

# IMMUNITY IN HUMAN SCHISTOSOMIASIS MANSONI

## Regulation of Protective Immune Mechanisms by IgM Blocking Antibodies

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Evidence is accumulating that antibody dependent, cell-mediated cytotoxicity (ADCC) plays a critical role in the mechanisms of defense against schistosomes (1, 2). In several experimental models and in the human situation, the participation of phagocytic cell populations, including macrophages, eosinophils, and platelets, has been demonstrated (3–6). Killing in vitro of schistosomula, the main targets of immune attack, is observed when these cells interact with the parasite in the presence of specific antibodies (3, 5–9).

During experimental rat schistosomiasis, or during human infection, large variations in the cytotoxic activity of eosinophils attributable to immune complexes may occur (7, 10), and it has been shown (10) that immune complexes isolated from rat infection serum can either activate or inhibit schistosomulum killing by normal eosinophils. More recently, the production of *Schistosoma mansoni*-specific mAbs has allowed an alternative explanation for variations in the capacity of different sera to support killing (11). A rat IgG2c mAb (IPLSm3), which did not exhibit any killing activity for schistosomula, specifically inhibited the eosinophil-dependent cytotoxicity mediated by an IgG2a mAb (IPLSm1) (11). The blocking effect of IgG2c was dual, both at the surface target antigen ( $M_r$  38,000) and at the effector cell level, by competition for the Fc $\gamma$  receptor (12, 13). IgG2c antibodies were also able to inhibit the in vivo protection conferred by IgG2a mAbs.

The demonstration of such mechanisms in the rat raises the question of the possible existence in human schistosomiasis of blocking antibodies modulating the efficiency of immune effector mechanisms (14). To test such a hypothesis, we have studied a group of infected children who are involved in epidemiologic and immunologic studies of schistosome infection (15, 16). Among this group, two subgroups have been distinguished. The first, one of 22 children showing

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high reinfection intensities (>100 eggs per gram of feces), were considered to be susceptible: the second, of 35 children who showed high levels of water contact but low reinfection intensities (<30 eggs per gram of feces), were considered resistant. Comparison of various parameters of the immune response in the two subgroups, including antibody-mediated eosinophil-dependent killing of schistosomula, antischistosomulum IgE antibodies, and eosinophil levels measured as an indirect estimate of eosinophil functional activity, showed no significant differences (16).

In the present work, we demonstrate the existence of blocking antibodies capable of inhibiting eosinophil effector function in human infection with *S. mansoni*, as previously shown for rat experimental schistosomiasis (11). A detailed statistical study by Butterworth et al.<sup>1</sup> suggests that these blocking antibodies may prevent the expression of immunity in young children.

### Materials and Methods

**Human Sera.** Human sera were obtained from *S. mansoni*-infected patients before and after treatment with oral oxamniquine (30 mg/kg). Subjects were living in an endemic area in Kenya (Machakos district), which has already been described in previous papers (15, 16). Normal human sera were prepared from *S. mansoni*-uninfected healthy donors. All sera were stored in frozen aliquots at  $-70^{\circ}\text{C}$ . When indicated, the sera were heated for 2 h at  $56^{\circ}\text{C}$ .

**Indirect Fluorescence Assay.** Surface-binding antibodies were detected by indirect fluorescence on sections of *S. mansoni* schistosomula prepared as previously described (17). The sections were overlaid either with total serum or fractions at various dilutions. After a 30-min incubation and three washings in PBS, FITC-conjugated anti-human immunoglobulin antisera at 1:20 final dilutions were added ( $\alpha$ ,  $\mu$ , or  $\gamma$  chain-specific; Cappel Laboratories, Cochranville, PA) for an additional 30 min. The slides were washed three times in PBS and counterstained with Evans blue (1:10,000 final dilution). Normal human sera or medium were used as negative controls. The sections were examined under fluorescence microscopy.

**Serum Absorption.** 100  $\mu\text{l}$  of serum diluted (final dilution 1:5) in MEM with penicillin (100 U/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ) were mixed in tubes with 100 mg protein A-Sepharose (PAS)<sup>2</sup> (Pharmacia Fine Chemicals, Uppsala, Sweden) previously swollen and equilibrated with medium. Tubes were loaded on a test tube rotator and agitated overnight at  $4^{\circ}\text{C}$ . The unabsorbed fraction (PAS effluent fraction) was recovered by centrifugation at 600 g for 10 min and tested as such in indirect fluorescence and cytotoxicity assays. The bound IgG fraction was washed extensively and eluted with 0.5 ml 1 M glycine, pH 2.8 (PAS eluate fraction) (18). The aliquots were immediately brought to neutral pH with 10 N NaOH dialyzed against PBS for 3 h and overnight against MEM.

**Serum Fractionation (Ig Isotype Separation).** A fast protein liquid chromatography (FPLC) (Pharmacia Fine Chemicals) system was used for separation of IgM and IgG from human sera (19). Briefly, after priming the gel filtration column (Superose 6) with PBS (50 mM, pH 7.2), 100  $\mu\text{l}$  of serum was filtered through a 0.2- $\mu\text{m}$  filter and loaded onto the column by a V-7 valve. The operating pressure was 1 millipascal, and the flow rate was set at 0.5 ml/min. The fraction collector was programmed to collect 1-ml samples (Fig. 1 A). Ig isotype was determined by solid-phase RIA on plates coated with anti-human immunoglobulin antisera specific for  $\gamma$  or for  $\mu$  chain (Cappel Laboratories) (Fig. 1 B).

<sup>1</sup> A. E. Butterworth, R. Bensted-Smith, A. Capron, M. Capron, P. R. Dalton, D. W. Dunne, J. M. Grzych, H. C. Kariuki, J. Khalife, D. Koech, M. Mugambi, J. H. Ouma, T. K. arap Siongok, and R. F. Sturrock. Immunity in human schistosomiasis mansoni. Prevention by blocking antibodies of the expression of immunity in young children. Manuscript submitted for publication.

<sup>2</sup> Abbreviations used in this paper: FPLC, fast protein liquid chromatography; PAS, protein A-Sepharose.

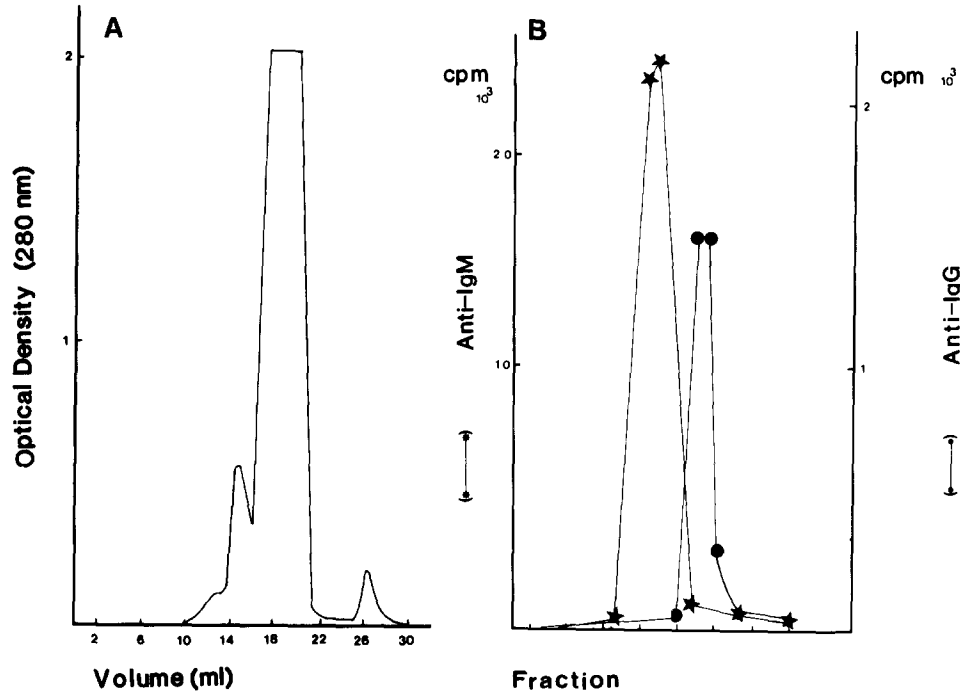


FIGURE 1. IgG and IgM separation from human sera by FPLC. (A) elution profile from the chromatography column. (B) detection of IgM and IgG fractions by RIA. Plates coated with anti-human IgM or anti-human IgG were incubated with 100  $\mu$ l of each fraction. After 2 h of contact, wells were washed and then incubated with  $^{125}$ I-labeled anti-IgG or anti-IgM for 1 h at 37°C and overnight at 4°C.

**Cytotoxicity Experiments.** Sera were tested for their levels of heat-stable antibodies mediating eosinophil-dependent damage to *S. mansoni* schistosomula according to methods described elsewhere (16). Eosinophils were prepared from eosinophilic individuals by centrifugation on discontinuous metrizamide gradients. Cell fractions collected from the 24–25% metrizamide layers were used in these assays. The experimental procedure for the cytotoxicity assay was similar to that previously described (5). Briefly, 50  $\mu$ l of the schistosomula suspension (1,000 schistosomula/ml in MEM/NHS [heat-inactivated normal serum]) were incubated either with 50  $\mu$ l of heat-inactivated total human serum samples or fractions at various dilutions. The effector cell suspension containing >90% eosinophils at an E/T ratio of 5,000:1 was then added. In some experiments, schistosomula were preincubated with PAS effluents for 2 h at 37°C. After this incubation period, the schistosomula targets were washed twice with medium before use in the cytotoxicity assay. The percentage of cytotoxicity was evaluated after 48 h contact by microscopic examination.

**Immunoprecipitation.** Schistosomulum proteins were radioiodinated using the lactoperoxidase technique (20). Surface antigens were extracted by treatment of labeled parasites with 0.5% NP-40 in Tris-HCl, pH 6.8, containing 100 U/ml aprotinin for 30 min at 0°C ( $10^5$  parasites per 0.5 ml), and immunoprecipitated with antibodies present in immune sera as previously described (21). Briefly, aliquots corresponding to 500 schistosomula were incubated with 10  $\mu$ l of serum for 2 h at 4°C and then transferred into tubes containing 10 mg of PAS previously swollen and equilibrated in adsorption buffer (5 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2% Triton X-100, and 100 U/ml aprotinin). PAS was sensitized with anti-human IgM before the addition of antigen-antibody complex to identify the antigen recognized by IgM antibodies. In all experiments,

tubes were agitated overnight at 4°C in a test tube rotator. The Sepharose beads were then washed with 3 ml of 5 mM Tris-HCl, 0.15 M NaCl and 3 ml of 5 mM Tris-HCl, pH 7.4. Fixed complexes were eluted with 40 µl of sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% sucrose, and 0.005% bromophenol blue) at 100°C for 3 min. The eluted labeled antigens were identified by SDS-PAGE, and autoradiography was performed according to the method described elsewhere (21).

**Radioimmunoassay.** Sera and Ig fractions (IgG, IgM) were tested for their capacity to inhibit the binding to antigen of two rat mAbs against the 38,000 *M<sub>r</sub>* schistosomulum surface antigen (11). Inhibition of binding of radiolabeled IgG2a (IPLSm1) and IgG2c (IPLSm3) mAbs was performed according to the technique previously described (22). Briefly, polyvinyl chloride (PVC) plates precoated with rat IgM (C<sub>3</sub>-109) mAb recognizing the 38,000 *M<sub>r</sub>* antigen were incubated with 100 µl of schistosomula NP-40 extract (1 mg/ml). After a 2-h exposure at 37°C, plates were washed twice in PBS/0.1% BSA buffer. For the inhibition test, 100 µl of sera or Ig fractions (1:10 final dilution) were added. After 2 h incubation at 37°C and three washings, plates were incubated with <sup>125</sup>I-labeled IgG2a or IgG2c for 1 h at 37°C, and overnight at 4°C, and the plates were then washed three times.

The crossinhibition between the Ig fractions purified from human sera by FPLC system was performed as described above. Wells precoated with schistosomulum NP-40 extract were preincubated with unlabeled IgM or IgG fraction for 2 h at 37°C. After three washings, plates were incubated with the homologous human <sup>125</sup>I-IgG or -IgM fraction (labeled by the chloramine T method; 1 µg/µCi) (23). After 1 h at 37°C and overnight at 4°C, the plates were washed three times. In all experiments, the wells were counted in a gamma counter. Percent inhibition of binding was calculated as  $[100 \times (\text{cpm without inhibitor}) - (\text{cpm in the presence of inhibitor})] / (\text{cpm without inhibitor})$ .

**Determination of IgM Antibodies.** A double-sandwich ELISA was used to detect IgM antibodies against the 38,000 *M<sub>r</sub>* antigen (24). Microtiter plates coated with anti-human IgM antibodies (Institut Pasteur Production, Marnes La Coquette, France) were washed with saline/0.4% Tween and incubated for 3 h at room temperature with 200 µl of serum diluted in PBS. After three washings, captured human IgM was incubated with 200 µl schistosomulum NP-40 extract (100 µg/ml) overnight at 4°C. The wells were then washed with saline/Tween and incubated for 3 h at 4°C with 200 µl of β galactosidase-conjugated IgG2a mAb. Substrate containing 0.014 M 2-nitrophenyl β-D-galactopyranoside (Merck, Darmstadt, Federal Republic of Germany), 0.003 M MgCl<sub>2</sub>, and 0.1 M 2-ME (Sigma Chemical Co., St. Louis, MO) was added to the test wells after a wash step, and the plates were incubated for 1 h at 45°C. The reaction was stopped by adding 500 µl of 1 M Na<sub>2</sub>CO<sub>3</sub>, and the absorbance was measured at 405 nm using a Titertek Multiskan (Flow Laboratories, McLean, VA). Each test was assessed in duplicate. Results are expressed as mean absorbance values at 405 nm.

**Statistical Comparisons.** The statistical significance of differences between means were analyzed by using Student's *t* test.

## Results

**Characterization of Antibodies Binding to Schistosomula.** Intense membrane fluorescence with FITC-anti-human IgG (Fig. 2A) or with FITC-anti-human IgM (Fig. 2B) was observed after incubation of schistosomulum sections with total serum from infected patients (*n* = 53; final dilution, 1:50). Occasional and weak fluorescence was seen with FITC-anti-human IgA (final dilution, 1:5). Identical fluorescence patterns persisted in the presence of the corresponding IgG or IgM antibodies fractionated on PAS or FPLC system. No crossreaction was observed between the purified fractions throughout the course of experiments. Control sections incubated with human normal serum or with Ig fractions purified from normal serum showed no reactivity either with FITC-anti-human IgG or with FITC-anti-human IgM.

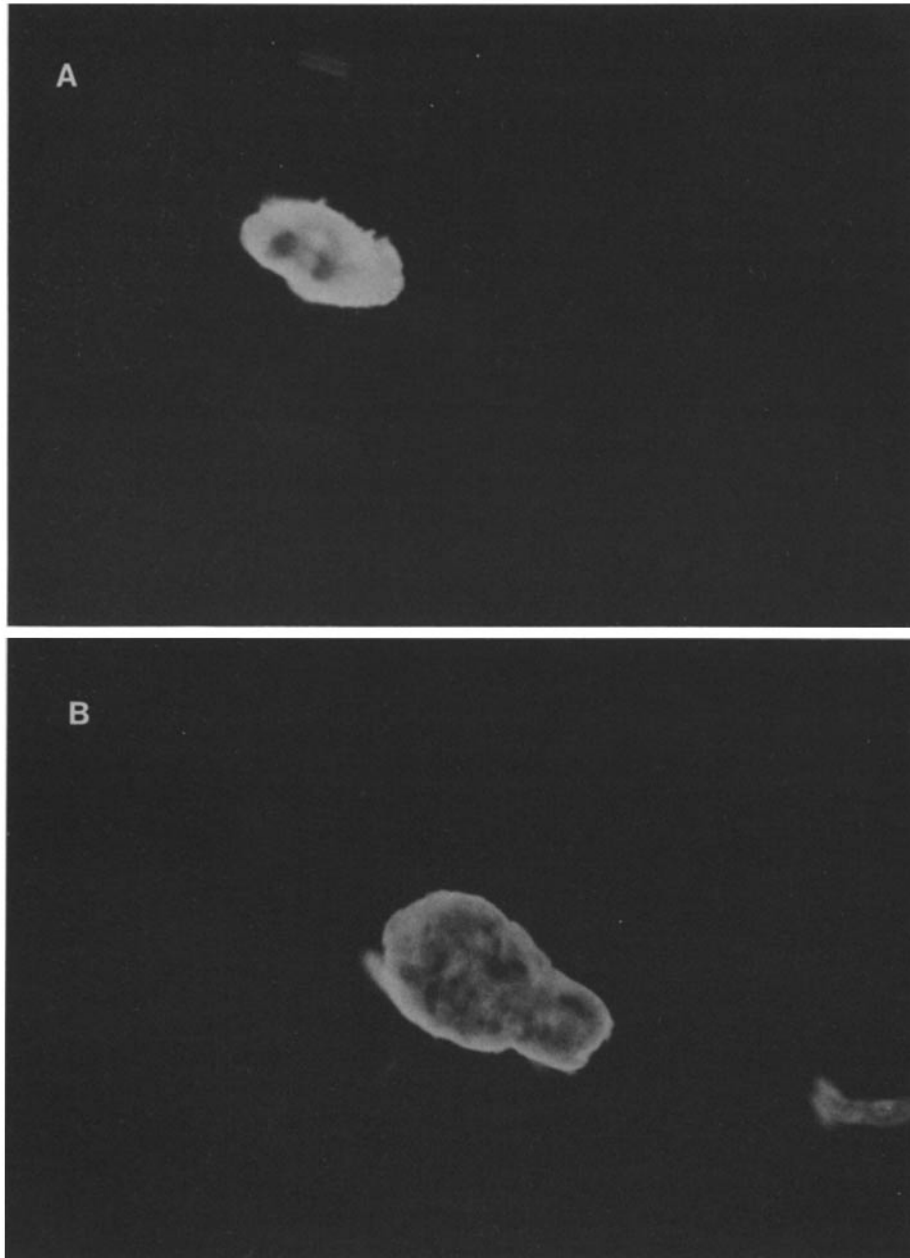


FIGURE 2. Localization of the antigenic sites recognized by total serum and Ig fractions. (A) indirect fluorescence on sections of *S. mansoni* schistosomula incubated with total serum and revealed by FITC-anti-human IgG. (B) same as A using FITC-anti-human IgM.

*Antibodies Mediating Eosinophil-dependent Killing of Schistosomulum.* To determine the antibody isotype required in the cytotoxicity assay, 20 infected sera and 6 uninfected sera were fractionated either by absorption on PAS or by Ig separation on chromatography column (FPLC system). Each fraction was con-

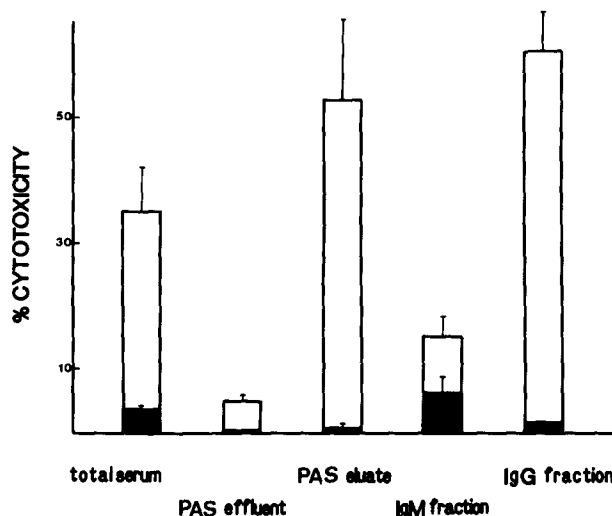


FIGURE 3. Eosinophil-dependent killing of *S. mansoni* schistosomula by various fractions of immune sera. The percentage of cytotoxicity was evaluated after 48 h of contact with eosinophils. Sera or fractions obtained by PAS or FPLC separation (1:20, final dilution) were heat inactivated for 2 h at 56°C. Open bars, immune serum; filled bars, normal serum (mean of six experiments +SD).

trolled by indirect fluorescence assay and tested for their capacity to mediate eosinophil-dependent killing of parasite larvae (Fig. 3). Detectable levels of cytotoxicity were observed when *S. mansoni* schistosomula were incubated with eosinophils purified from individuals with moderate eosinophilia in the presence of heat-inactivated total immune sera. When the serum was fractionated, the cytotoxic activity was detected in the IgG-containing fractions (PAS eluate fraction or purified IgG fraction). Moreover, the IgG fractions produced significantly higher levels of cytotoxicity than the total sera, when tested in the same conditions ( $p < 0.01$ ). Apart from some direct toxicity ( $<5\%$ ), no cytotoxic effect was observed either in the presence of PAS effluent fractions or with IgM fraction purified by FPLC. The control Ig fractions from normal serum did not lead to any significant killing activity.

**Inhibitory Activity of IgM Antibodies.** Since IgM antibodies were able to bind to target surface, it was interesting to study their activity in eosinophil-dependent cytotoxicity mediated by IgG antibodies. *S. mansoni* schistosomula were preincubated with various dilutions of PAS effluents for 2 h at 37°C. After two washings, total immune sera or PAS eluates and eosinophils were added. The percentage of cytotoxicity was compared to the killing activity induced by total immune serum or by PAS eluate fraction after preincubation of schistosomula with medium. As shown in Table I, preincubation of schistosomula with PAS effluents of immune sera markedly inhibited the IgG-dependent cytotoxicity mediated by total immune serum or PAS eluates in a dose-dependent manner ( $p < 0.01$ ). To rule out a possible nonspecific inhibition, schistosomula were preincubated with PAS effluents of normal serum. Results presented in Table I showed no inhibitory effect after the addition of total immune serum. Preincu-

TABLE I  
*Inhibitory Role of IgM-containing PAS Effluents on IgG-dependent Cytotoxicity  
 by Human Eosinophils*

Preincubation (2 h at 37°C)	Incubation (final dilution)	Percent cytotoxicity
Medium	Total normal serum	2.7 ± 0.0
Medium	Total immune serum (1:20)	36.5 ± 10.0
Medium	PAS effluent* immune serum (1:20)	4.9 ± 0.6
Medium	PAS eluate† immune serum (1:20)	45.5 ± 9.9
PAS effluent immune serum (1:10)	Total immune serum (1:20)	8.2 ± 3.9
PAS effluent immune serum (1:30)	Total immune serum (1:20)	20.4 ± 4.6
PAS effluent immune serum (1:10)	PAS eluate immune serum (1:20)	11.1 ± 1.5
PAS effluent normal serum (1:10)	Total immune serum (1:20)	30 ± 5.2
PAS effluent immune serum (1:10)‡	Total immune serum (1:20)	35.7 ± 6.5

Schistosomula were preincubated for 2 h at 37°C and washed twice with medium. The homologous serum or fractions were then added. Percent cytotoxicity was measured after 48 h contact with eosinophils (mean ± SD of seven experiments).

\* PAS effluent represents the unbound fraction to PAS.

† PAS eluate represents the bound fraction to PAS eluted with glycine (1 M).

‡ Eosinophils were preincubated with PAS effluent and then washed with medium.

bation of eosinophils instead of target incubation with PAS effluents of immune serum did not affect the killing activity of total immune serum.

*Antibody Response to Schistosomula Surface Antigens.* The target specificity of IgG and IgM antibodies was determined. Immunoprecipitation of <sup>125</sup>I-labeled schistosomulum antigens from infected human serum using protein A-binding antibodies (IgG antibodies) revealed 30,000–40,000 *M<sub>r</sub>* antigens (100% of sera tested; *n* = 17) (Fig. 4). However, additional bands in the 20,000–25,000 *M<sub>r</sub>* range were revealed (66% of sera tested). Occasional faint bands were also detected in the upper region of the gel (>67,000) (41% of sera tested). Surface antigens precipitated by IgM antibodies from infected human serum ranged from 30,000 to 40,000 *M<sub>r</sub>* (100% of sera tested) and also between 20,000 and 25,000 *M<sub>r</sub>* (20% of sera tested). However, the predominant IgM antibody response was against the 32,000 *M<sub>r</sub>* antigen. Adsorption experiments, in which the detergent extract was previously adsorbed by protein A-binding IgG antibodies demonstrated a crossinhibition at the level of the whole antigen between IgG and IgM antibodies (Fig. 4, lane 5).

*Epitopes Recognized by IgG and IgM Antibodies.* To investigate the respective epitopes recognized by IgG and IgM antibodies, we used an RIA in which the capacity of IgG and IgM fractions to inhibit antigen binding of two rat mAbs (IgG2a and IgG2c) was tested. These mAbs (IPLSm1 and IPLSm3) recognize the 38,000 *M<sub>r</sub>* antigen. This antigen was bound to plates by the use of a different rat mAb (C3-109) that does not interfere with the binding of either IPLSm1 or IPLSm3. Preincubation of wells precoated with schistosomulum antigen in the presence of IgG or IgM fractions of infected serum inhibited both the binding of <sup>125</sup>I-labeled IgG2a and IgG2c (Table II). In the same conditions, total serum led to a twofold inhibition, when compared with the inhibition induced either by the IgG or by the IgM fractions. The specificity of the epitopes recognized by IgG and IgM antibodies in infected serum was tested by crossinhibition

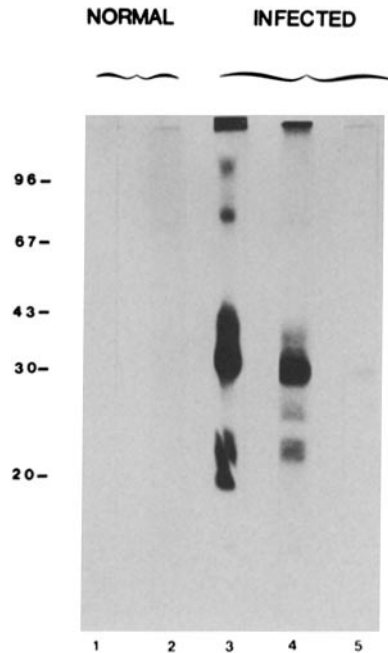


FIGURE 4. Immunoprecipitation of schistosomulum surface antigens by immune sera. Detergent extracts of 500 labeled skin schistosomula were incubated with 10  $\mu$ l of total serum or 100  $\mu$ l of PAS effluent fraction. Immune complexes of total serum were absorbed on PAS and were analyzed, in lanes 1 and 3. Protein A-unbound material from lane 3 was further incubated with PAS effluent fraction, and immune complexes were analyzed in lane 5. The immune complexes of PAS effluent fraction were absorbed on PAS previously sensitized with 10  $\mu$ l (1 mg/ml) of anti-human IgM (lanes 2, 4, and 5).

TABLE II  
Inhibition of Binding to Schistosomulum Antigen of Two Rat mAbs  
Directed against the 38,000 M<sub>r</sub> Antigen

Preincubation	Percent inhibition		n <sup>‡</sup>
	Labeled IgG2a*	Labeled IgG2c*	
IgG fraction <sup>§</sup>	30 ± 2.0	38 ± 5.7	17
IgM fraction <sup>§</sup>	23 ± 1.9	33 ± 3.7	17
Total serum	55.8 ± 2.9	54.6 ± 4.1	17

Plates precoated with antigen were preincubated with IgG, IgM fraction, or total serum from infected patients (1:10 final dilution) for 2 h at 37°C. Percent inhibition was calculated as described in Materials and Methods (mean ± SD). Inhibition by Ig fraction of normal serum or total normal serum has been subtracted.

\* Purified anti-*S. mansoni* IgG2a and IgG2c mAbs were labeled by <sup>125</sup>I by the chloramine T method.

‡ Number of sera tested.

§ IgG and IgM fractions were obtained from total serum by fractionation with an FPLC system.



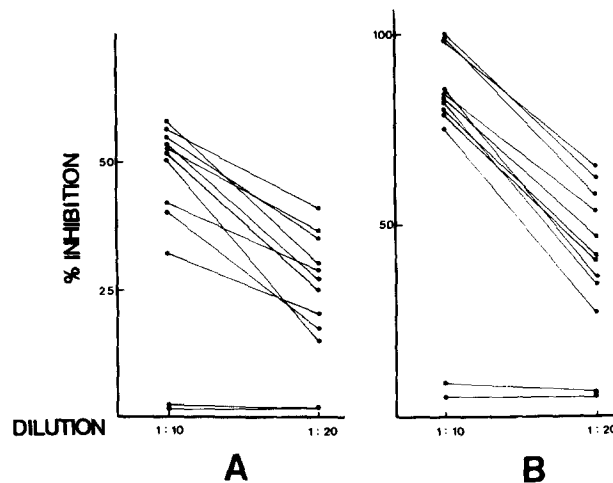


FIGURE 5. Crossinhibition of binding to antigen of IgG and IgM fractions from immune sera. Schistosomulum antigen bound to plates by using a rat mAb (C3-109) were preincubated with IgG fraction (A) or with IgM fraction (B). After 2 h of contact and three washes, homologous labeled IgM or IgG were added to A and B, respectively, and incubated for 1 h at 37°C and overnight at 4°C. Results are presented as percent inhibition (see Material and Methods) (mean of duplicate  $\pm$ SD). Dots represent the inhibition between the Ig fractions of immune sera; stars represent the inhibition of Ig fractions of infected sera by Ig fractions of normal sera.

experiments. As shown in Fig. 5 A, the preincubation of antigen-coated plates in the presence of IgG fraction inhibited the binding of homologous  $^{125}$ I-labeled IgM antibodies. In the same conditions, higher levels of inhibition were obtained when unlabeled IgM antibodies were preincubated before labeled IgG (Fig. 5 B), suggesting higher avidity for the targets.

*Role of These IgM Antibodies in Immunity to Reinfection.* The biological relevance of such IgM blocking antibodies was assessed by the evaluation of anti-38,000 IgM antibodies in the sera from individuals classified as resistant or susceptible to reinfection using previously defined criteria (16). Such antibodies were measured in an IgM capture assay (see Materials and Methods) in order to avoid competition with the IgG antibodies. Results presented in Table III showed that the mean levels of IgM antibodies in susceptible individuals were significantly higher than those present in resistant subjects in the pretreatment and 12-mo serum samples, but did not differ significantly at 5 wk after treatment. For the susceptible population (defined previously [16] as those individuals having >100 eggs per gram of feces) there was a positive association between the level of IgM antibodies in the pretreatment blood sample and the number of eggs at 12 mo after treatment. In addition, there was a positive association at any given time between IgM antibody levels and the actual egg counts at that time.

## Discussion

The numerous studies concerning the effector mechanisms directed against *S. mansoni* schistosomula have shown that eosinophils can act as killer cells in the presence of specific antibodies (1, 2). The factors directly involved in such

TABLE III  
*Relationship between Presence of IgM Antibodies against the 38,000 Schistosomulum Surface Antigen and Status of Immunity to Reinfection*

Blood sample	Before treatment	After treatment	
		5 wk	12 mo
Susceptible			
Optical density	1.5 ± 0.09* (19)	0.97 ± 0.12 <sup>‡</sup> (21)	1.38 ± 0.11 <sup>§</sup> (21)
eggs per gram	206	7	133
Resistant			
Optical density	1.19 ± 0.1* (34)	0.72 ± 0.1 <sup>‡</sup> (24)	0.77 ± 0.07 <sup>§</sup> (32)
eggs per gram	110	0	8

Sera were selected from susceptible and resistant individuals using previously defined criteria. IgM antibodies were measured by a capture assay (see Materials and Methods). Preliminary studies, in which varying concentrations of the serum (1:100–1:12,800) were used, indicated that a final dilution of 1:6,400 would be the highest dilution to which the normal and immune serum showed a significant difference. Results are presented as mean ± SEM. Number of sera used is shown in parentheses. Egg counts were calculated by examination of duplicate Kato preparations (geometric mean).

\*  $p < 0.01$  between the two groups.

<sup>‡</sup>  $p > 0.05$  between the two groups.

<sup>§</sup>  $p < 0.001$  between the two groups.

mechanisms include IgG and IgE antibodies (5, 7). However, in previous epidemiological studies (16) of human immunity to schistosome infection, comparison of the levels of IgG and IgE antischistosomulum antibodies between two groups classified as resistant and susceptible to reinfection, respectively, showed no significant difference. The demonstration in experimental models that defined antibody isotypes might block the expression of immunity prompted us to investigate the presence of such blocking antibodies leading to prevention of a given effector mechanism during human schistosomiasis.

Evidence from fluorescence studies on schistosomulum sections indicated the presence of IgG and IgM antibodies specifically directed against the schistosomulum surface in sera from infected patients. As previously reported (7), in vitro studies of the effector function of heat-inactivated immune serum revealed significant levels of eosinophil-dependent cytotoxicity against *S. mansoni* schistosomula. In the same conditions, when serum samples were fractionated and tested for their killing capacity, it was found that the activity was associated with IgG antibodies eluted from protein A, whereas no cytotoxic activity was detected in the PAS effluent preparation of IgM antibodies. Comparable results have been obtained with IgG and IgM antibodies fractionated by FPLC. Moreover, the IgG fraction produced significantly higher levels of cytotoxicity than total immune serum. These results confirm a previous report (7) in which the authors demonstrated the involvement of IgG antibodies. They suggest, in addition, that the depletion of some inhibitory factor not retained on protein A led to an increase in IgG-mediated killing.

The experiments showing that IgM-containing fractions were unable to exhibit any antiparasite killing activity in the presence of eosinophils, together with the demonstration (11) of blocking antibodies in rat experimental schistosomiasis,

led us to investigate the possible inhibitory role of IgM antibodies in eosinophil-dependent cytotoxicity mediated by IgG antibodies. Preincubation of schistosomula with PAS effluents or IgM-containing fractions of immune serum strongly inhibited the cytotoxicity mediated by total immune serum or PAS eluate fractions (IgG antibodies). The absence of inhibition with IgM purified from normal serum confirmed that the blocking effect was related to the antiparasite antibody specificity of IgM. In contrast, these IgM antibodies were unable to inhibit the killing capacity dependent upon specific IgE anti-*S. mansoni* antibodies and eosinophils (data not shown). The interpretation of these observations is that IgM blocking antibodies were crossreactive with the same target antigen as IgG antibodies, and that IgE antibodies were directed against another antigen, as previously described in rat schistosomiasis (25). In this context, previous studies (26) reported in human schistosomiasis showed the presence of blocking antibodies of IgG isotypes able to inhibit the histamine release induced by IgE-sensitized basophils. It was interesting to note that several investigations (27, 28) have demonstrated the presence of such blocking antibodies (IgG and IgA isotypes) in malignant diseases. More recently, in rat experimental schistosomiasis, it was shown (11, 12) that blocking antibodies (IgG2c) inhibited the IgG2a-dependent eosinophil activation and cytotoxicity at the level both of the target antigen and of the eosinophil Fc receptor. The present data showed that the preincubation of eosinophils with blocking IgM antibodies did not inhibit the cytotoxic effect of IgG antibodies, suggesting no crossinhibition between IgG and IgM antibodies at the Fc receptor level.

This consideration prompted us to examine the antigens recognized by IgG and IgM antibodies. The predominant surface antigens precipitated by protein A-binding IgG and by IgM antibodies in infected human sera ranged between 30,000 and 40,000  $M_r$ , with additional major bands at 20,000–25,000  $M_r$ . The antigens precipitated by the IgM antibodies could be removed by prior adsorption with protein A-binding IgG antibodies. In addition, competition between IgM and IgG antibodies to one of the major antigens, of 38,000  $M_r$ , could be directly demonstrated. The 32,000–38,000  $M_r$  complex represents a cluster of related antigens (29) and the preferential binding of IgM to the 32,000  $M_r$  antigen, in comparison with IgG, suggested the recognition of a nonidentical family of epitopes on the 32,000–38,000  $M_r$  complex.

To identify in more detail some of the epitopes recognized by IgG and IgM antibodies, respectively, we used two previously-described rat mAbs, IPLSm1 (a protective IgG2a antibody), and IPLSm3 (a blocking IgG2c antibody). Both of these antibodies recognize carbohydrate epitopes on the 38,000  $M_r$  major surface antigen, and show crossinhibition of binding. In addition, the IPLSm3 mAb that recognizes the 32,000 and 20,000  $M_r$  molecules (11), suggesting either that they recognize structurally-related but not identical epitopes on the 38,000  $M_r$  molecule, or that they compete sterically. The binding of both IPLSm1 and IPLSm3 to the 38,000  $M_r$  molecule could be inhibited by both IgG and IgM fractions of human infected sera.

These findings indicate that the families of IgM and IgG antibodies in human infected sera that have specificity to schistosomulum surface antigens include antibodies with specificity to the major 38,000  $M_r$  antigen. Such antibodies may

compete with each other, either by direct competition for the same or structurally-related epitopes, or by a steric inhibition of binding.

Our results revealed the existence, among the various antibodies specifically directed against schistosomula surface in infected human sera, of two particularly interesting antibodies. The first was of IgG isotype, and was able to induce the killing of *S. mansoni* schistosomula in the presence of human eosinophils; the second was of IgM isotype, and showed no cytotoxic effect, but demonstrated an effective inhibition of eosinophil-dependent cytotoxicity mediated by IgG antibodies. In the context of the in vivo relevance of such findings we wanted to know whether the presence of IgM antibodies could be used as a marker of reinfection intensities to *S. mansoni*. We performed a capture assay to measure, in human infected sera, the presence of IgM antibodies specific of the epitope present on the 38,000  $M_r$  antigen, as evaluated by using a second arm with IgG2a mAbs. Results revealed that the mean level of IgM antibodies in individuals classified as susceptible were significantly higher than those present in the resistant individuals; there was a positive association between the level of IgM antibodies in the pretreatment and the number of eggs counted in the susceptible group at 12 mo after treatment, suggesting that such IgM might be related to subsequent reinfection. The positive association between IgM levels and egg counts at any given time suggested, first, that these IgM antibodies may be associated with anti-egg responses, and second, that they fluctuate rapidly with time (16). These results suggest that the production of blocking IgM antibodies could be induced by the same epitope expressed on schistosomula and eggs. Further statistical analysis (Butterworth et al.)<sup>1</sup> supported this hypothesis.

In conclusion, although our evidence is derived from in vitro studies, these findings indicate that susceptibility to reinfection after treatment of *S. mansoni* infections in man might be explained in part by the presence of schistosomulum-specific IgM blocking antibodies. In addition, they suggest indirectly a major role played by the effector IgG antibodies directed against the  $M_r$  38,000 antigen. The fact that these antibodies are a necessary but not limiting factor in immunity, and the presence of blocking antibodies support the hypothesis that the acquisition of immunity might reflect the loss of a blocking response, rather than the acquisition of an effector response. However, besides IgM antibodies, statistical studies (Butterworth et al.)<sup>1</sup> raise the possibility that another antibody isotype may block the protective response. The availability of this type of information would be of considerable importance in defining the nature of a vaccine capable of inducing the production of both effector and blocking antibodies. However, another approach to explain the prevention of immunity may involve idiotypic regulation, whereby the protective antibodies may be blocked by antibodies directed against the antigen-specific combining sites of effector antibodies (i.e., anti-idiotypic antibodies). Experiments are now under way to test this hypothesis.

### Summary

After the demonstration of blocking antibodies during rat experimental schistosomiasis, the existence of such factors was investigated in human schistosomiasis. The depletion, in sera from *S. mansoni*-infected patients, of a given isotype (IgM) either by protein A-Sepharose (PAS) absorption or by fast protein liquid

chromatography (FPLC) induced a significant increase in IgG-mediated killing of *S. mansoni* schistosomula by human eosinophils. Inhibition experiments showed that IgM-enriched fractions (PAS effluents) were able to inhibit eosinophil-dependent cytotoxicity mediated by IgG fractions (total sera or PAS eluates). Both IgG and IgM antibodies from infected human sera immunoprecipitated antigens of 30,000–40,000  $M_r$  in the labeled detergent extracts of schistosomulum surface. The specificity of IgG and IgM for the 38,000  $M_r$  antigen was suggested by competition experiments using two radiolabeled mAbs (IPLSm1, IPLSm3) directed against this antigen. Moreover, crossinhibition between IgG and IgM antibodies for the  $M_r$  38,000 antigen could be directly demonstrated. The in vivo relevance of such IgM blocking antibodies in the context of human immunity to schistosomiasis was evaluated in two groups of children classified as resistant or susceptible to posttreatment reinfection. IgM antibodies specifically directed against the 38,000  $M_r$  antigen were measured by a capture assay. The mean levels of IgM antibodies were significantly higher in the susceptible than in the resistant group both before and after treatment. These results are consistent with the idea that immunity to schistosomiasis could be attributable not only to the existence of antibodies with defined effector function, but also to the absence of blocking antibodies. The description of the existence in human schistosomiasis of antibody isotypes blocking the effector response against defined surface targets might lead to a new understanding of the mechanisms regulating immunity to reinfection against schistosomes and possibly other parasites.

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### References

1. Capron, A., J. P. Dessaint, M. Capron, M. Joseph, and G. Torpier. 1982. Effector mechanisms of immunity to schistosomes and their regulation. *Immunol. Rev.* 61:41.
2. Butterworth, A. E., D. W. Taylor, M. C. Veith, M. A. Vadas, A. Dessein, R. F. Sturrock, and E. Wells. 1982. Studies on the mechanisms of immunity in human schistosomiasis. *Immunol. Rev.* 61:5.
3. Capron, A., J. P. Dessaint, M. Capron, and H. Bazin. 1975. Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature (Lond.)*. 253:44.
4. Butterworth, A. E., R. F. Sturrock, V. Houba, and A. A. F. Mahmoud. 1975. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature (Lond.)*. 256:727.
5. Capron, M., H. L. Spiegelberg, L. Prin, H. Bennich, A. E. Butterworth, R. J. Pierce, M. A. Ouassii, and A. Capron. 1984. Role of IgE receptors in effector function of human eosinophils. *J. Immunol.* 132:462.
6. Joseph, M., C. Auriault, A. Capron, H. Vorng, and P. Viens. 1983. A new function for platelets: IgE dependent killing of schistosomes. *Nature (Lond.)*. 303:810.
7. Butterworth, A. E., G. H. Remold, V. Houba, J. R. David, D. Franks, P. H. David,

- and R. F. Sturrock. 1977. Antibody-dependent eosinophil-mediated damage to <sup>51</sup>Cr-labeled schistosomula of *Schistosoma mansoni*: Mediation by IgG, and inhibition by antigen-antibody complexes. *J. Immunol.* 6:2230.
8. Capron, M., A. Capron, G. Torpier, H. Bazin, D. Bout, and M. Joseph. 1978. Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG2a antibody and role of mast cells. *Eur. J. Immunol.* 8:127.
  9. Capron, M., H. Bazin, M. Joseph, and A. Capron. 1981. Evidence for IgE dependent cytotoxicity by rat eosinophils. *J. Immunol.* 126:1764.
  10. Capron, M., G. Torpier, and A. Capron. 1979. In vitro killing of *Schistosoma mansoni* schistosomula by eosinophils from infected rats: role of cytophilic antibodies. *J. Immunol.* 123:2220.
  11. Grzych, J. M., M. Capron, C. Dissous, and A. Capron. 1984. Blocking activity of rat monoclonal antibodies in experimental schistosomiasis. *J. Immunol.* 133:998.
  12. Khalife, J., M. Capron, J. M. Grzych, H. Bazin, and A. Capron. 1985. Fc receptors on rat eosinophils: Isotype-dependent cell activation. *J. Immunol.* 135: 2780-2784.
  13. Grzych, J. M., M. Capron, H. Bazin, and A. Capron. 1982. In vitro and in vivo effector function of rat IgG2a monoclonal anti-*Schistosoma mansoni* antibodies. *J. Immunol.* 129:2739.
  14. Capron, A., M. Capron, M. Joseph, C. Dissous, and C. Auriault. 1983. Vaccination against schistosomiasis: Dream or reality? *In* New approaches to vaccine development. Rosemary Bell and G. Torrigiani, editors. Schwabe and Co. AG. Basel, Switzerland. 460-475.
  15. Butterworth, A. E., P. R. Dalton, D. W. Dunne, M. Mugambi, J. H. Ouma, B. A. Richardson, T. K. arap Siongok, and R. F. Sturrock. 1984. Immunity after treatment of human schistosomiasis mansoni. I. Sturdy design, pretreatment observations and the results of treatment. *Trans. R. Soc. Trop. Med. Hyg.* 78:108.
  16. Butterworth, A. E., M. Capron, J. S. Cordingley, P. R. Dalton, D. W. Dunne, H. C. Kariuki, D. Koech, M. Mugambi, J. H. Ouma, M. A. Prentice, B. A. Richardson, T. K. arap Siongok, R. F. Sturrock, and D. W. Taylor. 1985. Immunity after treatment of human schistosomiasis. II. Identification of resistant individuals, and analysis of their immune responses. *Trans. R. Soc. Trop. Med. Hyg.* 79:393.
  17. Clegg, J. A., and S. R. Smithers. 1972. The effects of immune Rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation in vitro. *Int. J. Parasitol.* 2:79.
  18. Lindmark, R., T. T. Kerstin, and J. Sjöquist. 1983. Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera. *J. Immunol. Methods.* 62:1.
  19. Sampson, I. A., A. N. Hodgen, and I. H. Arthur. 1984. The separation of immunoglobulin M from human serum by fast protein liquid chromatography. *J. Immunol. Methods.* 69:9.
  20. Marchalonis J. J., R. E. Cone, and V. Santer. 1971. Enzymatic iodination: a probe for accessible surface proteins on normal and neoplastic lymphocytes. *Biochem. J.* 124:921.
  21. Dissous, C., C. Dissous, and A. Capron. 1981. Isolation and characterization of surface antigens from *Schistosoma mansoni* schistosomula. *Mol. Biochem. Parasitol.* 3:215.
  22. Grzych, J. M., M. Capron, P. H. Lambert, C. Dissous, S. Torres, and A. Capron. 1985. An anti-idiotypic vaccine against experimental schistosomiasis. *Nature (Lond.)* 316:74.
  23. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine 131-labelled human growth hormone of high specific activity. *Nature (Lond.)* 194:495.
  24. Cesbron, J. Y., A. Capron, G. Ovlaque, and F. Santoro. 1985. Use of monoclonal

- antibody in a double-sandwich ELISA for detection of IgM antibodies to *Toxoplasma gondii* major surface protein (P30). *J. Immunol. Methods.* 83:151.
25. Auriault, C., M. Damonville, C. Verwaerde, R. Pierce, M. Joseph, M. Capron, and A. Capron. 1984. Rat IgE directed against schistosomula-released products is cytotoxic for *Schistosoma mansoni* schistosomula in vitro. *Eur. J. Immunol.* 14:132.
  26. Hofstetter, M., R. W. Poindexter, E. Ruiz-Tiben, and E. A. Ottesen. 1982. Modulation of the host response in human schistosomiasis. III. Blocking antibodies specifically inhibit immediate hypersensitivity responses to parasite antigens. *Immunology.* 46:777.
  27. Steele, J.R., J. Amkerst, H. O. Sjögren, H. Vang, and O. Lannerstad. 1975. Absorption of blocking activity from human tumor-bearer sera by *Staphylococcus aureus* cowan I. *Int. J. Cancer.* 15:180.
  28. Mathew, G. D., L. F. Qualtierre, H. B. Neel, and G. R. Pearson. 1980. IgA antibody, antibody-dependent cellular cytotoxicity and prognosis in patients with nasopharyngeal carcinoma. *Int. J. Cancer.* 27:175.
  29. Dissous, C., J. M. Grzych, and A. Capron. 1985. Biochemical studies on the 30–40 kDa *Schistosoma mansoni* surface antigen. *Mol. Biochem. Parasitol.* 16:277.