospecific rabbit serum was raised against an electroeluted 22-kDa AW band (Sm22) in the presence of Ribi adjuvant (Sigma) and used to screen a cDNA expression library prepared in λgt11 (16). A 932-bp cDNA clone, encoding a 22-kDa antigen (rSm22), was subcloned into the M13 vectors (New England BioLabs), and the nucleic acid sequence was determined by the dideoxy chain termination method of Sanger et al. (32). The deduced amino acid sequence of rSm22 was used to search the Swissprot, PIR, and BLAZE databases for homology to other proteins by using the FASTA package (29). The entire coding region for rSm22 was then subcloned into the expression vector pGEX-1λT (Pharmacia) and expressed as a fusion protein with glutathione-*S*-transferase (GST) of *Schistosoma japonicum*. The fusion protein was purified in a single step from *Escherichia coli* TG2 (35) transformed with recombinant pGEX-1λT, by affinity chromatography with glutathione-coated Sepharose 4B (Pharmacia) by a method based on that of Smith and Johnson (35). rSm22 was released from the GST carrier protein by specific cleavage with 10 U of thrombin (>4,500 enzyme units/mg of thrombin [Pharmacia]) per mg of fusion protein at room temperature for 2 h. Cleaved rSm22 was retrieved by electroelution from SDS-PAGE (14% polyacrylamide) (29) at 8 to 10 mA for 3 to 5 h in a model 422 electroeluter (Bio-Rad Laboratories, Ltd.).

Sera from human infections. The sera, obtained from human infections, used in ELISA were the same as those described previously (10). A total of 141 participating individuals aged between 6 and 66 years, who resided in Kangundo Location, Machakos District, Kenya, an area where schistosomiasis mansoni is endemic, were selected on the basis of the presence of *S. mansoni* eggs in six stool samples and high frequencies of observed water contact during April to August 1984. Sera were collected before treatment (bleed A), 6 months after treatment and approximately 6 months before significant reinfection had occurred (bleed B), and 18 months after treatment (bleed C). Sera from bleed B, which contained antibodies indicative of posttreatment but pre-reinfection immune responses to *S. mansoni*, were collected in April 1985. Corresponding intensities of reinfection for each individual were determined by counting the number of eggs per gram of stool collected at six time points after treatment. An overall reinfection score was calculated on the basis of all six stool surveys carried out between March 1986 and November 1988 (9). Normal human serum was obtained from a British individual with no history of schistosomiasis. Sera, obtained from human infections, used for Western blot analysis were selected from those obtained from 50 individuals resident in an area where schistosomiasis mansoni is highly endemic (Kinyungu in Kangundo Location, Machakos District, Kenya). Selection was based on a positive or negative IgE response to a 22-kDa band of AW by Western blot analysis (unpublished observations). Western blots to detect IgE antibodies reactive with rSm22 were performed by the method of Towbin et al. (38) with human sera at a 1/20 dilution.

ELISA. Anti-human isotype antibodies were used to quantify the rSm22-reactive IgG1, IgG2, IgG3, IgG4, IgM, IgA, and IgE present in individual sera from human infections by ELISA. rSm22 was used at 1/80, and the method was as described previously (9). Anti-Sm22 responses were determined in a similar manner with electroeluted native Sm22 at 1/80 (11a). Optimal antigen concentrations were determined by "checkerboard" titration. All human sera were used at 1/200 for the IgG1, IgG4, and IgA assays, 1/100 for the IgG2 and IgG3 assays, and 1/20 for the IgE assay. All ELISA results are expressed as absorbance units and correspond to the average results in triplicate wells assayed on different plates. The optical density values were adjusted for plate-to-plate variation by means of an internal standard.

Statistical analysis. Relationships between the various immune responses to rSm22 and Sm22 were examined by using Pearson's correlation coefficient. Relationships between the IgE response to rSm22 and reinfection and age were examined by using Spearman's rank correlation coefficient. Age and intensity of reinfection data were taken from reference 9. Potential confounding factors identified by nonparametric tests were subsequently controlled for by multiple regression analysis. Both age and age squared were used as explanatory variables when controlling for the effect of age. Statistical significance by any of these methods was inferred as $P < 0.05$.

RESULTS

Human isotype responses to rSm22. Following expression in pGEX-1λT, the rSm22/GST fusion protein was purified in a single step by affinity chromatography with glutathione-coated Sepharose and separated from the GST carrier by specific cleavage with bovine thrombin and electroelution. To determine if rSm22 was recognized by human IgE, antibodies reactive with rSm22 were detected qualitatively by Western blot analysis with sera from seven *S. mansoni*-infected individuals, four of whom showed anti-Sm22-IgE reactivity. As shown in Fig. 1, rSm22 was recognized by IgE only from the patients who showed anti-Sm22 IgE reactivity. Recombinant Sm22-reactive IgG1, IgG2, IgG3, IgG4, IgM, IgA, and IgE in individual sera from human infections were detected quantitatively by ELISA. IgG1, IgG4, IgA, and IgE isotype responses to rSm22 were compared with the isotype responses to Sm22 by statistical correlation. All showed positive correlations (IgG1, $r = 0.521$, $P < 0.001$; IgG4, $r = 0.771$, $P < 0.001$; IgA, $r = 0.282$, $P < 0.05$; IgE, $r = 0.813$, $P < 0.001$; minimum $n = 92$).

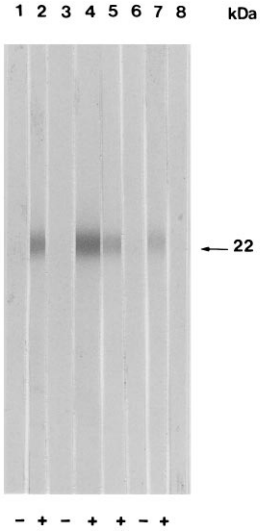

FIG. 1. Western blot from SDS-PAGE (14% polyacrylamide) of approximately 200 μl (total) electroeluted rSm22 probed with sera from human infections and anti-human IgE monoclonal antibody. Lanes 1, 3, and 6 contain sera from *S. mansoni*-infected patients with no anti-Sm22–IgE reactivity (−); lanes 2, 4, 5, and 7 contain sera from *S. mansoni*-infected patients who recognized Sm22 with IgE (+); lane 8 contains normal human serum.

Isotype responses to rSm22 associated with resistance to reinfection. To ascertain if human isotype responses to rSm22 were associated with resistance to reinfection after treatment, the IgG1, IgG3, IgG4, IgA, and IgE isotype responses to rSm22 were correlated with the intensity of reinfection following treatment, expressed as $\log_e$ (eggs per gram + 1) of the average stool count collected at six time points after treatment, which were taken from Dunne et al. (9). The IgG1 response to rSm22 did not correlate with the intensity of reinfection ($r = 0.02$, $n = 94$, not statistically significant), but statistically significant negative correlations were observed with IgG3, IgG4, IgA, and IgE isotypes, as shown in Table 1. Although these results indicated that IgG3, IgG4, IgA, and IgE responses to rSm22 may have been associated with resistance to reinfection following treatment for schistosomiasis mansoni, statistically significant positive correlations with age were also observed for the IgG3, IgA, and IgE isotype responses to rSm22 (Table 1). To test the significance of the correlations with the intensity of reinfection after taking into account the effect of age, since age itself correlates negatively with the intensity of reinfection (Spearman's ranked correlation coefficient $r = -0.776$, $n = 74$, $P < 0.001$), the IgG3, IgA, and IgE responses to rSm22 were subjected to multiple-regression analysis. The relationships be-

TABLE 1. Correlation between isotype responses to rSm22 and reinfection and age

Parameter	Correlation[a] for:				
	IgG1	IgG3	IgG4	IgA	IgE
Reinfection	0.020 (94, NS)	−0.242 (93, $P < 0.02$)	−0.257 (95, $P < 0.01$)	−0.318 (94, $P < 0.01$)	−0.417 (95, $P < 0.001$)
Age	−0.059 (104, NS)	0.359 (103, $P < 0.001$)	0.125 (105, NS)	0.304 (104, $P < 0.01$)	0.449 (105, $P < 0.001$)

[a] Expressed as Spearman's correlation coefficient. The number of observations and level of significance are given in parentheses; NS, not significant.

tween the IgG3, IgG4, and IgA responses to rSm22 and resistance to reinfection were lost after allowing for age ($F_{[\text{rSm22 response given age and age}^2]}$ for IgG3 = 0.889, for IgG4 = 0.231, and for IgA = 2.86), whereas the relationship between the IgE response to rSm22 and resistance to reinfection remained statistically significant at the 0.5% significance level ($F_{[\text{rSm22 IgE response given age and age}^2]} = 9.96$, $df_{1,91}$, $P < 0.005$). As shown in Table 2, all the isotype responses to rSm22 were correlated. However, the IgE response to rSm22 and the intensity of reinfection following treatment were independent of the IgG and IgA isotype responses to this protein, in particular IgG4, as shown by the regression analysis in Table 3. In fact, the correlation between anti-rSm22 reactivity and resistance to reinfection following treatment remained significant even after taking into account age and all other isotype responses ($F_{[\text{IgE to rSm22 given age, age}^2\text{, and other isotype responses}]} = 7.86$ [df 1, 84, $P < 0.01$]).

DISCUSSION

Following the identification of Sm22, whose IgE responses correlated with resistance to reinfection following treatment for schistosomiasis mansoni (9), the purpose of this work was to identify the antigen(s) within that band that was the target of this response. To do so, we raised polyclonal monospecific rabbit serum that recognized the 22-kDa band and isolated a cross-reactive 22-kDa antigen from an adult-worm cDNA expression library. The rSm22 was reactive with human IgE antibodies but only in sera that had previously been found to react to Sm22 with IgE (Fig. 1). rSm22 was also recognized by IgG1, IgG3, IgG4, and IgA antibody isotypes but not by IgG2 or IgM. A primary IgM anti-Sm22 response was not expected because the sera used were collected 6 months after treatment but before significant levels of reinfection had occurred. Similarly, an IgG2-reactive Sm22.6 response was not expected because IgG2 is directed predominantly against carbohydrate epitopes (9, 24), which are not present on recombinant proteins synthesized by prokaryotic expression systems incapable of posttranslational glycosylation. With the exception of IgA, there was a high degree of correlation between isotype responses to Sm22 and rSm22, indicating that the correct antigen had been cloned and that rSm22 was responsible for the majority of the antigen activity of Sm22. The low correlation observed between the IgA responses to rSm22 and Sm22 may have been due to the low quality of the IgA assay in comparison with other isotypes or, alternatively, to the presence of different IgA epitopes on the recombinant and native antigens.

IgG3, IgG4, IgA, and IgE against rSm22 correlated negatively with the intensity of reinfection following treatment for schistosomiasis mansoni, but only the relationship with IgE remained significant ($P < 0.005$) after allowing for age. This is the first description of IgE against a defined antigen being associated with resistance to reinfection following treatment. It is also the first time that a relationship of this kind has been shown to still be significant after allowing for age, thus demonstrating that the relationship between this response and low intensities of reinfection is not merely due to a general increase in the IgE level with age. IgE recognition of rSm22 therefore either constitutes part of the protective immune response against *S. mansoni* or is at least a marker for a "resistant-to-reinfection" phenotype. In this context, it is worth noting that Dunne et al. (9) found no correlation between the IgE response to the parasite egg and low intensities of reinfection, indicating that the relationship with resistance to reinfection is specific for IgE responses to AW. However, it is not clear if IgE responses to worm antigens other than that of rSm22 are associated with resistance to reinfection or if rSm22 is unique in this respect. We are in the process of isolating recombinant forms of several other *S. mansoni* AW antigens that are recognized by human IgE. Examination of the relationship between the IgE responses to a number of different defined AW antigens should reveal if human resistance to reinfection is associated with IgE responses to a specific antigen; with IgE responses to a group of functionally, structurally, or anatomically related antigens; or with IgE responses to the adult worm in general. If the last situation proves to be the case, it is likely that the target antigens will still be a restricted subgroup of antigens, because we have found that the number of *S. mansoni* AW antigens recognized by human IgE is much smaller than that recognized by IgG (9). Whatever further experimentation reveals about the involvement of other AW, the relationship between IgE responses to rSm22 and low intensities of reinfection is indicative of either a protective immunological response or a specific immunological response that is a marker for a resistant-to-reinfection human phenotype.

A role for IgA in human immunity to *S. mansoni* at the population level has been implicated by the results of Auriault

TABLE 2. Correlation between isotype responses to rSm22

Isotype	Correlation[a] for:			
	IgG1	IgG3	IgG4	IgA
IgE	0.475 (104, $P < 0.001$)	0.605 (103, $P < 0.001$)	0.532 (105, $P < 0.001$)	0.611 (104, $P < 0.001$)
IgG1		0.703 (102, $P < 0.001$)	0.705 (104, $P < 0.001$)	0.738 (104, $P < 0.001$)
IgG3			0.745 (103, $P < 0.001$)	0.773 (102, $P < 0.001$)
IgG4				0.704 (104, $P < 0.001$)

[a] Expressed as Pearson's correlation coefficient. The number of observations and level of significance are given in parentheses.

TABLE 3. Multiple regression analysis showing the effect of IgG and IgA isotype responses to rSm22 on the relationship between IgE to rSm22 and resistance to reinfection

Isotype	$F_{[\text{IgE to rSm22 given other responses}]}$[a]
IgG1	15.65 (1, 91, $P < 0.001$)
IgG3	9.47 (1, 90, $P < 0.005$)
IgG4	12.13 (1, 92, $P < 0.001$)
IgA	5.27 (1, 91, $P < 0.025$)

[a] Degrees of freedom and level of significance, shown in parentheses, indicate the significance of the relationship between IgE to rSm22 and resistance to reinfection after allowing for IgG1, IgG3, IgG4, and IgA to rSm22.

et al. (3) and Grzych et al. (14) showing that individuals with low intensities of reinfection following treatment had significantly higher IgA levels against Sm28GST, a GST of *S. mansoni*. The presence of IgA receptors on the surface of eosinophils (6), the degranulation of IgA-coated activated eosinophils (1, 6), the inhibition of anti-schistosome serum-mediated antibody-dependent cell-mediated cytotoxic killing of schistosomula by preincubation with aggregated IgA (6), and anti-NIP (5-iodo-4-hydroxyl-3-nitrophenacetyl) hapten chimeric mouse-human monoclonal antibody-mediated killing of hapten-coated schistosomula by eosinophils (11) provided supporting evidence for a role for IgA in mediating human immunity to *S. mansoni*. In the light of this recent evidence, it was interesting that the level of IgA-reactive rSm22 correlated with resistance to reinfection ($P < 0.01$ [Table 1]). Although the correlation between IgA-reactive Sm22.6 and resistance to reinfection did not remain significant at the 5% level when age was taken into account ($P < 0.1$), this result was still encouraging for future analysis of IgA responses.

Analysis of the association between IgE and IgG4 antibodies was imperative since the age dependence of resistance to reinfection with schistosomiasis may be a consequence of blocking antibodies of IgM, IgG2, and IgG4 isotypes (4, 5, 8, 15, 20, 31). IgG4 is considered an IgE-specific blocking antibody, since Demeure et al. (8) found that the effects of IgE and IgG4 on the intensity of reinfection with *S. mansoni* (negative for IgE and positive for IgG4) were not dissociable. In addition, Hagan et al. (15) found that schistosome-specific IgG4 levels peaked at the age of about 12 years and thereafter declined in the presence of increasing IgE levels and resistance to reinfection with age. In contrast, clear evidence has been presented here for the existence of a relationship between high levels of IgE specifically reactive with rSm22 and low intensities of reinfection, which was independent of other isotype responses to this antigen, especially IgG4 ($P < 0.001$).

Comparison of the deduced amino acid sequence for rSm22 with other proteins in various databases revealed that rSm22 was 100% identical to Sm22.6, an antigen cloned twice previously, first by Stein and David (37) and second by Jeffs et al. (18). rSm22 also showed partial identity (47%) to a 21.7-kDa *S. mansoni* adult worm protein, Sm21.7 (12) and myosin regulatory light chain (8% overall; 38% over a 40-amino-acid region) (13, 17, 26, 27). Although Jeffs et al. (18) did not speculate on the biological function of this antigen, Stein and David (37) suggested that Sm22.6 may have been involved in either maintenance of or gas exchange across the parasite tegument. The partial identity between rSm22 and myosin regulatory light chain, reported here for the first time, added support to the hypothesis that the biological role of this protein may indeed be connected to maintenance of the tegument. To summarize, we have shown here, for the first time, that Sm22.6 is recognized by human IgE antibodies and, more importantly, that IgE recognition of this antigen is associated with resistance to reinfection with schistosomiasis mansoni, an association that is still significant even after allowing for age and other isotype responses (especially IgG4) to this antigen.

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