The Immunopathology of Human Schistosomiasis-III. Immunoglobulin Isotype Profiles and Response to Praziquantel

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Immunoglobulin (Ig) isotype (IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgM, IgD and IgE) levels were investigated, both pre- and post-treatment with praziquantel (PZQ), in 43 adults and children chronically infected with Schistosoma mansoni, *by means of a two-site, isotype-specific immunoenzymometric assay. The patients were classified as responders (R) or non-responders (NR) on the basis of their circumoval precipitin test (COPT) results 12 months after treatment.*

In comparison with controls, pre-treatment R children showed significantly higher levels of IgG, IgG_1, IgG_4 ($p<0.001$) and IgE ($p<0.01$), and diminished IgG_2 ($p<0.05$), while NR children showed significantly elevated levels only of IgE ($p<0.05$). Twelve months after therapy, R children maintained significantly lower levels of IgG_2, but showed significantly decreased levels of IgG, IgG_1, IgG_4, and IgE, while the Ig isotype profile of NR children was unaltered.

Adult R and NR showed similar isotype profiles before chemotherapy, with the exception of significantly elevated IgM levels in R. Twelve months after therapy, R adults showed significantly decreased levels of IgG, IgG_1, and IgG_4, while NR adults showed only diminshed IgG_4 levels.

These results reveal different Ig isotype profiles in untreated adults and children chronically infected with S. mansoni. *The results further show that the pre-treatment Ig isotype profile may be significantly modified after an effective R to chemotherapy, accounted for by down regulation of the IgG_1 isotype in association with negative seroconversion of the COPT in R patients. The COPT reaction has been associated with the highly specific egg glycoprotein antigen w_1, which shows a significant reduction in reactivity six months after treatment. IgG_1 may thus play a main role in the response against the w_1 antigen.*

Key words: isotypes - immunoglobulin - *Schistosoma mansoni* - praziquantel

The role of the cellular and humoral immune response against *Schistosoma mansoni* infection has been well characterized by several laboratories (Colley et al. 1977, Butterworth 1987, Butterworth et al. 1987, Mendlovic et al. 1987, Aldrey et al. 1988, Benarroch et al. 1988, Butterworth et al. 1988). Hypergammaglobulinemia is a common feature of this response, with consistently elevated total serum IgG (mainly IgG_1 and IgG_4) and IgE concentrations in untreated, chronically infected individuals (Iskander et al. 1981, Jassim et al. 1987, Evengard et al. 1988, Boctor & Peter 1990).

Resistance to reinfection by *S. mansoni* after chemotherapy was investigated in Kenyan school children, in whom the existence of a specific acquired immunity was postulated. When patients susceptible to reinfection were compared with those who were able to eliminate the parasite, it was found that mean antibody levels were higher in the former group. After six months, the titre of specific antibodies against surface antigens declined and remained relatively constant over a period of 18 months in both groups. It was not clear, however, whether this finding was associated with, or independent of, reinfection (Butterworth et al. 1985).

It has also been demonstrated that IgM and certain IgG isotypes can act as blocking antibodies, preventing the expression of an efficacious protective immunity. These antibodies appear to be elicited in response to egg polysaccharide antigens, and cross-react with glycosylated epitopes located on the schistosomulum surface (Butterworth, 1987).

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Rihet et al. (1991) measured enhanced anti-parasite IgE levels in subjects resistant to reinfection with *S. mansoni*, and found that reinfection occurred when patients produced high levels of antibodies which could compete with IgE. A similar conclusion, suggesting a positive effect of IgG_4 in reinfection by *S. haematobium*, was proposed by Hagan et al. (1991), while Demeure et al. (1983) showed that resistance to reinfection after oxamniquine (OX) chemotherapy was associated positively with IgE and negatively with IgG_2 and IgG_4 anti-larval antibodies. These results suggest that IgE and IgG_4 may be antagonistic in protection against schistosoma infection.

Massive chemotherapy with OX or PZQ (Cline et al. 1982, Andrews et al. 1983, Cioli et al. 1993) has been one of the most important measures in the control of schistosomiasis in endemic areas. While resistance to OX is well documented, such has not been the case for PZQ. Nevertheless, there have been reports of possible failures in the treatment of humans infected with *S. mansoni* in Brazil (Tavares Neto & Prata 1988, Katz et al. 1991) and Senegal (Anonymous 1992), and of induction of resistance in mice (Fallon & Doenhoff 1994).

Although published reports suggest that PZQ alone is capable of killing or damaging adult schistosoma *in vitro* (Xiao et al. 1985), other evidence strongly indicates that anti-schistosome antibodies potentiate the effect of PZQ *in vivo* (Xiao et al. 1985, Brindley & Sher 1987, Mondha et al. 1990). However, a clear relationship between response to PZQ and the host Ig isotype profile has not yet been established. Studies in mice infected with *S. mansoni* have shown that the efficacy of PZQ against this parasite is probably linked to the host immune response. In fact, considerably less effective schistosomicide activity has been observed by Sabah et al. (1985) in adult thymectomized mice treated with anti-thymocyte sera, in comparison with intact animals.

As part of an ongoing research protocol on the immunopathology of *S. mansoni* (Aldrey et al. 1988, Benarroch et al. 1988, Alarcón de Noya et al. 1992, Noya et al. 1995a, b), we present herein the results of an immunoglobulin isotype level quantitation study undertaken in Venezuelan patients chronically infected with *S. mansoni*. Our results suggest a possible relationship between response to PZQ and a given isotype profile.

MATERIALS AND METHODS

Patients – Forty-three subjects chronically infected with *S. mansoni* were selected for the study, by means of a clinical, parasitological, and immunological protocol standardized by the Biohelminthiasis Section of the Institute of Tropical Medicine (Caracas, Venezuela). The subjects, including 22 children (aged 8-12 years) and 21 adults (aged 18-40 years), came from the town of Caraballeda, an isolated focus of schistosomiasis on the northern shore of Venezuela. The possibility of reinfection was avoided by subsequent elimination of schistosomiasis transmission by implementation of environmental sanitation and massive PZQ treatment programs.

All patients received PZQ in a single oral dose of 40 mg/kg, and were reevaluated 3, 6, 9, and 12 months after treatment (Alarcón de Noya et al. 1992, Noya et al. 1995a, b). Of the 43 patients, 27 (14 children and 13 adults) responded to PZQ (responders, R) while 16 patients (8 children and 8 adults) remained infected one year after treatment with PZQ (non-responders, NR), as assessed by the circumoval precipitin test (COPT) (Oliver-Gonzalez 1954, Alarcón de Noya et al. 1992, Noya et al. 1995a, b). Thirty-seven healthy individuals, including 12 children (aged 8-12 years) and 25 adults (aged 16-42 years) free of schistosomal infections, were selected as the control group after evaluation under the same protocol.

Parasitological and specific immunodiagnostic assessment – Stool examination was performed by a formol-ether assay (Martin & Beaver 1968) and quantified by the Kato-Katz technique (Katz et al. 1972). From each subject, two fecal samples were collected on different days before, and two after, treatment. Three conventional Kato tests were performed on each fecal sample. Antibodies to *S. mansoni* were detected in sera by both COPT (with a 10% cut-off level) and ELISA assays using soluble *S. mansoni* egg and adult worm antigens (Alarcón de Noya et al. 1992).

Immunoglobulin isotypes – The immunoglobulin isotypes were measured by a two-site immunoenzymometric assay, specific for each Ig isotype, as previously described by Black et al. (1988) and Reimer et al. (1988). Briefly, patients Igs were captured by a human isotype-specific mouse monoclonal antibody bound to the plastic surface of Immulon II 96-well microtest plates (Dynatech, Alexandria, VA). The presence of each isotype was quantitated using a mixture of peroxidase-conjugated monoclonal antibodies (kindly provided by the late Dr CB Reimer of the Centers for Disease Control, Atlanta, GA) to the kappa, lambda, and/or other appropriate Ig epitopes.

The World Health Organization (WHO) International Standard for human IgG, IgA and IgM, 67/97 (Rowe et al. 1972), was used to establish the numerical basis for the IgG, IgG_1, IgG_2, IgG_3, and IgG_4 assays, using the mass units assigned by Klein et al. (1985). For IgA and IgM, the mass units used were the International Units estimated for these two

analytes by Reimer et al. (1982). IgD and IgE calibration was performed using the British Research Standard, 67/37 (Rowe et al. 1970), and the WHO International Reference Standard, 67/204 (Rowe et al. 1973), respectively. Duplicates of the quality control preparation for each isotype were systematically included to estimate inter- and intra-assay variability.

Data analysis – Standard curves for each analyte were constructed using a public domain BASIC computer program, as described by Black et al. (1988), allowing an accurate computer interpolation of unknowns. Statistical analysis was performed by Student test for paired and unpaired samples; a linear correlation test and Wilcoxon range test were also used.

RESULTS

Parasitological and COP test – Results of the parasitological and standard immunodiagnostic evaluations of R and NR adults and children are presented in Table I. The parasitic load of these patients was low: only 2 of 22 children and 1 of 21 adults eliminated more than 100 eggs/g of feces. Posttreatment stool examinations were negative in both groups. The pretreatment COPT was positive in all the patients, while posttreatment values were below 10% in the R group and above 10% in the NR group.

Immunoglobulin isotype levels – a) Children vs. controls – In comparison with the control group, the pretreatment R children showed significantly higher levels of IgG, IgG_1, IgG_4, and IgE, and lower levels of IgG_2, while the NR children exhibited significantly elevated levels only of IgE (Table II). When pretreatment sera from R and NR children were compared, the former showed significantly higher levels of IgG and IgG_1 ($p<0.05$), and lower levels of IgG_2 ($p<0.05$); differences between other isotypes were not significant. *b) Adults vs. controls* – In comparison with controls, baseline pretreatment isotype levels in R and NR adults showed similarly high concentrations of IgG, IgG_1, IgG_3, IgG_4, and IgE. IgM was significantly higher only in R patients. After treatment, the R patients maintained high levels of IgG, IgG_1, IgG_3, and IgE, while only IgG and IgE remained significantly elevated in NR patients (Table III). *c) Baseline vs. post-treatment comparison* – When the pretreatment baseline levels of isotypes were compared with the values at 12 months after therapy, R children showed significantly decreased levels, although still significantly higher than controls, of IgG ($p<0.0001$), IgG_1 ($p<0.05$), IgG_4 ($p<0.05$), and

TABLE I

Immunoparasitological characteristics of chronic *Schistosoma mansoni*-infected patients

Groups of patients	n	Intensity of infection eggs/g feces x ± s bt/at	COPT (%) x ± s bt	COPT (%) x ± s at
R children	14	57±62/0	44±14	3±3
NR children	8	33±23/0	34±14	25±8
R adults	13	44±23/0	42±20	1±2
NR adults	8	51±55/0	47±15	33±13

COPT: Circumoval Precipitin Test; bt/at: before/after praziquantel treatment; x ± s: mean ± standard deviation; R/NR: responder/non-responder to praziquantel chemotherapy.

TABLE II

Immunoglobulin isotypes in children before and after treatment

Ig	Controls	Responders		Non-responders	
		bt	at	bt	at
	x ± s	x ± s	x ± s	x ± s	x ± s
IgG	13.2 ± 2.6	16.2 ± 2.6[a]	13.7 ± 2.6	14.4 ± 2.4	13.2 ± 1.9
IgG_1	9.3 ± 2.1	12.8 ± 2.8[a]	10.3 ± 2.7[b]	9.7 ± 1.7	9.5 ± 1.3
IgG_2	2.5 ± 1.0	1.7 ± 0.7[b]	1.7 ± 0.7[b]	2.1 ± 0.5	2.3 ± 0.6
IgG_3	0.6 ± 0.4	0.7 ± 0.31	0.7 ± 0.4	0.5 ± 0.3	0.6 ± 0.4
IgG_4	0.6 ± 0.4	1.7 ± 1.4[a]	0.8 ± 0.5[b]	1.6 ± 1.8	0.8 ± 0.9
IgM	1.3 ± 0.4	1.2 ± 0.6	1.3 ± 0.7	1.2 ± 0.6	1.2 ± 0.5
IgA	2.3 ± 0.7	1.9 ± 0.6	1.7 ± 0.7	1.8 ± 0.7	1.7 ± 0.6
IgD	0.07 ± 0.04	0.108 ± 0.09	0.109 ± 0.08	0.07 ± 0.05	0.08 ± 0.04
IgE	211 ± 72	465 ± 416[c]	375 ± 199[b]	363 ± 217	310 ± 199

p value in comparison with the control group: *a*: $p<0.001$, *b*: $p<0.05$, *c*: $p<0.01$; bt/at: before/after treatment; IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgM, and IgA are expressed in mg/ml, IgE in IU/ml, and IgD in µg/ml.

TABLE III

Immunoglobulin isotypes in adults before and after treatment

Ig	Controls	Responders		Non-responders	
		bt	at	bt	at
	x ± s	x ± s	x ± s	x ± s	x ± s
IgG	11.3 ± 1.9	16.9 ± 2.7[a]	13.4 ± 2.6[c]	16.1 ± 3.5[a]	14.1 ± 3.2[c]
IgG_1	7.4 ± 1.5	10.4 ± 2.3[a]	8.8 ± 1.8[b]	10.4 ± 2.8[a]	9.6 ± 3.6
IgG_2	2.3 ± 1.1	2.6 ± 1.6	2.9 ± 2.0	2.8 ± 1.2	2.6 ± 0.6
IgG_3	0.4 ± 0.2	0.7 ± 0.2[a]	0.7 ± 0.3[a]	0.8 ± 0.4[a]	0.7 ± 0.3
IgG_4	0.6 ± 0.4	1.9 ± 1.3[a]	0.8 ± 0.8	2.4 ± 1.3[a]	1.8 ± 1.2
IgM	1.2 ± 0.5	1.7 ± 0.7[b]	1.9 ± 0.9[b]	1.3 ± 0.7	1.1 ± 0.7
IgA	2.4 ± 0.8	2.1 ± 0.6	2.1 ± 0.3	2.1 ± 0.5	1.9 ± 0.5
IgD	0.06 ± 0.03	0.08 ± 0.06	0.06 ± 0.03	0.08 ± 0.06	0.06 ± 0.03
IgE	188 ± 41	607 ± 621[c]	432 ± 279[b]	747 ± 777[c]	371 ± 299[c]

p value in comparison with the control group: *a*: $p<0.001$, *b*: $p<0.05$, *c*: $p<0.01$; bt/at: before/after treatment; IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgM, and IgA are expressed in mg/ml, IgE in IU/ml, and IgD in μg/ml.

IgE ($p<0.01$), with consistently lower levels of IgG_2 ($p<0.05$). In NR children, only IgG_4 levels were decreased ($p<0.05$).

The adult R patients had decreased levels of IgG ($p<0.001$), IgG_1 ($p<0.01$), and IgG_4 ($p<0.001$), while in NR adults, the levels of IgG ($p<0.001$), IgG_4 ($p<0.001$), and IgE ($p<0.05$) were significantly decreased.

Correlations between immunoglobulin isotypes and immunoparasitological parameters – Among R children, a significant positive correlation was observed before treatment between COPT and both IgG_4 ($p<0.001$) and parasitic load ($p<0.001$), while NR children showed a significant positive correlation before treatment between COPT and the levels of IgM ($p<0.01$), IgD ($p<0.025$), and IgE ($p<0.025$).

Among adults, a positive correlation before treatment was found only in R patients, between parasitic load and IgG_4 levels ($p<0.05$).

DISCUSSION

To gain further insight into the possible relationship between Ig isotype levels and response to PZQ therapy, we evaluated, by means of a high performance, two-site immunoenzymometric assay (Black et al. 1988, Reimer et al. 1988), the pre- and post-PZQ treatment serum immunoglobulin isotypic profiles of a group of 43 patients (both children and adults) chronically infected with *S. mansoni*.

This investigation was conducted in an isolated focus of schistosomiasis in northern Venezuela, where the patients presented with prevailing chronic infections and low egg counts. It was possible to halt the spread of *S. mansoni* infection in this area by eradicating the intermediate host of *S. mansoni* by both the application of molluscicides and the introduction, into the river snails of the *Thiaridae* family, of competitors capable of reducing, and even eliminating, the population of *B. glabrata* (Pointer & McCullough 1989). Over the course of the study, *B. glabrata* was not detected in the river, and no new infections were identified in individuals under four years of age. We therefore assume that the subjects included in our protocol were not affected by episodes of reinfection during the post-treatment evaluation period. Thus, effects on isotype levels were dependent on the natural progression of the chronic infection by *S. mansoni* and on the action of both the host immune response to the parasite and the PZQ therapy.

In patients eliminating more than 100 eggs/g of feces, stool examination has conventionally been considered the best measure of the effectiveness of chemotherapy (WHO 1995). The sensitivity of the parasitological methods diminishes, however, when individuals excrete less than 100 eggs/g of feces (Mott & Cline 1980, Alarcón de Noya et al. 1992). In this situation, the immunodiagnostic tests seem to better assess the presence of the parasite. Among this kind of test, the COPT and methods based on the detection of circulating antigens have been extensively used. However, the antigen-detecting techniques are not yet of sufficiently high sensitivity in cases of low parasitic burden (De Jonge et al. 1991). The COP test, however, is both highly specific and sensitive, and has the additional advantage of demonstrating negative seroconversion after successful treatment, as has been shown in both mice (Cancio et al. 1967) and humans (Rifaat et al. 1969, Alarcón de Noya et al. 1992).

We have previously demonstrated that patients over five years of age and with an excretion rate lower than 100 egg/g of feces were correctly identified by COPT, with sensitivities higher than 90%. In children under five years of age, COPT sensitivity was 86% (Alarcón de Noya et al. 1992). In the current study, all of the patients were positive by COPT before treatment, and on initial observation, we could not establish any baseline differences among patients. Twelve months after treatment, all of the patients were negative for *S. mansoni* eggs. However, 48.4% of the patients dropped below the COPT cut-off value of 10%, while 48.5% remained positive. On this basis, we identified two main groups of patients: "responders" (R), characterized by negative COPT conversion, and "non-responders" (NR), who remained COPT-positive.

Previous studies (Rihet et al. 1991, Hagan et al. 1991, Demeure et al. 1993) of resistant and susceptible subjects revealed that resistance to reinfection was associated with enhanced IgG and IgE levels, and occurrence of reinfection, with high levels of IgG_4 and IgG_2. It has also been postulated that IgG_4 and IgG_2 may compete with effective isotypes, such as IgE and IgG_1, thereby blocking the host immune response against the parasite.

Jassin et al. (1987) found that Sudanese children from an area highly endemic for *S. mansoni* had elevated levels of IgG and IgE in comparison with a normal European population. Additionally, they reported that a significant part of the overall IgG increase was accounted for by IgG_1, IgG_3, and IgG_4, associated with an increase of IgA and IgM. The IgG isotype profile in response to PZQ was not evaluated.

Demeure et al. (1993) suggested that resistance to reinfection is influenced by the balance between a protective effect of IgE and a negative action of IgG_4 and IgG_2 antibodies to carbohydrate determinants on schistosomula. Rihet et al. (1992) demonstrated that certain antibodies present in the sera of chronically infected subjects compete with IgE antigen-binding, and that IgG_4 accounts for most of this blocking activity. They identified the immunoglobulin isotypes IgE, IgG_4, and IgG_2 as having a significant role in the human response to *S. mansoni* infection.

In the present study, evaluation of the immunoglobulin isotype profile in both R and NR patients showed a particular pattern of expression. At presentation, the NR children showed significantly elevated levels of IgE ($p<0.05$) and IgG_4 ($p<0.01$), suggesting a predominant TH-2 response. In the R children, who presented with significantly increased levels of IgG_1 ($p<0.001$), IgG_4 ($p<0.01$), and IgE ($p<0.05$), and a decreased level of IgG_2, there was no clear-cut predominance of either a TH-1 or TH-2 response, suggesting a possibly major difference between the two groups of children at presentation (Gascan et al. 1991). Although IgE has been considered to be protective against parasitic infections (Demeure et al. 1993), our findings indicated that increased levels of IgE alone were not sufficient for protection in NR children.

Moreover, in comparing the behavior of the isotype profile after treatment in both groups of children, the diminishment of the level of total IgG ($p<0.001$) in R patients was found to be due primarily to decreases in IgG_1 ($p<0.001$) and IgG_4 ($p<0.001$), with IgG_2 levels remaining low, while in NR patients, diminishment of the total level of IgG was due primarily to a reduction in IgG_4 ($p<0.001$). These observations may indicate a specific protective response in R patients, dependent on the IgG_1 and IgE isotypes, which competes with the blocking effect of IgG_4, and that the balance between IgG_4 and IgG_1 may downregulate the synthesis of IgG_2, as suggested by its diminished levels in R children.

The diminishment of most of the elevated isotype levels found in R children is consistent with previous studies of the specific response against *S. mansoni* egg antigens, which showed weaker recognition, after cure, of most of the electrophoretic bands. In fact, after successful treatment, the majority of the patients did not recognize the Sm-25 molecule (Noya et al. 1995b).

Isotype expression among the group of adult patients did not show differences before treatment between R and NR individuals, with the exception of increased levels of IgM in the R group ($p<0.02$). Twelve months after treatment, IgG ($p<0.001$), IgG_1 ($p<0.001$), and IgG_4 ($p<0.001$) showed significantly diminished levels, while the increased IgM remained unaltered in the R group. In the NR group, only the IgG_4 level was downregulated ($p<0.001$). These findings may also be indicative of the importance of IgG_1, and IgG_4 in the efficacy of PZQ treatment.

The simultaneous downregulation of IgG_1 and IgE levels and negative seroconversion of the COPT in post-treatment R children suggest that both parameters may have a common antigen specificity. The COPT reaction has been associated with a very specific egg glycoprotein antigen, designated ω_1, which has been postulated as one of the most promising antigens for diagnosis, because it also correlates with cure (Dune et al. 1981, 1988, 1991, McLaren 1981). The ω_1 antigen is one of the most important components of the *S. mansoni* egg antigen, which showed, in immunological tests, a significant reduction in reactivity six months after treatment (Mott & Dixon 1982). The

downregulation of IgG_1 in the groups of R patients is very suggestive that this isotype could act synergistically with PZQ, contributing to an effective therapy, as well as contributing to the negative seroconversion of the COPT by participating in reactions with antigens such as ω_1.

In conclusion, our results are in agreement with those of previous studies (Jassin et al. 1987, Hagan et al. 1991, Rihet et al. 1991, Demeure et al. 1993), in demonstrating the participation of IgG, IgG_1, IgG_2, IgG_4, and IgE in the response to *S. mansoni* infection, and suggest that the assessment of Ig isotype profile may help to understand the regulatory mechanism of the anti-parasite response in humans subjected to praziquantel treatment.

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