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HUMAN RESISTANCE TO *Schistosoma mansoni* IS ASSOCIATED WITH IgG REACTIVITY TO A 37-kDa LARVAL SURFACE ANTIGEN[1]

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The aim of this work was to determine whether human resistance to *Schistosoma mansoni* was associated with increased antibody reactivity to certain larval surface Ag. To this end, young residents of a hyperendemic area were selected for their low or high susceptibility to reinfection after parasitologic cure, and the reactivity of their sera to individual larval surface Ag was determined at different times before and after treatment. The data showed that six Ag: 202, 165, 90 to 92, 85, 72, and 37 kDa are the principal targets on the larva of IgG in the sera of resistant subjects. The comparative study, by immunoblotting and ELISA on purified Ag, of the sera from high and low susceptibility subjects indicates that IgG reactivity toward the 37-kDa Ag may be associated with resistance. This work and ongoing vaccination trials carried out in mice suggest that the 37-kDa Ag may have vaccinating potentials.

Schistosoma mansoni is a major health problem for a number of developing countries (1). Eradication of this parasite by vector control and chemotherapy has been attempted with some successes. The scaling up of these programs, however, is faced with numerous difficulties, and an efficient vaccine would represent a major step toward the control of this parasitic disease (2–11).

With the aim of identifying vaccinating Ag, a number of studies have been conducted in permissive and nonpermissive animals analyzing animal protective immunity against *S. mansoni* (reviewed in Reference 12). Several groups produced protective mAb or polyclonal antibodies (13–18), which have allowed the identification of a number of parasite Ag. These have been characterized and their vaccinating potentials are being tested (17, 19–21). Other studies have also identified parasite Ag that may stimulate protective cell-mediated immunity in animals (22, 23).

In contrast to progress achieved in animal models, little is known about human protective immunity to *S. mansoni*. Although resistance has long been suspected in humans living in areas endemic to schistosomiasis, it was only recently that a study by Butterworth and colleagues (24) reported resistance to *S. mansoni* in Kenyan children. The immunologic response of these children has been extensively analyzed. These studies failed, however, to reveal major differences in the immune response of children with different susceptibilities to infection (24, 25).

Four years ago, we started a similar study in a hyperendemic area for *S. mansoni* in Brazil, where we assessed the ability of adolescents to resist reinfection following parasitologic cure with oxamniquine. The aims of that study were to identify subjects with low or high susceptibility to infection by *S. mansoni* and to determine whether the most resistant subjects exhibited enhanced immunologic reactivity against certain parasite Ag. The results presented here show marked differences in resistance to infection among adolescents with high levels of contact with infected waters. Analysis of the schistosomular Ag that react with IgG in the sera of these individuals show that a 37-kDa surface Ag reacts preferentially with antibodies in the sera of the most resistant subjects.

MATERIALS AND METHODS

Chemicals

MEM, HEPES, penicillin, and streptomycin, were purchased from GIBCO (Paisley, Scotland); *n*-octyglucoside (1-O-*n*-octyl-β-D-glucopyranoside), pepstatin, PMSF, α_2-macroglobulin, leupeptin, and dithiothreitol were from Boehringer-Mannheim Biochemicals (Mannheim, W. Germany); aprotinin, protein A, hemoglobin, Ponceau red stain, *p*-nitrophenyl phosphate, and diethanolamine were from Sigma Chemical Co. (St Louis, MO). The Bolton and Hunter reagent was from Amersham Corp. (Les Ulis, France); x-ray films were from Fuji-Japan; acrylamide and SDS were from Fluka AG Chemische Fabrik (CH-9470, Buchs, Switzerland); avidin-coated plates were from Immunotech (Marseille, France); mouse monoclonal anti-human IgG antibodies were from ICN K & K Laboratories Inc. (Eschwege, W. Germany); G-25 Sephadex was from Pharmacia Fine Chemicals (Uppsala, Sweden).

Epidemiologic Study

Caatinga do Moura. Caatinga do Moura is a village of a northeastern state of Brazil (Bahia), located in a semi-arrid area on the banks of a minor river that is the principal source of water for agricultural and domestic uses. The river is densely populated by *Biomphalaria glabrata* snails that are infected by *S. mansoni*, and Caatinga do Moura is a high transmission area for this parasite as indicated by prevalence, egg counts, and morbidity (26). Other human parasites common in this area include *Ascaris lumbricoides*, *Entamoeba histyolytica*, and *Giardia lamblia*. Caatinga do Moura is free of malaria, leishmaniasis, and Chagas disease.

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Study subjects. Study subjects were selected from young residents of Caatinga do Moura (aged 10 to 19) housed within 200 meters of the river whose parents had accepted regular medical checkups in the past. The study sample was chosen so as to include at least 25% of individuals with egg counts <150 eggs/g and 25% of subjects with egg counts >400 eggs/g. Of the 115 subjects in this study, 92 (80%) were totally cured by oxamniquine, 81 (49 girls) out of the 92 had regular parasitologic examinations during the 4 years of the study (data presented in Figs. 1 and 2), and water contacts could be assessed accurately for 69 subjects (43 girls) (data presented in Figs. 3 through 6 and in Table I).

Program of control of S. mansoni. The program was started in the area in December 1982: a molluscicide (niclosamide) was spread four times over an 11-mo period, and more than 90% of the residents of the village were given (orally) 15 to 25 mg/kg oxamniquine after the first molluscicide application. The molluscicide program produced a marked reduction in the snail population. Subsequent infection trials carried out with mice immersed in the river (1 h at noon, 3 days in a row) failed to detect infective cercariae 7 mo after the initiation of the program. Similar trials carried out before the initiation of the program had resulted in the infection of up to 50% of the mice (27). The effects of oxamniquine treatment was assessed by three Kato's examinations performed on stools from three different days at 2 to 2.5 mo after drug administration.

Assessment of the levels of water contact. Several activities required standing in the river for a lengthy period of time. These included: washing dishes, clothes, and hair; taking baths; irrigating fields; and, occasionally, fishing and playing. Short duration water contacts such as washing hands or crossing the river were not considered here. These activities, other than irrigating and fishing, were carried out at four different river sites; three of these sites were located 50 meters apart on the river, a fourth site was a water hole on a main irrigation channel 100 meters from the other sites. All sites were shown to be populated by snails and to be infective for experimental mice. Irrigation was strictly regulated by local rules and was carried out for 5 to 12 h once a week or, occasionally, twice a week.

Sites where adolescents entered the river were visited four consecutive days every 6 mo at 8 a.m., 10 a.m., 2 p.m., and 5 p.m. Subjects standing in the water, their activities, and the average duration of these activities, were recorded. One week later, study subjects were interviewed separately at the local health facility. Details of their daily activities were recorded including the frequency, duration, time, and location of their water-related activities. Each interview was repeated a second time a week later and was counterchecked by interviews of relatives, friends, and local persons living by the river near the sites. These observations allowed the classification of study subjects in three groups: a group of low water contact included subjects spending less than 1 h/wk in the river; a group of intermediate water contact included subjects spending 1 to 3 h once or twice a week in the river; and a group of high water contact included the adolescents who spend 1 to 3 h/day, every day, in the river. Individuals in the low and intermediate water contact groups had access to noninfected underground water for their domestic uses; individuals in the high water contact group had not.

Parasitologic Methods

Egg counts. These were performed by Kato's method (28, 29) on at least three stool samples collected on different days. Individual egg counts are the arithmetic mean of these determinations. Because 1:24 g of stool was examined on each examination, this method allowed the detection of $24 \div 3 = 8$ eggs/g of stool for three examinations.

Parasite. The Puerto Rican strain of *S. mansoni* used in this study was obtained from the Harvard Medical School colony and was maintained by passage through CBA/J mice and *B. glabrata* snails. Schistosomula were prepared by the method of Ramalho Pinto as modified by Lazdins et al. (30) and cultured for 3 to 5 h at 37°C in MEM supplemented with 10 mM HEPES, 100 IU/ml penicillin, and 100 UG/ml streptomycin.

Immunologic and Biochemical Procedures

Tegument extraction. Schistosomular tegument was extracted by resuspending schistosomula (100.000/ml) in 1% *n*-octylglucoside containing 10 mM phosphate buffer (pH, 7.1), 4 mM $MgCl_2$, 140 mM NaCl to which the following protease inhibitors were added just before use: 50 μM PMSF, 50 μg/ml aprotinin, 1 μM pepstatin, 20 μg/ml leupeptin, and 10 μg/ml α2-macroglobulin. After 15 min at 4°C in the extraction solution, somula bodies were pelleted (800 g, 3 min, 4°C) and the supernatants were centrifuged at 100.000 × G for 35 min at 4°C. Then the supernatants of ultracentrifugation were stored aliquoted at −70°C until use.

Labeling procedures. Schistosomula were labeled with the Bolton and Hunter reagent (31, 32) in 25 mM borate-buffered saline, pH 8.0, using 0.5 mCi for 20.000 schistosomula in 100 μl.

Western blotting. Schistosomular membrane extracts were heated (3 min at 100°C) in the presence of 2-ME and electrophoresed on SDS-PAGE (33). Protein transfer was carried out at 40 V for 12 h (34). Quality of the transfer was assessed by staining with Ponceau red; only blots with distinguishable bands in the whole range of 25 to 200 kDa were used. Nitrocellulose strips were cut and soaked at 4°C for 24 h in two changes of a 10-mM Tris buffer, pH 7.2, containing 150 mM NaCl, 3% hemoglobin, and 0.3% Tween 20 (Tris/Hb). Then the strips were incubated, with gentle agitation, for 3 h at room temperature in 5 ml of a 1/100 dilution of serum in Tris/Hb. After this incubation, strips were washed five times in Tris/Hb over a 3-h period and reacted for 1 h at 4°C with 5 ml of ^{125}I-protein A in Tris/Hb (5×10^5 cpm/ml). At the end of the incubation period, strips were washed six times over a 12-h period and exposed for autoradiography on x-ray films.

Ag purification by electroelution from acrylamide gels. Electroelution of the Ag of interest from polyacrylamide gels was carried out according to Hunkapiller et al. (35) with few modifications: soaking of acrylamide bands was carried out in 0.5% SDS, 5 mM dithiothreitol; no SDS was added to electroelution buffer. To identify the bands of interest, half of the acrylamide gel was stained with Coomassie blue and half was blotted on nitrocellulose. Half of the resulting nitrocellulose blot was stained with Ponceau red stain and kept as such; the other half was cut in strips and reacted with human sera and ^{125}I-protein A. The autoradiogram allowed clear identification of the bands of interest on the nitrocellulose blots (stained with red Ponceau) and then on the acrylamide gel. This procedure allowed the preparation of 0.1 to 0.2 μg of Ag (37, 72, and 165 kDa) from 1000 schistosomula. Electroeluted Ag yield one sharp band after rerunning on acrylamide gels and staining by the Silver Stain method (36). Mice immunized with three injections of 37-kDa preparation in CFA produced antibodies that reacted with one band (37 kDa) on Western blots of schistosomular extracts (data not shown). Biotinylation of the purified Ag was carried out according to Hofman et al. (37) after filtration of the Ag preparation on G-25 Sephadex columns equilibrated in 10 mM phosphate buffer pH 7.2, 150 mM NaCl, 10 mM NaN_3.

Antibody titration. Titration of the human sera was performed by ELISA (38) on plates coated with Ag 37, 72, and 165 kDa electroeluted from acrylamide gels. Avidin-coated plates were reacted for 16 h at 4°C with biotinylated Ag 37, 72, or 165 kDa (500 ng/ml, 50 to 100 μl/well) in 10 mM borate buffer, pH 8.5, containing 150 mM NaCl. Then Ag-coated plates were washed in 150 mM NaCl, 0.5% Tween 20 (washing solution) and used within 48 h. For titration, sera diluted in 10 mM phosphate buffer, pH 7.2, 150 mM NaCl, 10 mM NaN_3, 2 mg/ml BSA, were reacted for 3 h at room temperature with the plates. Then the plates were washed four times in the washing solution and incubated for 1 h at room temperature with a 1/1000 dilution of alkaline phosphatase coupled mouse monoclonal anti-human IgG antibody. After three washes, the substrate (*p*-nitrophenyl phosphate, 1 mg/ml in 10 mM diethanolamine buffer, pH 9.8) was added, and the colored reaction was read at 410 nm with a spectrophotometer 1 h later. Results are expressed as the difference between sample OD and OD of wells reacted with control sera. Because all subjects in Caatinga have been in contact with infected waters, controls were adolescents and young adults from Jacobina (30 miles from Caatinga) or from Marseille. These individuals had no history of schistosomiasis and did not excrete *S. mansoni* eggs.

Statistical Analysis

Statistical analysis of the results has been carried out with analytical methods that do not require a normal distribution of the epidemiologic data. These statistical methods are indicated in the figure legends. p values < 0.01 were considered to be significant.

RESULTS

Reinfection after parasitologic cure with oxamniquine. Oxamniquine treatment caused a marked reduction of egg excretion in all 115 adolescents we had selected; 92 (80%) of them were totally cured, and 81 out of these 92 completed all examinations and interviews for the 4 yr after treatment. In spite of the schistosomiasis control program enacted in the area (see *Materials and Methods*), 48%, 54%, and 68% of these adolescents were

reinfected within 15, 27, and 43 mo, respectively, post-treatment (Fig. 1). Subjects excreting more than 200 eggs represented 5, 9, and 31% of the adolescents 15, 27, and 43 mo post-treatment, respectively, vs 46% before treatment. Adolescents with heavy infections (>400 eggs/g) represented 0, 2.5, and 16% of the study subjects at 15, 27, and 43 mo, respectively, vs 29% before treatment. Thus, infection levels were low after 15 and 27 mo as compared with pretreatment levels. Infection intensities had, however, markedly increased after 43 mo and approached pretreatment infection levels. This indicates that the control program (mass chemotherapy + molluscicide) probably caused a significant reduction in parasite transmission for the first 2 yr but that parasite transmission reverted to previous high levels during the third and fourth year of the study.

Influence of sex, age, and water contacts on reinfection. Reinfection, as assessed by egg excretion or by prevalence, was not statistically different for boys and girls 15, 27, and 43 mo after treatment ($p = 0.1$, data not shown). Egg excretion was maximal in the 10- to 13-yr age group and decreased with age ($p = 0.05$), as shown in Figure 2 with data obtained 43 mo after treatment. The statistical significance of this association was increased ($p < 0.01$) when data from older individuals (aged 20 to 40, $n = 15$) were included in this analysis. The geometric mean egg excretion for the individuals older than 17 yr was 8 to 10 times lower than that in the 10- to 11-yr age group. The reduction of egg excretion with age did not merely result from a decrease of water contacts with age

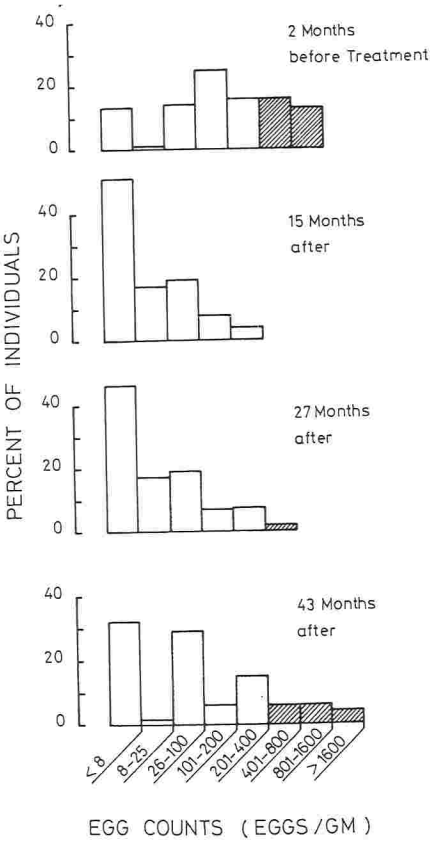


Figure 1. Egg excretion patterns for the 81 study subjects 2 to 2.5 mo before and 15, 27, and 43 mo after oxamniquine treatment. Only adolescents (aged 10 to 19) who were totally cured by oxamniquine treatment were included. Eight eggs/g of stool is the detection threshold of Kato's method performed on three stool samples.

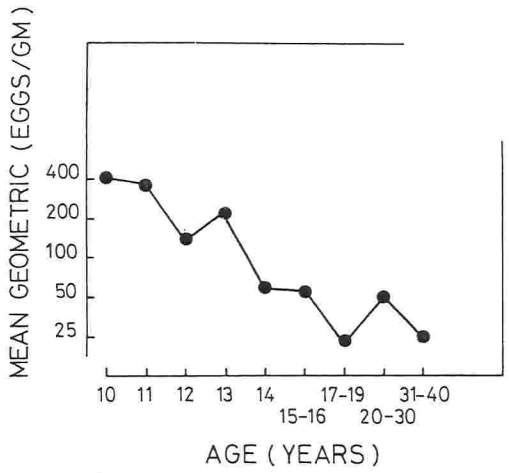


Figure 2. Influence of age on reinfection 43 mo after oxamniquine treatment. Results are represented as the geometric mean of individual egg counts for each age group. Spearman rank correlation tests yield $p = 0.05$ for the association between egg counts and age for the 10- to 19-year-old subjects ($n = 81$) and $p < 0.01$ when older subjects ($n = 15$) were included.

TABLE I

Influence of water contacts on postinfection egg counts 15, 27, and 43 mo after oxamniquine treatment[a]

Mo after Treatment	Level of Water Contact			p[b]
	Low	Intermediate	High	
15	<8/64	<8/160	14/270	0.01
27	<8/150	<8/100	25/780	0.01
43	24/150	54/330	250/3600	<0.01

[a] Results, indicating intensity of infection (eggs/g), are represented as the median and the maximal egg counts for each water contact group.
[b] Kruskal-Wallis statistic test.

for the 10- to 19-yr age group, because there was no significant association between age and water contacts for these individuals (data not shown).

The percentage of infected individuals was significantly greater ($p < 0.01$) for adolescents with high water contact, 15 and 27 mo after treatment, than for those with low and intermediate water contact. The percentage of reinfected adolescents was not significantly different in the intermediate (60%) and high (75%) water contact groups 43 mo after treatment. The intensity of reinfection increased with water contacts 15, 27, and 43 mo after treatment (Table I). This finding is also illustrated in Figure 3 with egg counts 43 mo after treatment: adolescents excreting >25 eggs/g 43 mo after treatment represented 5, 60, and 73% of the subjects in the low, intermediate, and high water contact group, respectively. Most subjects with intermediate water contact had low to medium infections with egg excretion <200 eggs/g; none excreted more than 400 eggs. In contrast, most infected adolescents in the high water contact group excreted >200 eggs/g, and 28% had heavy infections (>400 eggs/g).

Reinfection in the group of adolescents with high water contact: evidences for different susceptibilities to infection. Among the 40 adolescents whose water contacts were high during the 43 mo of the study, 14 and 6 were reinfected within 15 and 27 mo of treatment, respectively (during the period of reduced parasite transmission). Infection intensities in 18 of these 20 subjects increased further during the third and fourth year, and 11 of 20 had heavy infections (range 400 to 2600 eggs/g)

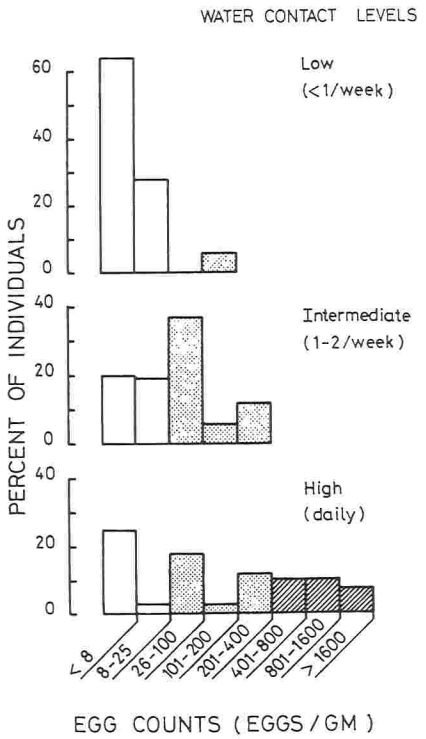


Figure 3. Influence of water contact levels on intensity of reinfection 43 mo after oxamniquine treatment. The number of adolescents in each group was: $n = 14$, low water contact; $n = 15$, intermediate water contact; $n = 40$, high water contact. Only subjects who did not change water contact group during the whole study period were included.

after 43 mo. Infection intensities remained low and stable in two individuals in the third and fourth year (Fig. 4*A*, *open circles*). Nine of the 20 adolescents who were not reinfected during the period of reduced parasite transmission, in spite of high water contacts, still had negative stool exams after 43 mo; nine developed low infections (<150 eggs/g) and two acquired medium level infections (<300 eggs/g). These findings are presented in Figure 4*A* and *B* together with pretreatment egg counts. *Panel A* corresponds to adolescents who were reinfected during the period of reduced parasite transmission. *Panel B* represents those who had negative stool examinations during that same period. Infection intensities before and 43 mo after treatment differ significantly ($p < 0.01$) between these two groups of adolescents: the geometric mean egg counts before and 43 mo after treatment were 8 to 10 times higher in group A than in group B.

Finally, egg counts of these two groups of adolescents for the whole study period are represented in Figure 5. Egg counts in groups A and B differed significantly ($p < 0.01$) at all points, except for 2.5 mo after treatment when no subject excreted eggs. These results altogether suggested that these two groups of adolescents have different susceptibilities to infection by *S. mansoni*.

Figure 6 illustrates further the different susceptibilities to reinfection among study subjects with observations made on adolescents from two families. These adolescents had very similar (high) water contacts and had living habits as similar as it is possible to find in such a study. Figure 6*A* shows the egg excretion patterns of two dizygotic twins (age 14), and *B* represents observations on two brothers (aged 14 and 15) and one sister (age 13). One child in each family had low or negative egg excretion

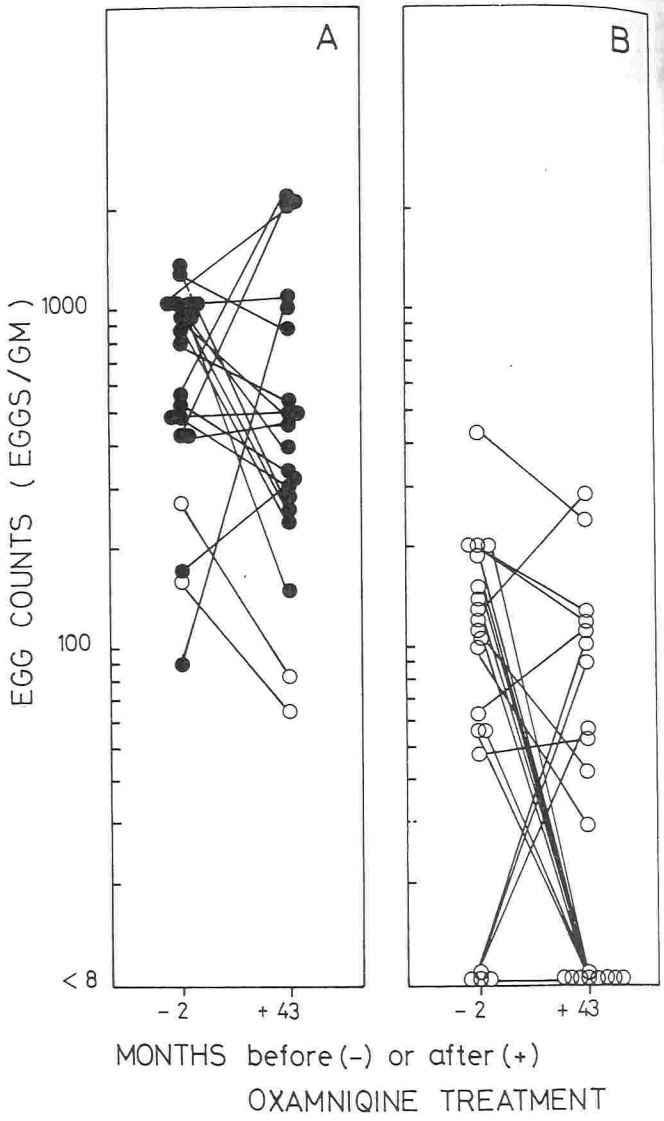


Figure 4. Infection levels before oxamniquine treatment and 43 mo after for adolescents with high water contact level ($n = 40$). Subjects who were or were not reinfected during the period of lowered parasite transmission (15 to 27 mo post-treatment) are represented in *A* and *B*, respectively. Open circles on *panel A* represent the two individuals with low and stable infections in the third and fourth year.

levels before treatment and for the 43 mo thereafter, whereas his brother (*family A*), or his brother and sister (*family B*), was (were) reinfected shortly after treatment and acquired high infections. Because two of these children (*family B*) were at risk of developing hepatosplenomegaly, they were treated again with oxamniquine 2 yr after the first drug administration; nevertheless, these children developed heavy infections within 16 (Fig. 6*B*) and 22 mo (data not shown) of this second treatment.

Schistosomular surface Ag identified by sera from subjects with low susceptibility to infection. One aim of this study was to determine whether the most resistant subjects had developed enhanced immunologic reactivity to certain *S. mansoni* Ag. Because studies in vitro and in laboratory animals have shown that schistosomula are likely to be a major target for antibody mediated protective immunity (reviewed in Reference 12), we assessed the reactivity of adolescent sera against larval Ag. In another study, to be published elsewhere, we observed

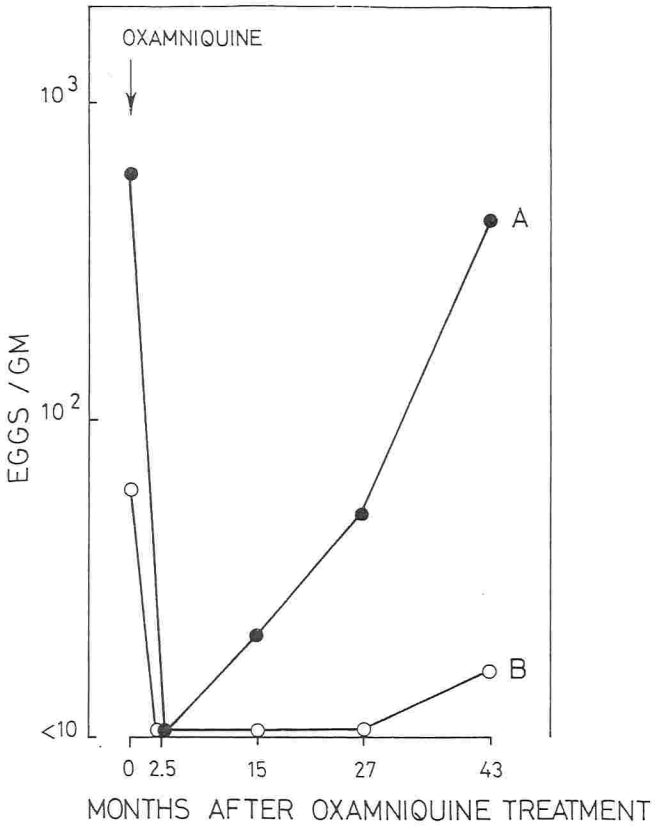


Figure 5. Egg excretion of the low and high susceptibility groups 2 mo before oxamniquine treatment and for the 43 mo after. The two curves differ significantly 2 mo before and 15, 27, and 43 mo after treatment ($p < 0.01$ Kruskal-Wallis statistic test).

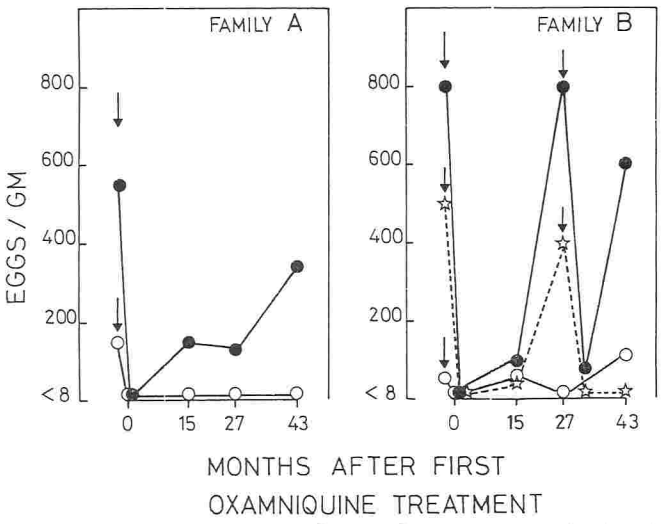


Figure 6. Susceptibility to reinfection after oxamniquine treatment among adolescents with comparable high water contact levels. Data from two male twins (aged 14), two brothers, and one sister (aged 13, 14, 15) are represented on *A* and *B*, respectively. Oxamniquine treatments are indicated by *arrows*.

no marked differences in the IgG antibody response of the most resistant and susceptible subjects when the sera were titrated by ELISA on schistosomular total membrane extracts. Because this approach may detect antibodies against the most abundant or the dominant Ag in the extracts, we also determined sera reactivity by methods allowing a separate evaluation of sera reactivity to the different schistosomular Ag. Adolescents with low and high susceptibility to infection were defined in Figure 4*A* and *B* and in Figure 5. Living schistosomula, labeled with the Bolton and Hunter reagent, were reacted at 4°C with sera from three subjects with low susceptibility to infection; unbound antibodies were washed out and the surface bound Ag-antibody complexes were extracted and precipitated with protein A. The precipitated Ag were characterized by acrylamide gel electrophoresis and autoradiography. This procedure identified several larval Ag with apparent molecular masses of 37, 41, 47, 52, 54, 72, 85, 90, 92, 135, 165, 202 kDa and a high molecular mass component that barely entered the gel (Fig. 7).

Then the reactivities for larval Ag of sera from subjects with different susceptibilities to infection were compared by immunoblots. Figure 8 shows two representative immunoblots that were reacted with sera collected 2 mo before (*A*) and 15 mo after (*B*) treatment. Boxes on the top of the gels indicate the susceptibility of the serum donors, open for low, closed for high susceptibility. Because certain subjects changed water contact groups during the study, their susceptibility to infection could not be defined (strips without boxes). Most immune sera reacted with a large number of schistosomular Ag in the 30- to 200-kDa range. Most of the bands on the blots corresponded in molecular mass to surface Ag identified in Figure 7, in particular, the dense bands 202, 165, 90 to 92, 85, 72, and 37 kDa. The Ag 202, 165, 85, 72, and 37 kDa reacted with sera of at least 30% of the most resistant subjects and were selected for further analysis (Fig. 9). Sera reacting with the 165-kDa Ag also reacted, although in some cases less strongly, with the 202-kDa Ag and several minor bands with apparent molecular mass in the 120- to 220-kDa range; experiments in progress indicate that these Ag bear cross-reactive determinants and may belong to the same family of molecules. Because sera reactivity against minor bands cannot be accurately assessed on immunoblots we only analyzed sera reactivity against the two major 202- and 165-kDa Ag.

Two Ag, 165 and 37 kDa, reacted with sera of >80% of the most resistant individuals and therefore represent a major target on schistosomula for IgG antibodies in these resistant individuals. The 72- and 85-kDa Ag reacted with the sera of 30 to 60% of these same individuals. Comparison of the pattern of reactivity of the most resistant and susceptible individuals shows that reactivity toward the 37-kDa Ag is associated ($p < 0.005$) with resistance because sera from 80 to 90% of the most resistant subjects reacted with this Ag vs 14 to 33% for the most susceptible individuals.

Sera reactivities to Ag 37, 72, and 165 kDa were also assessed by ELISA. Somular Ag were electroeluted from acrylamide gels, biotinylated, and reacted with avidin coated plates. These plates were used to titrate IgG antibodies in adolescent sera taken 15 and 43 mo after treatment (Fig. 10): no marked differences were observed between sera from the most resistant and the most susceptible subjects when titrated on plates coated with Ag 72 and 165 kDa. Although sera taken 15 mo post-treatment from the most resistant subjects were on average more reactive to the 72 kDa Ag than sera from the most susceptible subjects ($p = 0.05$), this difference was of small magnitude and was no longer observed with sera taken at 43 mo. ELISA was less sensitive than immuno-

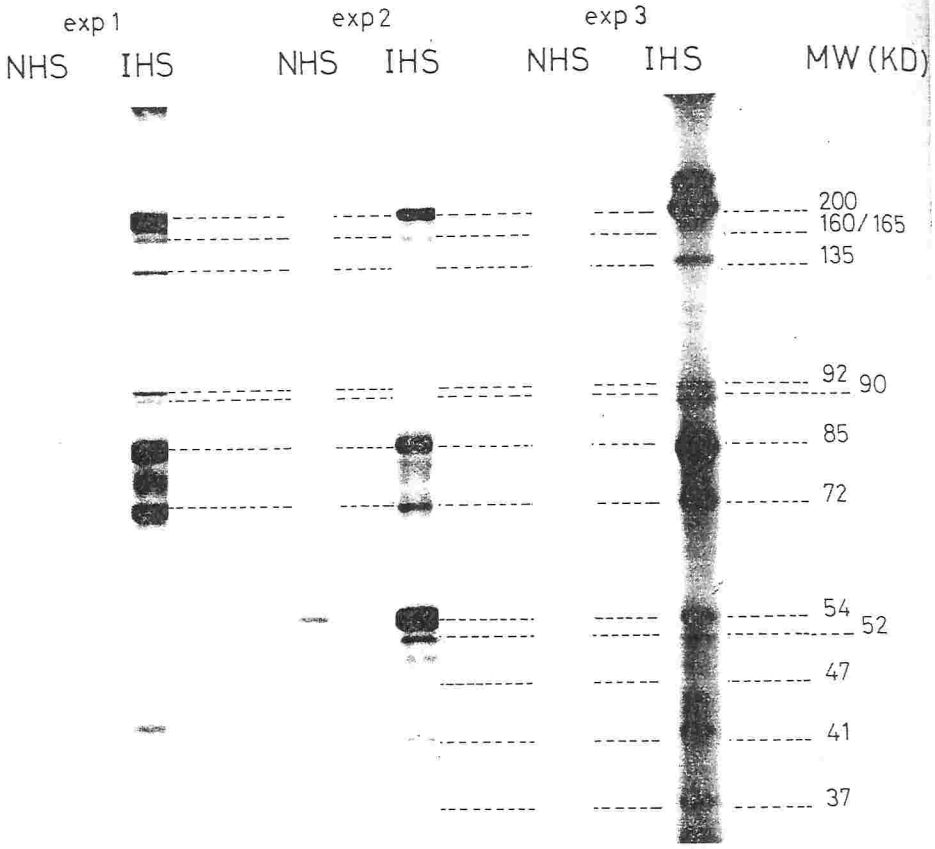


Figure 7. Schistosomular surface Ag identified by IgG from the most resistant subjects. Larvae labeled with the Bolton Hunter reagent were reacted with a 1/20 dilution of normal human serum (*NHS*) or sera from three resistant subjects (*IHS*) for 12 h at 4°C. The larvae were then washed three times and the larval bound immunocomplexes were extracted in 1% Nonidet P-40 with protease inhibitors and precipitated with protein A coupled to sepharose beads. The precipitates were characterized by electrophoresis on 7.5% acrylamide gels and autoradiography.

blots to detect antibodies to the 165-kDa Ag, possibly because of the low amount of Ag bound to the plates (10 to 50 times less than the amount bound to nitrocellulose) or because of modifications of certain epitopes by biotinylation.

Eight of 11 sera taken at 15 mo, and seven of 10 sera taken at 43 mo after treatment, from a total of 19 adolescents with low susceptibility to infection reacted strongly in ELISA with the 37-kDa Ag, whereas only one serum from 20 subjects with high susceptibility to infection exhibited a similar reactivity. Fourteen of 19 sera taken at 15 mo, and nine of 10 sera taken at 43 mo, from a total of 20 susceptible subjects, yield values within 2 SD of the values obtained with control sera.

Thus the results of ELISA confirmed the observations made on immunoblots and showed that sera from subjects with low susceptibility to infection demonstrate a higher IgG reactivity to the 37-kDa Ag than sera from individuals with high susceptibility.

DISCUSSION

The aim of this study was to assess the susceptibility to infection by *S. mansoni* of young residents of a hyperendemic area. Our goals were to identify subjects of low and high susceptibility to infection and, as a long term project, to evaluate the role of acquired immunity in human protection against *S. mansoni*.

This study was based on observations made in a hyperendemic area for *S. mansoni* that suggested that residents develop a certain resistance to infection during the first two decades of life (39–42). These observations included 1) the decrease of egg counts and prevalence after 15 to 20 yr of age that is not fully accounted for by a reduction in water contact levels at this age (40, 41); 2) the low occurrence of acute schistosomiasis among residents and its frequent occurrence together with heavy infections among immigrants recently settled in the infected area (41); 3) the differences in reinfection intensities, after treatment, among subjects with apparently similar habits with regard to infected waters (42).

Adolescents (aged 10 to 19) were selected for this study because this age period was likely to be a critical transition period for the development of resistance to *S. mansoni*, increasing the probability of encountering within the same age group subjects differing markedly in their ability to resist reinfection. Moreover, this age group develops the most severe infections and therefore runs the higher risks of developing hepatosplenomegaly (43, 44), allowing for the analysis of relationships between the mechanisms of resistance and those involved in the pathogenesis of severe schistosomiasis.

These adolescents were treated with oxamniquine to eliminate established infections. The effectiveness of this treatment, comparable with that reported in a previous study (45), was tested 2 to 2½ mo later. This length of time was sufficient to detect worms that would have been temporarily "silenced" and not destroyed by the drug. These adolescents were then followed for up to 43 mo with periodic stool examinations and assessments of their water contact levels. Any subjects excreting more than 400 eggs/g were given oxamniquine.

As expected from previous studies in the same area, the chemotherapy and snail control program did not interrupt parasite transmission: half of the adolescents

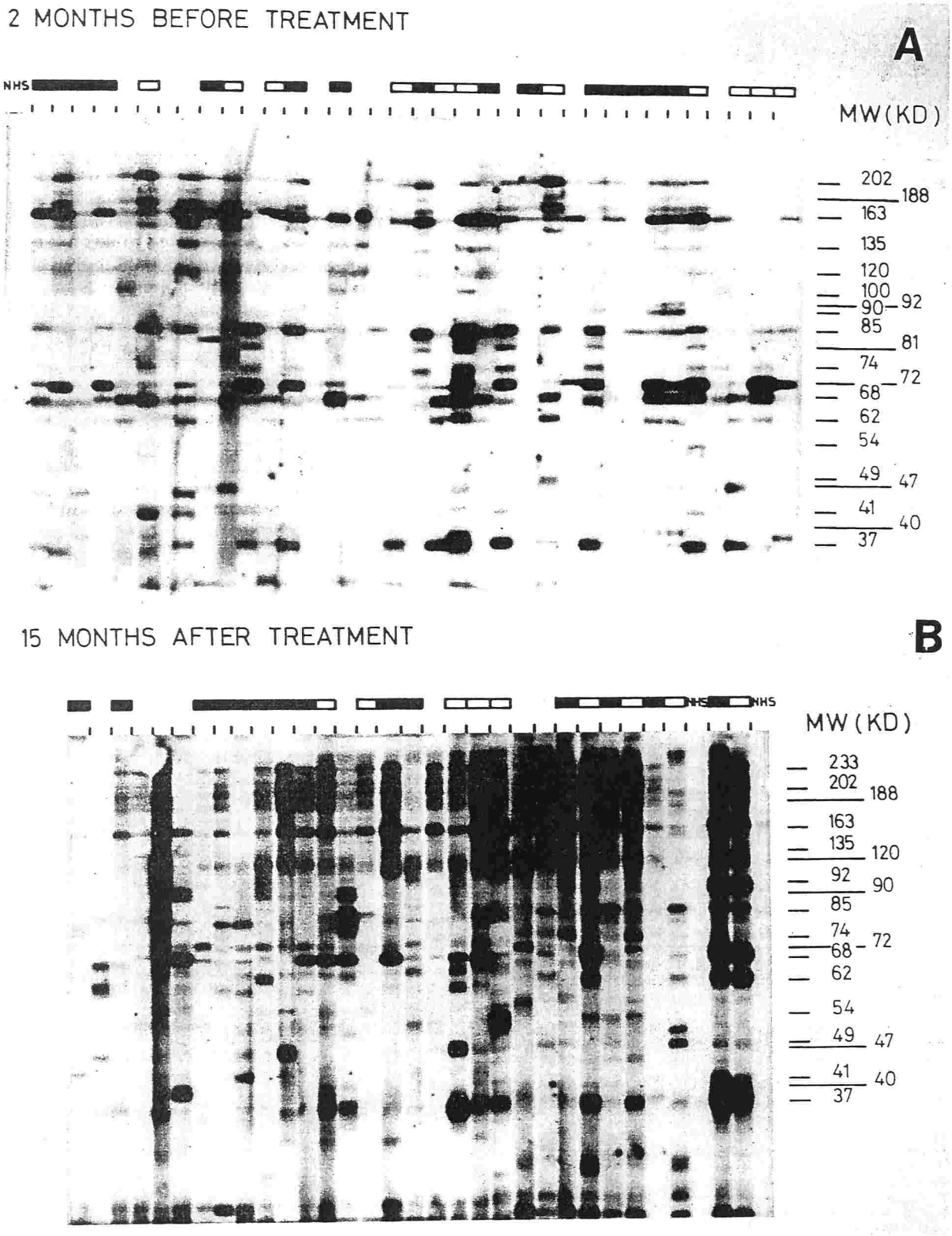
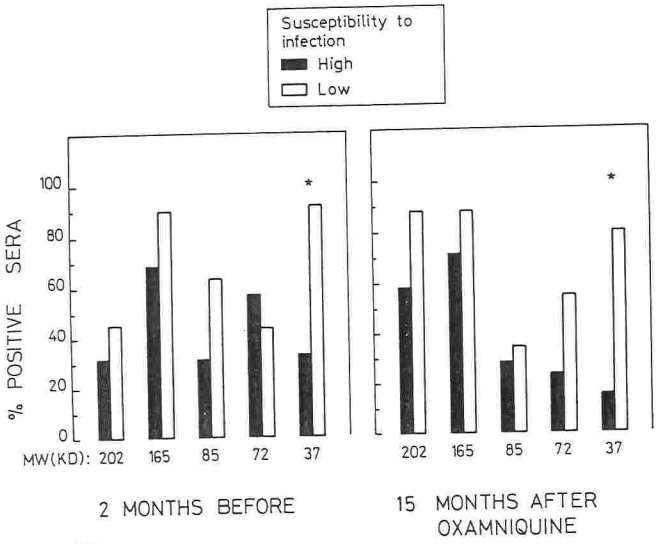


Figure 8. Western blot analysis of schistosomular Ag with sera from subjects with low and high susceptibility to infection. Sera were taken either 2 mo before (*A*) or 15 mo after (*B*) treatment. Low and high susceptibility to infection are indicated by an *open* or *closed box*, respectively. Strips without boxes were reacted with sera of subjects who changed water contact groups during the study.

were reinfected within 15 mo of oxamniquine treatment. Reinfection could have occurred as early as 3 to 4 mo after treatment because infection trials carried out with mice immersed in the river detected infective cercariae within 3 to 5 mo of the initiation of the program. Infection intensities remained low and stable for 27 mo and only



* $p < 0.01$

Figure 9. Percentage of sera reacting on western blots with Ag 37, 72, 85, 165, and 202 kDa in the low and high susceptibility groups. Results are from a total of 19 subjects with low susceptibility to infection 2 mo before ($n = 11$) and 15 mo after ($n = 12$) treatment, and 19 individuals with high susceptibility to infection 2 mo before ($n = 19$) and 15 mo after ($n = 15$) treatment; * $p < 0.01$ (χ^2 test).

increased in the third and fourth year, thus indicating that parasite transmission was low for the first 25 to 27 mo of the study and increased thereafter.

Data in Figure 3 and Table I show that reinfection depended markedly on water contact levels confirming that resistance studies should be performed with subjects having similar water contact levels. The high water contact group seemed the most suitable for this analysis of resistance: 1) it was the most homogeneous for water contacts, 2) frequent exposure to infected waters was likely to randomize cercarial "challenge" conditions, and 3) previous epidemiologic studies on other parasite diseases have suggested that resistance increases in subjects experiencing the highest rates of parasite challenge (46).

Three different reinfection patterns were observed among the adolescents with high levels of water contact: 1) Certain subjects were reinfected during the period of LPT[3] and acquired heavy infections at the end of the study, probably during a high parasite transmission period. 2) Certain individuals were not reinfected for the 43 mo of the study or were only reinfected at low levels, at 43 mo. 3) Few adolescents had a mixed pattern of infection; they were infected during the LPT period. Their egg counts, however, remained low and stable during the whole duration of the study, suggesting that these subjects were able to control additional infections.

Subjects who showed greater resistance to reinfection after treatment also had the lowest egg counts before treatment, whereas those who were reinfected during the LPT period and acquired heavy infection after 27 mo had the highest egg counts before treatment. These observations altogether indicate that these two groups (1 and 2) of patients have different susceptibility to infection and that some resistance had probably developed before the initiation of this study. It is also possible, however, that susceptibility to reinfection after treatment was influenced by previous infections; i.e., heavy infections could cause the production of blocking factors favoring host reinvasion, as suggested by others (25, 47).

A similar study on Kenyan schoolchildren also showed marked differences in susceptibility to reinfection among children living in hyperendemic areas (24). That study was performed over a 21-mo period with no marked modification of the condition of parasite transmission, because only a small fraction of the population was treated with oxamniquine and no snail control was attempted. The most resistant children had low or negative egg counts for the 21 mo after parasitologic cure, in spite of high water contact levels. The most susceptible children had egg counts >100 eggs/g (100 to 800) and low to

[3] Abbreviation used in this paper: LPT, lowered parasite transmission.

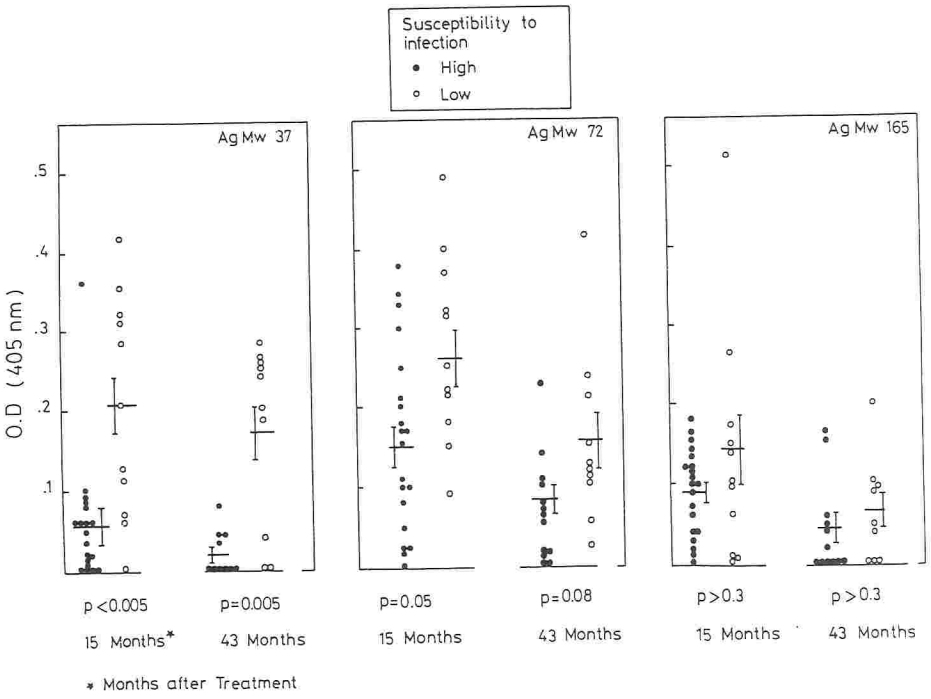


Figure 10. Reactivity of IgG from high and low susceptibility subjects for Ag 37, 72, and 165 kDa as assessed by ELISA. Plaques were coated with 100 ng/well of 37- and 72-kDa Ag and 50 ng/well of 165-kDa Ag. The results represented here were obtained at a 1:400 dilution of the sera from 19 low and 20 high susceptibility subjects taken 15 and/or 43 mo after treatment. Results are presented as the difference between OD_{405} (sample) and OD_{405} (control sera). Control sera were from two subjects with no previous contacts with infected waters. SD on OD measurements made with control sera varied from 0.015 to 0.025.

high water contact levels. This differs from our study, in which the most resistant and susceptible groups had comparable high water contact levels for reasons mentioned above. This may explain why no difference was observed between pretreatment egg counts of resistant and susceptible subjects in the Kenyan study (susceptible children with low water contact may not acquire high infections).

Because age and water contacts were not associated, at least for the 10- to 19-yr-old subjects of the study, the reduction of reinfection intensities with age suggests that resistance increases with age in the second decade of life. This was also the conclusion of Butterworth's study (24), in which a significant age difference between susceptible and resistant children was shown. We failed, however, to observe a significant age difference between the most resistant and susceptible adolescents; moreover, the data did not show an increase in resistance in the most susceptible group of subjects for the 4 yr of this study. This may indicate that resistance develops very slowly in these adolescents.

Although no conclusive evidence of protective acquired immunity has been reported in humans, it is generally thought that concomitant immunity produced by established, egg-laying infections plays an important role in human protection against *S. mansoni* (48–50). This study shows that a number of subjects resisted reinfection and had negative stool examinations for the 43 mo of this study. This indicates that effective human resistance to *S. mansoni* probably does not necessarily involve concomitant immunity-like mechanisms. This raises the possibility that aborted infections may be a sufficient stimulus to maintain resistance in certain subjects, as it has been reported in animal models vaccinated with irradiated cercariae (51 and reviewed in 12).

Animal studies and in vitro work with human material have shown that IgG and IgE antibodies, together with C or cytotoxic effector cells, take a significant part in the protection of animals against *S. mansoni* and could play a role of equal importance in humans (12, 41). Those studies have also shown that the young larvae of *S. mansoni* are very susceptible to antibody-mediated immune attack. For these reasons, we have analzyed the anti-schistosomular IgG and IgE antibody response of the most resistant and susceptible subjects. We reported here our findings on IgG antibodies: IgG in the sera of 30% or more of the most resistant subjects reacted with five schistosomular Ag 202, 165, 85, 72, and 37 kDa that were located on the surface of the larva and were bound by IgG on the intact schistosomula. These results were obtained with sera taken before treatment as well as 15 and 43 mo after treatment. As also noticed by Butterworth et al. in their study (24), the most resistant subjects who excreted no eggs for 43 mo after drug administration maintained high levels of antibodies against the larvae (this study and manuscript in preparation); this confirms that these individuals were regularly boosted by cercarial challenge.

Sera from susceptible subjects also reacted with the five Ag identified by the sera from the most resistant subjects on schistosomula. However, the proportion of sera from the most susceptible patients that reacted with the 37-kDa Ag in Western blot was significantly ($p < 0.01$) lower than for the sera from resistant subjects, i.e., 14 vs 80%, 15 mo after treatment. This finding was confirmed by ELISA using plates coated with the 37-kDa Ag electroeluted from acrylamide gels. These Ag preparations yield only one band when rerun on an acrylamide gel, and sera of mice immunized with three distinct 37-kDa Ag preparations reacted with only one band (37-kDa) on Western blots with schistosomular extracts and precipitated a 37-kDa Ag labeled by Bolton Hunter reagent on living schistosomula (data not shown). Experiments are presently being carried out to determine whether most "susceptible" subjects fail to produce high levels of IgG antibodies against the 37-kDa Ag or whether their sera contain specific factors that interfere with their detection by Western blotting and ELISA. It is unlikely, however, that immunocomplexes trapped IgG anti-37-kDa Ag in sera of susceptible subjects because sera taken 15 mo post-treatment, when infection intensities were low, did not show higher IgG reactivity against this Ag.

Several groups have described mAb directed toward a 38-kDa schistosomular surface Ag and capable of partially protecting mice or rats against *S. mansoni* infection (15, 16, 52, 53). Dalton et al. have also identified a 38-kDa Ag as a likely target of protective immunity in mice vaccinated with irradiated cercariae (54). More recently, blocking antibodies against the 38-kDa Ag that react with mAb have been detected in the sera of susceptible Kenyan children (47). We are presently testing whether the Ag identified in this study and the Ag identified by mAb are different or are the same molecule. We know already that the 37-kDa Ag does not disappear during parasite development and is synthesized by adult schistosome. Preliminary observations indicate that mice immunized with this purified Ag are less susceptible than control animals to infection by cercariae.

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