Circulating anodic antigen levels in two areas endemic for schistosomiasis mansoni indicate differences in worm fecundity

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Abstract

Serum levels of the adult schistosome circulating anodic antigen (CAA) were compared in 2 populations, both living in an area with extremely high transmission levels of *Schistosoma mansoni*. In one focus (Maniema, eastern Zaire) transmission has been established for several decades, while in the other focus (Ndombo, northern Senegal) transmission started only recently. While parasite egg counts in the 2 populations were virtually similar, including analogous age-related distributions, serum levels of CAA were approximately 5 times higher in the chronically exposed community. This difference in antigen level was most pronounced in adolescents and adults. As the level of CAA is assumed to be a direct reflection of worm burden, these findings suggest higher parasite fecundity in the recently exposed community. It is not very likely that these observations could be explained solely by differences in clearance mechanisms caused by a variation in experience of infection. The relationship between circulating antigen levels and egg counts was consistent for all age groups in the Maniema population, while in the Ndombo population only children showed a pattern similar to that in the chronically exposed community.

Keywords: schistosomiasis, *Schistosoma mansoni*, circulating anodic antigen, fecundity

Introduction

Most of the communities living in an area where *Schistosoma* infection is endemic show a peak in faecal egg counts early in the second decade of life, followed by a rapid decline in adults. This characteristic convex shape of intensity of infection has been interpreted as being due to the slow acquisition of partial protective immunity following years of infection (BUTTERWORTH *et al.*, 1992). However, whether this immunity is directed against newly incoming or existing parasites, or against worm fecundity, is still a matter of debate (CAPRON, 1992; GRYSEELS, 1994; DUNNE *et al.*, 1995).

Animal studies have shown that immunization with the antischistosome vaccine candidate Sm28-GST not only affected worm loads but also resulted in a reduction of egg production and viability. This 'anti-fecundity immunity' was associated with an immunoglobulin A (IgA) response, inhibiting the enzymatic activity of this antigen (XU *et al.*, 1991; GREZEL *et al.*, 1993). Moreover, in human studies a correlation was demonstrated between anti-Sm28-GST IgA antibody levels and the amount of reinfection, suggesting that 'acquired immunity' is partly directed against worm fecundity and the cause of the typical age-related egg count pattern seen in endemic areas (CAPRON, 1992; GRZYCH *et al.*, 1993).

In human populations, research on this issue is hampered by the fact that the number of *Schistosoma* worms in the infected host cannot be directly counted because of their intravascular localization. Alternatively, worm loads may be assessed by measuring one of the excretory or secretory products of the parasite. Until now, most research has focused on 2 of these antigens: circulating anodic antigen (CAA) and circulating cathodic antigen (CCA). Both CAA and CCA are glycoconjugates associated with the gut of the adult worm and released in large amounts into the bloodstream of the infected host (DEELDER *et al.*, 1994). Determination of these antigens has mainly been used for the diagnosis of an active *Schistosoma* infection. However, because of the good correlation between CAA or CCA concentration and the number of parasites (shown in animal models), and their rapid clearance from the circulation following successful chemotherapy in humans, CAA or CCA levels can also be interpreted as a reflection of current worm burdens in humans (DEELDER *et al.*, 1994; AGNEW *et al.*, 1995).

Recently, we used CAA and CCA determination for the assessment of worm burden in a community from Maniema, an area in eastern Zaire, endemic for *S. mansoni* for several decades (VAN LIESHOUT *et al.*, 1995). We found serum CAA and CCA levels to be closely related to faecal egg counts, independent of age. This indicated that, for this particular community, a reduction in egg count in adults reflects a genuine reduction in worm burden (VAN LIESHOUT *et al.*, 1995).

In another study, we determined serum CAA and CCA levels for diagnostic purposes in the community of Ndombo, a village in an area in northern Senegal where a recent outbreak of *S. mansoni* infection has occurred (POLMAN *et al.*, 1995). Due to the epidemic increase of schistosomiasis in this region, most individuals in Ndombo have become infected relatively recently. Thus, the pattern of infection should not be related to age (GRYSEELS *et al.*, 1995). Also, according to the current hypothesis that resistance develops only slowly following several years of infection, acquired immunity should not yet be present in this area. To our surprise, egg count patterns in Ndombo did not differ from those regularly observed in established endemic foci. In addition, we found circulating antigen levels to follow a pattern closely related to faecal egg counts (POLMAN *et al.*, 1995), but the influence of age on this relationship has not yet been investigated extensively.

The aim of the present study was to see whether the relationship between worm burden (reflected by antigen level) and egg output is influenced by the history of exposure on a population level. We therefore further analysed circulating antigen data from Ndombo similarly to the previous analysis in Maniema. In addition, we compared the relation between circulating antigen levels and egg output in the 2 communities. Despite the differences in transmission history, there was a striking similarity in faecal egg counts, ranging from low to extremely high levels. This made the comparison of circulating antigen levels even more interesting.

Materials and Methods

Origin of data sets

Parasitological data and serum circulating antigen levels were compared in 2 populations, both living in an area with intense transmission of *S. mansoni*. Both data sets were collected according to the same standardized methods, with similar protocols of parasitological examination and circulating antigen determination.

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The first data set was collected in Maniema (eastern Zaire), an area where *S. mansoni* had been endemic for several decades (VAN LIESHOUT *et al.*, 1995). The area and the local epidemiology of schistosomiasis mansoni have previously been described (POLDERMAN *et al.*, 1985a, 1985b). Faecal egg counts were calculated from 2 stool examinations, each consisting of duplicate 25 mg Kato–Katz smears (KATZ *et al.*, 1972; POLDERMAN *et al.*, 1985a), and expressed as eggs per gram of faeces. Data from 508 individuals, for whom both CAA and CCA levels were available, were used in the analysis.

The second data set was collected in Ndombo (northern Senegal), a recently established focus where the first case of schistosomiasis was reported only a few years before sample collection (TALLA *et al.*, 1990; STELMA *et al.*, 1993; POLMAN *et al.*, 1995). To prevent any bias caused by differences in the amount of stool examined, only those individuals were selected for further analysis from whom 2 stool samples were examined by duplicate 25 mg Kato–Katz smears, as in Maniema. In addition, serum levels of both CAA and CCA had to be available. This reduced the number of subjects analysed from 422 to 246. However, these procedures did not lead to any statistically significant change in age, egg counts or antigen levels.

CAA and CCA levels were determined by monoclonal antibody 'sandwich' assays, as described by DEELDER *et al.* (1989) and DE JONGE *et al.* (1990), with some minor modifications (POLMAN *et al.*, 1995; VAN LIESHOUT *et al.*, 1995). Both circulating antigen assays have a specificity of at least 98% (KRIJGER *et al.*, 1994). In accordance with previous studies, the levels of CAA and CCA were found to be highly correlated (VAN LIESHOUT *et al.*, 1992, 1995; POLMAN *et al.*, 1995). For this reason we focused exclusively on the CAA analysis.

Data analysis

The data sets from the 2 areas were analysed in exactly the same manner as described in our previous paper (VAN LIESHOUT *et al.*, 1995). In short, as circulating antigen levels and egg excretion have skewed distributions, data were normalized by $\log_{10}$ transformation of all positive values. Ranges and geometric means were calculated for all positive values only. Initially, the 2 populations were compared with the aid of Student's *t* test, while the association between circulating antigen concentrations and egg counts was examined by Pearson's correlation test.

To study the effect of age on the relationship between antigen levels and egg excretion, the populations were divided into 4 age groups. Regression analysis according to Deming was used, an extension of conventional linear regression analysis allowing for variation in both the dependent and the independent variables (CORNBLEET & GOCHMAN, 1979; WARD & CORNISH, 1992). The few cases with negative results for both egg excretion and CAA determination were considered to be uninfected and excluded from the regression analysis (one individual in Ndombo and 17 in Maniema). Individuals with negative results for only one of these indicators were included, and a value equal to half the detection limit of the assay concerned was assigned to them (VAN LIESHOUT *et al.*, 1995). A limited number of outlying points, i.e. those more than 3 times the standard deviation from the regression line, were rejected from the analysis (5 in Ndombo and 14 in Maniema). The effect of age was examined by covariance analysis.

Table 1. Comparison of two data sets, one from Maniema (Zaire), where intense, year-round transmission of *Schistosoma mansoni* has been occurring for several decades, the other from Ndombo (Senegal), where the transmission of schistosomiasis has been established only recently

	Maniema	Ndombo	*P*
No. of subjects	508	246	–
Males	51%	46%	NS
Age (years)[a]	30(1–66)	18(1–77)	<0·0001
Eggs in faeces			
Positive	95%	96%	NS
Count (eggs/g)[b]	653(10–13183)	675(10–10328)	NS
Circulating anodic antigen			
Positive	90%	94%	NS
Level (ng/mL)[b]	31·4(0·06–514)	5·8(0·02–339)	<0·0001

[a]Median (range in parentheses).
[b]Geometric mean excluding zero values (range in parentheses).

Results

The 2 data sets are summarized in Table 1. All ages were represented in both groups, but on average the study population from Zaire was older. Based on parasitological examination, *S. mansoni* infection levels were extremely high and remarkably similar in both communities. In contrast to egg counts, CAA concentrations differed significantly between the 2 groups, the mean antigen levels being higher in Maniema than in Ndombo, even after stratification into egg output classes (Table 2).

Both communities showed the characteristic age-related pattern of egg counts, except for a more pronounced peak in Ndombo in the age group of 10–14 years (Figure, A). Similarly, CAA levels showed a rapid increase in young children and a peak level around 5–15 years in both populations, but thereafter antigen levels in the Ndombo population decreased to a much lower plateau (Figure, B). This sharp drop of CAA level in adolescents from Senegal suggests higher worm fecundity in adult hosts in this recent focus.

For further comparison, the Ndombo population was divided into 4 age groups and regression analysis was performed in exactly the same manner as previously described for the Maniema data (VAN LIESHOUT *et al.*, 1995). The parameters of the regression lines describing the relation between antigen levels and egg counts are summarized in Table 3. While the previous analysis revealed no effect of age in the Maniema population (VAN LIESHOUT *et al.*, 1995), significant differences were found between the 4 age groups in Ndombo. However,

Table 2. Percentage of subjects with detectable circulating anodic antigen and its concentration according to egg count class in two communities, chronically (Maniema) and recently (Ndombo) exposed to *Schistosoma mansoni*

Egg counts (per g)	Maniema			Ndombo		
	No. of subjects	CAA[a] Positive (%)	CAA[a] Level (ng/mL)	No. of subjects	CAA[a] Positive (%)	CAA[a] Level (ng/mL)
0	25	32	4·8	10	90	0·4
1–100	46	74	4·9	29	79	0·4
101–400	104	90	14·6	41	88	2·5
401–1000	138	96	36·3	61	95	4·3
≥1001	195	97	62·5	105	100	21·0
Total	508	90	31·4	246	94	5·8

[a]Circulating anodic antigen. 'Level' is the geometric mean level excluding zero values.

Table 3. Intercepts (α) and slopes (β) of the regression lines describing the relationship between egg counts and circulating anodic antigen levels in two communities, chronically (Maniema) and recently (Ndombo) exposed to *Schistosoma mansoni*[a]

Community	Age (years)	n	α[b]	β[b]
Maniema	All ages	477	2·1 (2·0–2·1)	0·56 (0·51–0·60)
Ndombo	1–9	66	1·9 (1·8–2·1)	0·72 (0·61–0·83)
	10–19	66	2·9 (2·7–3·1)	0·24 (0·13–0·36)
	20–39	65	2·6 (2·5–2·7)	0·48 (0·37–0·59)
	≥40	43	2·5 (2·3–2·7)	0·93 (0·68–1·18)

[a]Log (eggs/g) = α+β log (antigen concentration, ng/mL).
[b]95% confidence interval in parentheses.

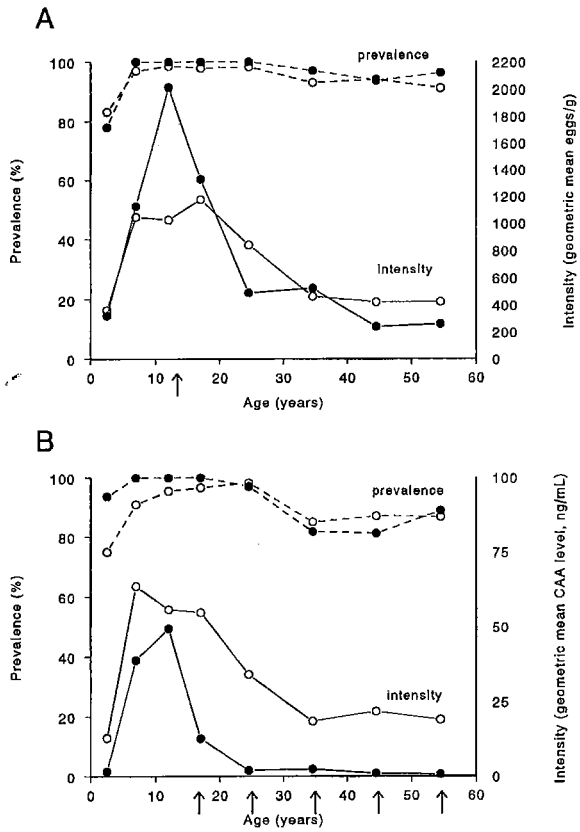

Figure. Comparison of *Schistosoma mansoni* prevalence and intensity of infection (geometric mean excluding zero values) in 2 endemic areas, Maniema in Zaire (○) and Ndombo in Senegal (●), in relation to age. Infections were measured by faecal egg count (A) or serum circulating anodic antigen (CAA) concentration (B). The arrows indicate values of egg count or antigen level for the 2 populations of which the 95% confidence intervals do not overlap.

this effect of age seemed to be a combination of several factors, and not clearly attributable to any one parameter. Most remarkable was the fact that, above the age of 10 years, the intercept α was significantly higher in Ndombo than in the Maniema population, indicating higher egg production at any given antigen level. The slope β was significantly lower than 1·0, indicating a non-linear relationship between egg counts and serum CAA levels (VAN LIESHOUT *et al.*, 1995), in all age groups below 40 years, with its lowest value in the 10–19 years age group.

Discussion

Several animal studies have shown a significant correlation between the levels of CAA and the number of *Schistosoma* worms in a host (BARSOUM *et al.*, 1990; AGNEW *et al.*, 1995). In this study, we found relatively lower circulating antigen levels in a population living in an area where *Schistosoma* transmission started only recently (Ndombo) than in a community in a more chronic situation (Maniema), while egg counts were closely similar in the 2 groups. Interpreting circulating antigen levels as a reflection of worm burden, these results indicate higher worm fecundity in the recent focus. As a consequence, our results suggest that, if anti-fecundity immunity is of any importance in *S. mansoni* infections, it seems to be related more to the history of transmission in the total population than to exposure experienced on an individual level. However, we have no explanation for the fact that worm fecundity in young children in Ndombo seemed to be relatively low compared to that in adults in this area, and was more similar to that in the study population in Maniema.

In mice, the level of serum CAA was found to be proportional to worm burden irrespective of the intensity of infection or the immune status of the host (AGNEW *et al.*, 1995). However, it cannot be ruled out that the production and/or clearance of CAA is affected by several, in part host-related, mechanisms. For example, the efficiency of the immune system in clearing this antigen may be influenced by previous experience of infection in combination with the age or health status of the host. Our findings could thus be interpreted as indicating relatively enhanced efficacy in adults from Ndombo compared to adults from Maniema in clearing CAA from the circulation, probably due to the recent nature of the infections in Ndombo. Consequently, it should be expected that children in Maniema, who also have limited experience of infection, would show the same effect as the population of Ndombo. However, we observed the opposite, with no effect of age in the Maniema population and no difference between the 2 populations in the younger age groups.

In addition to that, in our previous study we found urine CAA levels in Ndombo to be extremely low at all ages and not only in children (POLMAN *et al.*, 1995). Unfortunately, urine samples were not collected from the Maniema population, but determination of CAA in a chronically exposed community in Egypt showed a comparable ratio in serum and urine CAA levels, as in the Ndombo population (VAN LIESHOUT *et al.*, 1992). This argues against a major effect due to renal clearance efficacy. However, possible involvement of other clearance mechanisms, e.g. formation of immune complexes followed by their clearance by Kupffer cells in the liver, cannot be excluded.

Alternatively, the differences we found between the 2 populations could be explained by a difference in antigen production by the parasites themselves. This could be due to geographical strain differences, but it is also possible that, due to the recent nature of the outbreak of schistosomiasis in the Ndombo population, most infections consisted of relatively young worms whose CAA and CCA production had not yet reached its maximum level (VAN DAM *et al.*, 1996).

To gain a better understanding of the mechanisms of antigen production and clearance, further research is needed. Studies should preferably be undertaken in animal models imitating as closely as possible the infection patterns seen in humans, as experimental infections differ in many respects from the human situation, e.g.

worm load in relation to body mass and the continuous exposure to reinfection in humans (CHEEVER, 1969).

The low levels of slope (β) found in the Ndombo population in all age groups below 40 years (with the lowest value in those 10–19 years old) indicated a non-linear relationship between CAA levels and egg counts in these age groups. This non-linear relationship was suggested in the original study (POLMAN *et al.*, 1995), and was also seen in the Maniema population (VAN LIESHOUT *et al.*, 1995). These findings agree with the theory of density-dependent reduction of fecundity, i.e. decreased egg production by female worms in the presence of high parasite numbers (MEDLEY & ANDERSON, 1985). However, scattergrams of the individual Ndombo data showed that the relatively strongest density-dependent fecundity in the 10–19 years age group was caused mainly by a few subjects with high egg counts and low antigen levels, and not by reduced egg production in individuals with high antigen levels (data not shown). The phenomenon of density-dependent fecundity is still subject to debate (WERTHEIMER *et al.*, 1987; GRYSEELS & DE VLAS, 1996). Recently, AGNEW and colleagues (1996) showed a linear relationship between serum CAA levels and faecal egg counts in a community infected with *S. mansoni* in Kenya, but the intensity of infection in their population was not as high as in our study groups.

In conclusion, we found significant differences in the relation between circulating antigen levels and egg counts after detailed comparison of 2 data sets, both collected from foci with comparable extremely high intensities of *S. mansoni* infection but with a different history of transmission. The relatively lower serum levels of CAA in adolescents and adults living in Ndombo suggested lower worm burdens, and consequently higher worm fecundity, in this area where transmission commenced only recently. However, other mechanisms affecting production and clearance of these circulating antigens cannot be completely excluded.

Acknowledgements

We are grateful to F. Stelma, I. Talla, M. Niang, A. Polderman and P. de Caluwé for providing serum samples and parasitological data. We also thank S. de Vlas for helpful discussions. This study received financial support from the Research and Development Programme of Life Science and Technology for Developing Countries (STD-3; contracts no. TS3-CT91-0040 and 0041) of the European Community and from the Netherlands Organization for Scientific Research in the Tropics (NWO/WOTRO).

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Received 10 June 1997; accepted for publication 17 July 1997

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The conference will mark the 50th anniversary of the publication by MacCallum *et al.* describing the disease and the bacterium; the paper originated from the University and the Alfred Hospital, Melbourne.

The conference will be followed by a two days' bus tour of the two most important endemic areas in Victoria, incidentally passing through some of the most beautiful scenery in southern Australia.

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