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Changes in specific anti-egg antibody levels following treatment with praziquantel for *Schistosoma haematobium* infection in children

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SUMMARY

Fifty-seven children 6–15 years old resident in a Schistosoma haematobium *endemic area in eastern Zimbabwe were treated with praziquantel at 40 mg/kg body weight. Levels of IgA, IgE, IgG1, IgG2, IgG3, IgG4, and IgM antibodies against soluble egg antigen (SEA) were assayed by ELISA before treatment and at 18 and 36 weeks following treatment. Prevalence of infection (as determined by urine egg counts) was 65% before treatment, all children were confirmed egg negative six weeks after treatment, and re-infection prevalence was 4% at 18 weeks and 21% at 36 weeks after treatment. At 18 weeks after treatment, there was a massive increase in IgG1 levels and significant increases in IgE and IgG4 levels and significant decreases in IgA and IgG2 levels. Similar patterns occurred at 36 weeks after treatment. Egg positive children showed a more marked increase in IgG1 and (for older children) a more marked decrease in IgG2 levels. There were no other effects of age or sex. IgA and IgG1 levels fell significantly between 18 and 36 weeks following treatment but not to pretreatment levels. The results show that specific anti-egg antibody responses are highly sensitive to the effects of praziquantel treatment. A possible consequence is that the susceptibility of children to infection with* S. haematobium *is altered by chemotherapy; this requires further investigation.*

Keywords *antibody, immunity, treatment, re-infection, Schistosomiasis, Zimbabwe*

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INTRODUCTION

Various recent epidemiological studies of human schistosome infection have incorporated a re-infection aspect in their study design and have followed infection intensities and prevalences for a period of time following treatment. These studies have shown that for both *S. mansoni* (Butterworth *et al*. 1985, Sturrock *et al*. 1987, Kresmer *et al*. 1994) and *S. haematobium* infections (Wilkins *et al*. 1987, Etard *et al*. 1995), re-infection levels a year after chemotherapy had not recovered to pretreatment levels. Wilkins (1989) showed that levels of re-infection experienced after treatment were a result of the effects of several factors such as seasonal variations and host personal attributes such as age which would affect transmission of infection. Low levels of re-infection following treatment of a large fraction of the population have been attributed largely to a reduction in transmission.

However, studies conducted in Kenyan school children showed that despite levels of transmission remaining relatively high following treatment of some school children, re-infection among treated children remained low (Sturrock *et al*. 1983). They suggested that this might be due to infection conferring protection to re-infection following treatment. They reported this protection to be associated with high levels of eosinophils and cytotoxic antibodies. Dunne *et al*. (1992a) reported that the re-infection intensities in people whose IgE antibodies recognized a 22 kDa antigen 6 months post-treatment correlated negatively with the levels of their IgE response. They proposed that since this antigen is (specifically) located in the tegument of the adult worm and of the 'lung' and 'liver' stages it would have become available following worm death caused by the treatment. In this case, treatment augmented an immune response which is a negative predictor of re-infection.

Chemotherapy has been demonstrated to be accompanied by changes in both humoral (Grogan *et al*. 1996a) and

cellular immune responses (Kimani *et al*. 1985, Roberts *et al*. 1993, Grogan *et al*. 1996b). Characterization of these changes will enable a better understanding of the effects of chemotherapy on immune responses and may help in interpreting any immuno-epidemiological differences that may occur between endemic populations and populations which have experienced a chemotherapeutic intervention. A study of the changes occurring following treatment may also improve current understanding of the development of acquired immunity to schistosome infections. This study was therefore carried out to investigate any changes in the amounts of circulating antibodies that occur in children following chemotherapy.

MATERIALS AND METHODS

The study was conducted in the eastern highlands of Zimbabwe in the Burma Valley, an area where *S. haematobium* is endemic. The area was chosen as there is little or no infection with other helminths and the *S. mansoni* prevalence was low. The study took place from August 1994 to June 1995. *S. haematobium* transmission in this region occurs mainly between September and May.

A cross sectional survey was conducted during August-September 1994 as described in detail elsewhere (Mutapi *et al*. 1997). During this survey, at least two urine samples, a stool sample and 10 ml of blood were collected from all compliant participants (320 people). This was followed by treatment of all compliant participants with praziquantel at 40 mg/kg body weight. The efficacy of the treatment was checked six weeks after treatment. A cohort of school children was then selected for a follow up study. The children had to come from families which were permanent residents of the area and had to have given at least two urine samples over three consecutive days, a stool sample and single blood sample during the cross sectional survey. In addition, the children had to have accepted treatment and been confirmed egg negative for both *S. haematobium* and *S. mansoni* infection after treatment. Fifty-seven children were followed up 18 weeks after treatment. Of these children, 24 were female and 33 were male. The children were aged between six and 15 (31 children between six and ten and 26 children between 11 and 15 years old). Of these 57 children, 38 children were followed up 36 weeks after treatment. However, not all of the 38 children gave enough blood for all assays and therefore, the serology sample sizes were lower than 38 for some assays.

Urine specimens were processed by the method of Mott (1983), while stool samples were processed by the Kato/Katz method (Katz *et al*. 1972). Sera were processed and assayed by ELISA methods for the following isotypes; IgA, IgE, IgM, IgG1, IgG2, IgG3, and IgG4 reacted against soluble egg antigen (SEA) as already described elsewhere (Mutapi *et al*. 1997).

Statistical methods

All statistical procedures were carried out using the statistical package SPSS unless otherwise stated. The effects of treatment on antibody levels were analysed using a repeated measures ANOVA on the antibody levels before and after treatment. This procedure tested the hypothesis that there was no difference between the pre-treatment and post-treatment antibody levels. The *P*-value was set at $P < 0{\cdot}01$ to reduce the probability of making type 1 errors. This test was performed for antibody levels both at 18 weeks and 36 weeks following treatment.

The effects of age (two categories: 1, 6–10 years old; and 2, 11–15 years old), sex (male and female), pre-treatment infection status (egg positive and egg negative) and the interaction between age and pre-treatment infection status on the change in antibody levels 18 weeks following treatment were tested using an ANOVA performed on the untransformed differences. Differences were obtained by subtracting the antibody level 18 weeks after treatment from the pretreatment antibody level. Sequential sums of squares were used to calculate the *F*-value with the independent variables entered into the model in the order: sex, age, pretreatment infection status, and the interaction between age and pretreatment infection status last (see Norusis 1994). ANOVA assumptions were confirmed by residuals and homogeneity plots. This procedure was not carried out at 36 weeks after treatment as the small sample size reduced the power of the statistical test.

More detail about the statistical methods is given in a statistics appendix included in the manuscript.

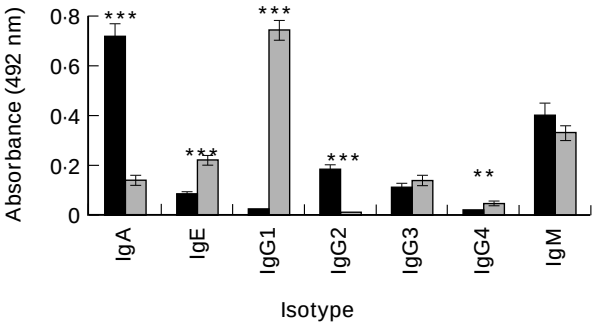

Figure 1 Antibody levels before treatment and 18 weeks after treatment. ■ represents the mean pre-treatment antibody level and □ represents the mean post-treatment antibody level, both untransformed. Bars represent standard error of the mean. ** represents $P < 0{\cdot}01$ and *** represents $P < 0{\cdot}001$.

RESULTS

Infection prevalence

Before treatment, 37 of the 57 children were positive for *S. haematobium* eggs (infection prevalence of 65%). Six weeks following treatment, all children were confirmed egg negative and at 18 weeks, two out of 57 children were egg positive (prevalence of 4%). At 36 weeks following treatment, 12 of the 57 children were egg positive (prevalence of 21%).

Effects of treatment on antibody production

At 18 weeks after treatment, there was a significant increase in levels of IgE, IgG1 and IgG4 and a significant decrease in levels of IgA and IgG2. Antibody levels at 36 weeks showed a similar pattern, with levels of IgA remaining significantly lower and IgG1 levels remaining significantly higher than pretreatment levels. Table 1 shows the *F*-values from the repeated measures ANOVA for each of the isotypes at 18 weeks and at 36 weeks following treatment while, Figure 2 shows the antibody levels at 18 and 36 weeks. A paired *t*-test showed that the change in levels of IgA and IgG1 was significantly greater at 18 weeks than at 36 weeks following treatment (Table 2)

Further analysis of the change in antibody levels at 18 weeks post-treatment showed that both age and sex alone did not have a marked effect on these levels. Children who had been egg positive before treatment showed a greater change in antibody levels, particularly for IgG1 where the change in egg positive children was significantly greater than in egg negative children. The change in levels of IgG2 depended on both the age and the pretreatment infection status of the child, so that the decrease was greater in older children who had been egg positive before treatment (Table 3).

Table 1 *F*-values for the levels of antibody levels produced before treatment and 18 and 36 weeks after treatment from the repeated measures ANOVA

	At 18 weeks		At 36 weeks	
Antibody	*F*-value	*n*	*F*-value	*n*
IgA	122·54***	57	121·17***	38
IgE	29·76 ***	56	2·54	38
IgG1	343·35***	57	129·88***	38
IgG2	74·76 ***	57	–	–
IgG3	1·97	55	2·39	35
IgG4	4·54	43	1·51	30
IgM	1·36	36	4·64	23

For each isotype *n* represents sample size, d.f. = 1 ($n-1$); ***$P < 0{\cdot}001$.

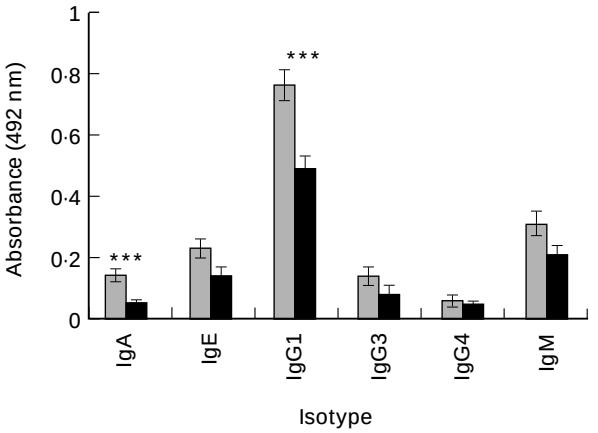

Figure 2 Comparison of the antibody levels at 18 and at 36 weeks for children who gave blood samples at both time points. Therefore sample sizes are lower than in Figure 1. There is no result for IgG2 as most children did not give enough sera for the assay 36 weeks following treatment. □ represents the untransformed mean antibody level at 18 weeks and ■ at 36 weeks. ***$P < 0{\cdot}001$.

DISCUSSION

Praziquantel treatment of children resulted in marked changes in *S. haematobium*-specific antibody responses directed against soluble egg antigen. Eighteen weeks after treatment, mean IgE, IgG1 levels and IgG4 levels increased significantly while mean levels of IgA and IgG2 decreased significantly. These changes persisted to at least 36 weeks post-treatment. These changes are largely independent of age (over the range of 6–15 years) and sex. For most isotypes, the change is also independent of infection status before treatment, egg negative children showing similar changes to egg positive children. The exceptions are that the increase in IgG1 was significantly greater in egg positive children, although the increase in egg negative children was still significant, and that the decrease in IgG2 was significant in older, egg positive children. This is possibly because some of the egg negative children were harbouring light, single sex or immature infections which could not be

Table 2 *t*-values for the comparison of between the change in antibody levels at 18 and 36 weeks post-treatment

Antibody (*n*)	*t*-value
IgA (38)	−0·71***
IgE (38)	−2·36
IgG1 (38)	−5·14***
IgG3 (35)	−1·81
IgG4 (30)	−1·38
IggM (23)	−2·30

d.f. = ($n-1$); ***$P <$ or $= 0{\cdot}001$.

Table 3 *F*-values and significance levels of factors affecting the change in antibody levels 18 weeks following treatment in all children.

Antibody (*n*)	Sex	Age	Pre-treatment infection status	Age × pretreatment infection status
IgA (57)	3·09	0·0	0·25	5·37
	m > f	1 > 2	0 > 1	
IgE (56)	2·14	0·46	0	0·27
	f > m	1 > 2	–	
IgG1 (57)	0	0·10	10·72***	1·80
	–	1 > 2	1 > 0	
IgG2 (57)	0·31	0·23	0·78	8·07**
	m > f	2 > 1	1 > 0	
IgG3 (55)	0·18	0	1·29	6·65
	m > f	–	0 > 1	
IgG4 (43)	0·07	1·62	0·78	
	0 > 1	2 > 1	1 > 0	
IgM (36)	0·14	0·11	0	3·50
	m > f	1 > 2	–	

n = sample size;. f, m = female, male; For age, 1 = 6–10 yrs, 2 = 11–15 yrs; pre-treatment status, 0 = egg negative children, 1 = egg positive. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

detected by egg counts (de Vlas & Gryseels 1992). The overall impression is that specific immune responses are highly sensitive to the effects of praziquantel treatment even if subjects are only lightly infected.

The changes in anti-egg antibody levels following treatment are likely to be related to changes in levels of antigen in circulation caused by the anti-helminthic action of the drug. Praziquantel penetrates the tegument of schistosomes and cestodes and rapidly moves through the tissues. The two primary effects of the drug are tegument damage and worm paralysis (Harnett 1988). Tegument damage results in antigen exposure and in the mouse host, the host's immune system has been shown to act synergistically with the drug to clear the infection (Brindley & Sher 1987, Fallon & Doenhoff 1995). The action of the drug results in a transient increase in antigens from damaged worms, resulting in an increase in the specific immune responses (both humoral and cellular) which assist in killing the damaged and paralysed worms. In the absence of re-infection, the levels of both worm and egg antigen would eventually decline. The effects of drug treatment are on the worm stage of the infection, but this study present results for anti-egg responses before and after treatment. Adult worms damaged and killed by praziquantel treatment will release both adult worm and egg antigens since adult female worms contain eggs. Changes in responses to both worm and egg antigens may then be expected. Analyses of the anti-worm and anti-egg responses showed a significant positive correlation between responses directed against both antigens for most isotypes. However, the extent to which there is cross-reactivity between antigens derived from worms and eggs and whether this influences subsequent responses to each cannot be determined from the data collected in this study.

Work on the biochemical effects of praziquantel in mice has shown that tegumental damage in schistosomes can occur as early as 15 min after treatment and that worms take about 14–18 days to disintegrate (Andrews 1986). Therefore, antigen from disintegrating worms is in circulation for a long enough period to induce changes in levels of antiegg antibodies. This study suggests that IgE, IgG1 and IgG4 antibodies are stimulated by antigens released from the worms after chemotherapy. This is because the levels of these antibodies increased significantly following chemotherapy and their high levels were maintained for at least 36 weeks. There is some evidence to support the localization of IgE stimulating antigen beneath the worm tegument, Dunne *et al*. (1992) reported that the 22 kDa antigen in S. mansoni stimulating the IgE response in their study population was located beneath the tegument. There was also an increase in IgG4 whose production is regulated by the same cytokines which regulate IgE production (King & Nutman 1993). The activity of the two antibodies is believed to be related, with IgG4 antibodies thought to regulate the anaphylactic responses related to IgE (Holfsetter *et al*. 1982, Hussain *et al*. 1992).

The antigens stimulating IgG1 have not been studied extensively but the isotype has been reported to be directed against both carbohydrate and peptide epitopes (Langley *et al*. 1994) and therefore it is likely that, in this population, this response is also directed against antigens beneath the tegument.

The significant decline in IgA and IgG2 antibody responses could be due to a removal of the main stimulus of these antibodies. IgG2 antibodies (together with IgM antibodies) are thought to be produced against the glycanic antigens on the surface of the adult worms and cercariae as well as on the eggs which are the main immunogens in the early phase of schistosome infections in children (see Butterworth *et al*. 1992). Treatment may remove these antigens, leading to

decline in the IgA and IgG2 responses. Furthermore, the antigens released from disintegrating worms may alter the cytokine environment resulting in a change in the antibodies produced. A treatment re-infection study in *S. mansoni* in Kenya (Gryzch *et al*. 1993) showed an increase in IgA antibodies directed against glutathione-S-transferase, but no changes in total IgA levels. The significance of any differences between these studies is not yet clear. A comparison of responses to individual defined antigens before and after treatment would help to clarify the effects of treatment on these responses.

It is unclear low long antigen from the damaged worms remains in circulation, but results from this study show that antibody levels at 36 weeks were lower than at 18 weeks post-treatment suggesting that the antigen stimulating the immune responses declined over this period. Two possible explanations for the persistence of the changes in antibody levels up to 36 weeks following treatment are that the antibodies have a long half life or that the children continued to be exposed to infective water which may or may not have resulted in infection. In the latter case, cross-reactive antigens between the cercarial stage and the adult stage could result in high antibody levels being maintained.

Treatment has been associated with changes in both humoral and cellular immune responses to schistosomiasis. Lympho-proliferative responses were shown to increase following chemotherapy in *S. mansoni* and *S. haematobium* infected communities (Roberts *et al*. 1993, Grogan *et al*. 1996b). Grogan *et al*. (1996b) also reported that levels of IL-4 (necessary for IgE production) increased following chemotherapy in *S. haematobium* infected people in Gabon. Grogan *et al*. (1996a) reported that levels of anti-SEA IgE, antiwhole worm homogenate (WWH) IgE and IgG4 increased following treatment. Satti *et al*. (1996) reported a significant increase in anti-WWH IgG1 and IgG4 following treatment in Sudanese canal cleaners carrying *S. mansoni* infections. The results reported here for anti-SEA responses are consistent with results from both studies.

The effects of such changes of levels of immune responses on re-infection have not been studied before and from this study, the full implications of these changes cannot be shown. It may be that the changes result in reduced susceptibility to re-infection as suggested by Sturrock *et al*. (1983) and Dunne *et al*. (1992). In this study, re-infection rates at 18 weeks (4%) and at 36 weeks (21%) did not reach the pretreatment levels of 65%. A further study of how the changes in some of the antibodies relate to adult-antibody profiles is reported elsewhere (Mutapi *et al*. 1998). An attempt was made to study the relationship between the changes in antibody levels at 18 and 36 weeks and the levels of re-infection at both times. But the levels of re-infection (both prevalence and infection intensity) at both time points were low, so no clear conclusion was reached. The long time taken for schistosome infections to re-establish, also makes it difficult to study the effects of the changes in immune responses.

Two main points emerge from this study: (1) The study has shown that treatment with praziquantel caused significant changes in levels of anti-egg IgA, IgE, IgG1, IgG2 and IgG4 produced 18 weeks following treatment when the effects of age and pretreatment infection status were allowed for, and that the changes are maintained for 36 weeks. This demonstrates that praziquantel treatment altered the natural immune responses of these children. Thus, apart from the immediate effects of reducing the worm load, praziquantel treatment may have long-term effects on specific immune responses. Furthermore, the results emphasize the need to consider that immunological changes induced by chemotherapy may influence levels of subsequent re-infection. The long-term effects of such immunological changes needs further investigation. (2) The observation that levels of antibodies change following praziquantel treatment calls for a re-examination of the use of the treatment re-infection study design in studying the nature and development of naturally acquired immune responses.

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Statistical appendix

The effects of praziqauntel treatment were analysed using a repeated measures analysis of variance (ANOVA). This procedure is used when the same variable is measured on several occasions for each individual. In this case, the variable was the level of antibodies produced before and after treatment by each individual. The procedure provides a control on the differences among individuals so that variability due to differences between individuals can be eliminated from the error sum of squares.

Factors affecting the change in antibody levels following treatment were tested using a general linear model (GLM). The variables of interest were sex, age, pretreatment infection status, and the interaction between age and pretreatment infection status.

All these variables were categorical. Age was categorical instead of continuous because the age-antibody relationship is not linear and GLM tests assume a linear relationship between the dependent and independent variables.

For both the repeated ANOVA and the GLM, the *F*-ratio

was calculated using the sequential rather than adjusted sums of squares. When adjusted sums of squares are used, all effects are adjusted for all other effects in the model, while in the sequential method, an effect is adjusted only for the effects that precede it in the model. The latter is useful when working with unbalanced designs, i.e. the number in all cells is not equal, or when working with models where the effects share information about the dependent variable. It is the second effect (shared information) which was corrected for in the analysis presented in this manuscript. Therefore, to find out the effects of pretreatment infection status, the effects of age and sex were accounted for before the effects of infection status were tested. As the repeated ANOVA and GLM tests are parametric tests, the residuals resulting from fitting the models to the data must be normally distributed. Normality of residuals was tested using homogeneity plots. For both tests, the *P*-value was $P < 0{\cdot}01$ instead of the more widely used $P < 0{\cdot}05$. This was to reduce the probability of making type 1 errors, i.e. accepting an effect to be significant when the apparent relationship arises purely by chance.

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