Parasitol Res (1988) 74:356–362

Parasitology Research



# *Schistosoma mansoni*: impaired clearance of model immune complexes consisting of circulating anodic antigen and monoclonal $IgG_1$ in infected mice

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**Abstract.** The clearance of schistosome-specific model immune complexes (IC) consisting of circulating anodic antigen (CAA), a gut-associated excretory-secretory antigen, and radiolabeled monoclonal antibody ($IgG_1$) was investigated in mice with a light and heavy *Schistosoma mansoni* infection and in noninfected control animals. The size analysis of the in vitro prepared and injected IC, as determined by density gradient centrifugation, revealed a wide peak at 11S. In infected animals the injected IC were cleared at a significantly lower rate than in control mice. This was attributed to a decreased uptake of IC by the liver in infected mice. In heavily infected mice, 30 min after injection of 11S IC, 8S, 11S, and >11S IC were present in the serum, whereas only small 8S IC were detected in the serum of lightly infected animals and noninfected controls. Immune complexes were also present in the serum of heavily infected mice 30 min after injection of antibody and were detectable as 11S and >11S IC. The importance of this study is twofold. First, these results show that schistosome-specific monoclonal antibodies can be used in the production of model immune complexes applicable in clearance studies. Second, our findings might be of importance when the possible pathogenicity of circulating IC in schistosomiasis is considered.

An important group of schistosome antigens is constituted by excretory and secretory antigens. Several of these antigens have been detected in *Schistosoma mansoni*-infected humans and in a variety of infected laboratory animals as free circulating antigens and as antigens complexed in circulating immune complexes (IC) (Carlier et al. 1975; Deelder et al. 1976; Santoro et al. 1979). One of these antigens, described for the first time by Berggren and Weller (1967), has been characterized as a heat-stable anionic antigen (Gold et al. 1969) with characteristic proteoglycan properties (Nash et al. 1974, 1977). This genus-specific antigen (Nash et al. 1974; Qian and Deelder 1983) has been found to be associated with the esophagus and gut of adult worms (Von Lichtenberg et al. 1974; Nash 1974). Ultramicroscopically, this antigen has been observed in the gut lumen and covering the gut epithelium of adult worms (De Water et al. 1986). In terms of the mobility of the antigen in an electric field, its localization in the worm gut, and its presence in the host serum, it has been designated as circulating anodic antigen (CAA) (Deelder et al. 1978), gut-associated schistosome proteoglycan (GASP) (Nash 1978), and gut-associated schistosome circulating anodic proteoglycan (GASCAP) (Nash and Deelder 1985), respectively.

CAA has been reported in kidney eluates of infected hamsters (Deelder et al. 1980), and the same antigen has been demonstrated with indirect immunofluorescence in the glomeruli of infected mice and hamsters (Deelder et al. 1980; El-Dosoky et al. 1984). In mice, glomerular localization of CAA was accompanied by deposits of immunoglobulins and complement (Van Marck et al. 1981). The glomerular localization of CAA and immunoglobulins was found to be similar, suggesting the involvement of CAA in IC. Consequently, CAA has been put forward as a possible factor that might be involved in the genesis of glomerulopathies in schistosomiasis (Van Marck 1983).

In studies with radiolabeled GASP, it has been shown that its clearance rate from the circulation of infected mice is faster in lightly infected mice

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*Abbreviations:* IC, Immune complexes; CAA, Circulating anodic antigen, SDG, Sucrose density gradient; S, Svedberg unit

than in heavily infected mice (Nash 1982), influenced by the size of the IC formed in vivo (Nash 1984).

The production of schistosome-specific anti-CAA monoclonal antibodies enabled the preparation of well-defined and highly reproducible model IC containing CAA. The aim of the present study was to investigate the clearance kinetics of these model IC, as this might contribute to a better understanding of the putative pathogenicity of circulating CAA IC.

## Materials and methods

*Animals.* Six- to eight-week-old female Swiss albino mice (Catholic University of Leuven, Belgium) weighing approximately 20 g were used throughout the experiments. The animals were fed on standard diet pellets (Hope Farms, the Netherlands) and water was available ad libitum. The mice were infected with *S. mansoni* cercariae according to the ring method described by Smithers and Terry (1965). A light infection was obtained by infecting each animal with 30 cercariae, and a heavy infection by infecting each animal with 150 cercariae. Golden hamsters (*Mesocricetus auratus*) were infected with 1000 *S. mansoni* cercariae by the same method.

*Monoclonal antibodies.* The production of $IgG_1$-anti-CAA (54-5G10-A) has been described in detail by De Water et al. (1986). Briefly, spleen cells of *S. mansoni*-infected Swiss mice were fused with SP2/0 mouse myeloma cells. Culture supernatants were screened for the presence of antibody activity against gut-associated polysaccharide antigens by indirect immunofluorescence (IFA) on paraffin sections of Rossman's fixed adult *S. mansoni* worms (according to Nash 1974). Positive culture supernatants were tested by immunoelectrophoresis against trichloroacetic acid-soluble adult worm antigen (AWA-TCA). One strongly positive line (54-5G10-A) giving a CAA precipitation line was selected for this study.

For the production of this monoclonal antibody, ascitic fluid was harvested from F1 Balb/C × Swiss mice, which had been intraperitoneally injected with $5 \times 10^6$ hybridoma cells. $IgG_1$ was isolated from the ascitic fluid by Protein A Sepharose CL-4B (Pharmacia, Belgium) according to the procedure indicated by the manufacturer.

*Radiolabeling of the monoclonal antibodies.* The purified antibody was dialyzed against TRIS-HCl (0.1 *M*, pH 7.4), concentrated up to 1 mg/ml, and radiolabeled ($Na^{125}I$, IRE, Belgium) according to the chloramin-T method (Hunter and Greenwood 1962), with small modifications as described by Hudson and Hay (1980). The specific activity of the radiolabeled antibody was 53.65 kBq (1.45 μCi)/μg.

*Preparation of antigen.* Adult *S. mansoni* worms were obtained from golden hamsters after perfusion of the liver (Radke et al. 1961) 45 days following infection. The worms were collected, washed three times in phosphate-buffered saline (PBS; 0.2 M; pH 7.3), and frozen at −70° C. AWA-TCA was prepared according to the method described by Deelder et al. (1976).

*Preparation of immune complexes with $IgG_1$-anti-CAA and AWA-TCA.* In order to determine the antigen-antibody equivalence, a dilution series of AWA-TCA ($OD_{280} = 1.6$) varying between 1:4 to 1:1024 was made in PBS containing 1% bovine serum albumin, (BSA, Janssen Chimica, Belgium). To each 100 μl antigen dilution, an equal volume of labeled monoclonal antibody (50 μg/ml; 50000 CPM/ml) was added, and the mixture was allowed to incubate at 37° C for 2 h. Subsequently, 200 μl Veronal-buffered saline (0.0032 *M* barbitone, 0.0018 *M* Na-barbitone, 0.15 *M* NaCl) containing 5% polyethylene glycol (PEG-6000, Merck, FRG) was added and the mixture was incubated overnight at 4° C, followed by centrifugation at 1200 *g*. The radioactivity in the pellet was measured in an automatic well-type gamma counter (Intertechnique CG-4000, France).

Immune complexes intended for injection were prepared at 10 × antigen excess. A single dose of IC consisted of 0.2 μg $IgG_1$-anti-CAA, 3 μl AWA-TCA ($OD_{280} = 1.6$), and 0.33 mg mouse serum albumin (0.1% MSA, Nordic Laboratories, the Netherlands) in 0.3 ml PBS. The IC mixture was allowed to react overnight at 4° C before it was injected. For the determination of antibody clearance, the same concentration of $IgG_1$-anti-CAA was used as for the IC production.

*Immune complex size analysis.* The size of IC, prepared in vitro and present in the serum after injection, was analyzed by SDG ultracentrifugation. For this purpose, 4 ml linear borate-buffered (0.2 *M* borate, 0.15 *M* NaCl, pH 8.3) gradients were prepared. Of each sample (in vitro prepared IC or serum), 100 μl was layered on top of the gradient. The gradients were centrifugated in a swing-out rotor (S. 9750 omega, Heraeus Christ, FRG) at 100000 *g* during 16 h at 4° C. The gradients were harvested from the top, and 25 fractions (165 μl/fraction) were obtained. Radiolabeled human serum albumin (HSA; 4.4 S), IgG (6.6 S) and IgM (19 S) were used as gradient markers. The sedimentation coefficients were calculated with the formula given by Martin and Ames (1961).

*Blood clearance studies.* Radiolabeled antibody and IC were injected intravenously in mice in 0.3 ml PBS. At regular time intervals (1, 2, 5, 10, 15, and 30 min, and 1, 2, 5, 8, 24, 48, and 96 h), 20 μl blood samples were taken from the retro-orbital plexus using calibrated, heparinized 20-μl capillaries (Bilbate Ltd, England). The blood samples were poured in 12 × 75 mm plastic test tubes containing 1 ml 0.1 *M* HCl. The amount of radiolabel in each sample was measured. The clearance results were subjected to nonlinear regression analysis according to the iterative Marquardt-Levenberg method (Draper and Smith 1966). The amount of radioactivity that would be present in the blood 0 min after injection was subsequently obtained by extrapolation and considered as 100% of the injected dose. The results are expressed as the mean ± asymptotic standard deviation.

*Organ localization.* Mice were sacrificed at 30 min and at 96 h after injection. Blood was collected by cardiac puncture and was allowed to clot at room temperature. The liver, lungs, spleen, and kidneys were removed, and the accumulation of antibody and IC was calculated by measuring the amount of radioactivity in each organ in relation to the total amount injected. The results are given as the mean ± 95% confidence limits. Different mean values obtained for blood clearance and organ localization were evaluated for significance with the two-tailed Student's *t*-test.

## Results

### *Molecular size composition*

The molecular size of the immune complexes prepared with 54-5G10-A ($IgG_1$-anti-CAA) depended

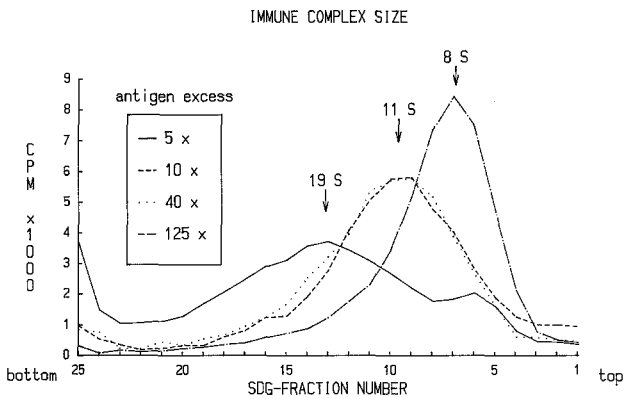


**Fig. 1.** SDG centrifugation of IC, prepared in vitro with monoclonal $IgG_1$-anti-CAA and excess antigen (AWA-TCA). At 5 × antigen excess, mainly 19S IC were formed. At 10 × and 40 × antigen excess, 11S IC were produced, whereas at 125 × antigen excess, small 8S IC were obtained

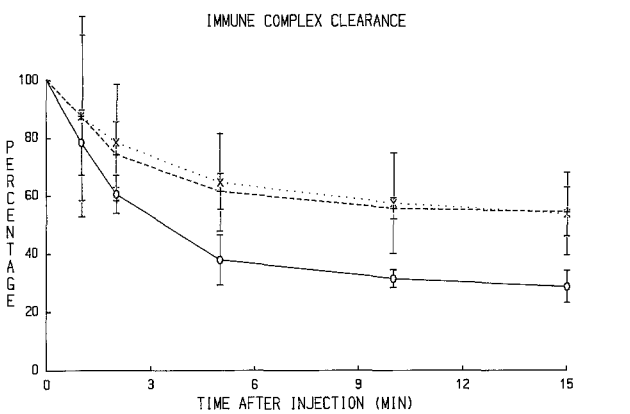


**Fig. 2.** Initial clearance pattern in *S. mansoni*-infected mice and in noninfected controls of 11S IC, prepared in vitro with monoclonal $IgG_1$-anti-CAA and AWA-TCA. The clearance rate was significantly lower in infected mice. The difference was maximal 15 min after injection and decreased at later time intervals. Differences were no longer significant after 24 h. Noninfected controls (——); light infection (---); heavy infection (·····)

upon the antigen: antibody ratio (Fig. 1). At 125 × antigen excess, a homogeneous group of 8S IC were produced. Using a ratio between 40 × and 10 × antigen excess, 11S IC were obtained, whereas at 5 × antigen excess, 75% of the formed IC consisted of ±19S IC and 25% consisted of small 8S IC.

### *IC clearance*

IC prepared with $IgG_1$-anti-CAA at 10 × antigen excess consisted predominantly of 11S complexes. The clearance of such IC is given in Fig. 2. The initial clearance rate of the injected IC was lower in mice infected with *S. mansoni* than in noninfected control mice. Table 1 summarizes the results of the regression analysis of the clearance data. In noninfected controls, 70.2% ± 5.6% of the injected dose was cleared from the circulation within 15 min, whereas significantly less ($P<0.005$) was cleared in heavily infected mice (38.2% ± 4.8%). The disappearance of the injected IC from the circulation was comparable in lightly and heavily infected mice.

Antibody was removed faster in infected mice than in noninfected controls, and the disappearance rate was greatest in heavily infected mice (Fig. 3). In mice infected with 30 cercariae/animal, the injected IC were initially removed at a higher rate than antibody, but in animals infected with 150 cercariae/animal, the clearance of antibody was comparable with the clearance of IC.

The size composition of the immune complexes 30 min after injection is presented in Fig. 4. Only small 8S IC were detected in the serum of control mice and of lightly infected mice. In addition to 8S IC, 11S and >11S immune complexes were also detected in heavily infected mice. In the latter, IC were formed after the injection of antibody, which were detectable in the serum and were larger than 8S (Fig. 5). The accumulation of IC in the liver 30 min after its injection was significantly higher ($P<0.05$) in control animals than in lightly and heavily infected mice (Table 2). Following the injection of antibody, however, significantly less ($P<0.02$) of the injected radiolabel was found in the liver of control mice than in the liver of lightly or heavily infected mice. The accumulation of the injected immune complexes and antibody in the liver, spleen, lungs, and kidneys 96 h after injection was small and lower than 3% of the injected dose (Table 3).

## Discussion

In the present study, the clearance kinetics of schistosome-specific model immune complexes containing CAA and monoclonal $IgG_1$-anti-CAA were investigated. Using the monoclonal antibody line 54-5G10-A, 75% precipitability of the radiolabel was obtained at equivalence. The production of a precipitation arc in immunoelectrophoresis and the formation of large IC both indicate that the 54-5G10-A line recognizes a repetitive epitope on the CAA molecule.

The molecular size composition of the prepared immune complexes depended upon the antigen: antibody ratio. The smallest IC were obtained at 125 × antigen excess and were 8S IC. The narrow peak of 8S IC seen at this antigen: antibody ratio

**Table 1.** Clearance of antibody and immune complexes in *Schistosoma mansoni*-infected mice and in noninfected controls

| | *n* | A<br>% ± ASD | Ta (min)<br>$t_{1/2}$ ± ASD | B<br>% ± ASD | Tb (h)<br>$t_{1/2}$ ± ASD | C<br>% ± ASD | Tc (days)<br>$t_{1/2}$ ± ASD |
|---|---|---|---|---|---|---|---|
| Controls | | | | | | | |
| IC | 3 | 70.21 ± 5.56 | 1.75 ± 0.20 | 21.84 ± 0.91 | 3.88 ± 0.37 | 7.94 ± 0.89 | 6.69 ± 2.94 |
| AB | 6 | 27.34 ± 2.43 | 8.84 ± 1.99 | 33.12 ± 2.09 | 4.96 ± 0.83 | 39.53 ± 1.71 | 6.17 ± 0.85 |
| Light infection (30 cercariae) | | | | | | | |
| IC | 3 | 42.76 ± 16.69 | 1.63 ± 0.67 | 24.06 ± 3.24 | 1.40 ± 0.38 | 33.18 ± 3.30 | 1.24 ± 0.15 |
| AB | 2 | 27.44 ± 2.16 | 4.38 ± 0.73 | 40.14 ± 2.22 | 0.67 ± 0.06 | 32.43 ± 1.13 | 0.99 ± 0.04 |
| Heavy infection (150 cercariae) | | | | | | | |
| IC | 3 | 38.16 ± 4.81 | 1.84 ± 0.42 | 34.48 ± 2.20 | 0.68 ± 0.11 | 27.36 ± 1.77 | 0.84 ± 0.08 |
| AB | 3 | 35.57 ± 3.95 | 1.84 ± 0.36 | 42.30 ± 1.45 | 0.68 ± 0.07 | 22.13 ± 1.47 | 0.79 ± 0.08 |

Clearance of monoclonal $^{125}$I-IgG$_1$-anti-CAA (AB) and of immune complexes (IC) prepared in vitro with this antibody and AWA-TCA. The parameters of the nonlinear regression curve were estimated by means of the iterative Marquardt-Levenberg curve-fitting method. The percentage of the injected dose remaining in the circulation at time $t = A\exp(-0.693t/Ta) + B\exp(-0.693t/Tb) + C\exp(-0.693t/Tc)$. In this model, the parameters A, B, and C can be thought of as the percentages of the injected dose ($A+B+C=100\%$) cleared from the circulation with the corresponding half-lives Ta, Tb, and Tc. The results are given as the mean ± asymptotic standard deviation (ASD). *n* = number of animals

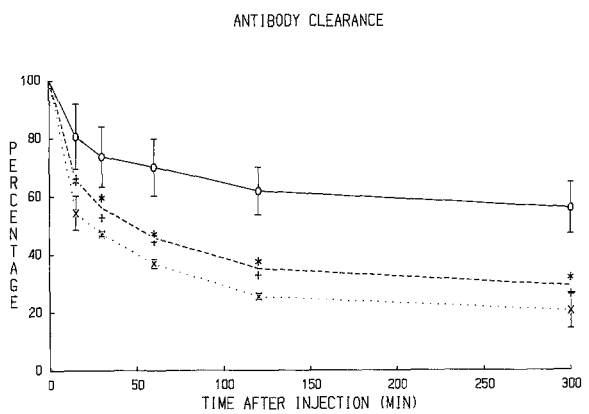


**Fig. 3.** Clearance pattern of monoclonal IgG$_1$-anti-CAA in *S. mansoni*-infected mice and in noninfected controls (——). Antibody was cleared at a higher rate in infected mice. In heavily infected mice (·····), antibody disappeared faster than in lightly infected mice (---)

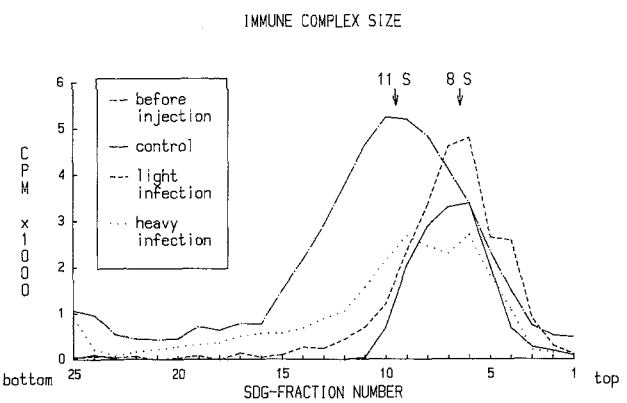


**Fig. 4.** SDG analysis of IC, prepared with IgG$_1$-anti-CAA and AWA-TCA in serum from *S. mansoni*-infected mice and from noninfected controls 30 min after injection. In heavily infected mice, 11S IC could still be detected in the serum, whereas in lightly infected mice and in control mice, only small 8S IC were present. However, a significantly larger quantity of 8S IC was found in the circulation of lightly infected mice than in controls

points toward a limited variability in molecular weight of CAA. This finding does not support the large molecular weight heterogeneity of CAA estimated by ultrafiltration (>100000; Nash et al. 1974), column chromatography (<50000–300000; Deelder et al. 1980), and SDS acrylamide gel electrophoresis (66000–350000; Nash et al. 1981). From a calibration curve, the following molecular weights of the IC were determined: 19S, 900000; 11S, 440000; and 8S, 260000. As mouse IgG$_1$ has a molecular weight of 150000, a mol. wt. of ±70000 may be assumed for CAA, in which case the IC would be composed of $Ag_4Ab_4$, $Ag_2Ab_2$, and a mixture of $Ag_2Ab_1$ and $Ag_1Ab_1$, respectively.

The injected IC, prepared with IgG$_1$-anti-CAA and AWA-TCA, were cleared at a lower rate in *S. mansoni*-infected mice than in control mice during the first 15 min. These findings do suggest that the clearance of such IC is impaired in infected mice. In former studies we have been able to demonstrate an impaired clearance of injected IC prepared with human serum albumin (HSA) and IgG-anti-HSA in *S. mansoni*-infected mice (Kestens et al. 1983; Kestens and Gigase 1984). The clearance of soluble IC is very much determined by the size of the IC (Mannik and David 1981). In our study, 11S and >11S IC could still be detected in the serum of heavily infected mice 30 min after injection. Therefore, it is unlikely that the de-

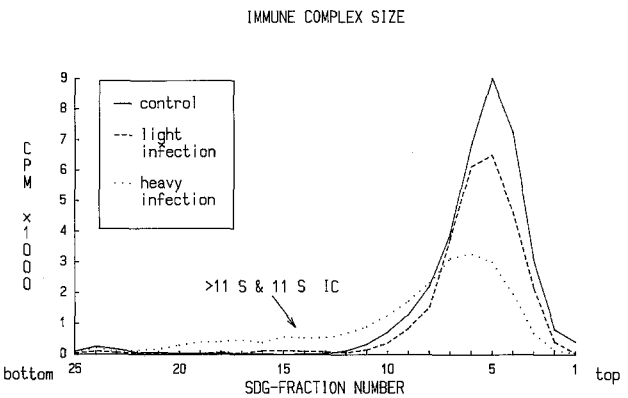


**Fig. 5.** SDG analysis of serum from *S. mansoni*-infected mice 30 min after injection of $IgG_1$-anti-CAA. In heavily infected mice, large (11S and >11S) IC were detected, indicating that large IC are produced and remain in the circulation. In lightly infected mice, only small IC and free antibody was demonstrated

creased clearance in infected mice was caused by a rearrangement of the 11S IC into smaller 8S IC induced by free circulating CAA. Interaction of the injected IC with circulating antibody and CAA, however, is very likely to occur. Free antibody was cleared faster in infected mice than in noninfected control mice. The difference between lightly and heavily infected mice was small, but antibody tended to disappear at a higher rate in heavily infected mice than in lightly infected animals. The results obtained from SDG analysis pointed out that in infected mice and in particular in heavily infected mice, IC were formed with the injected antibody. In heavily infected mice, the presence of 11S and >11S IC could clearly be demonstrated in the serum 30 min after injection.

In heavily infected mice, the clearance of $IgG_1$-anti-CAA antibody was comparable with the clear-

**Table 2.** Relative tissue distribution of antibody and immune complexes 30 min after injection in *S. mansoni*-infected mice and in noninfected controls

| | *n* | Blood $x \pm 95\%$ | Liver $x \pm 95\%$ | Spleen $x \pm 95\%$ | Lungs $x \pm 95\%$ | Kidneys $x \pm 95\%$ |
|---|---|---|---|---|---|---|
| Antibody (AB) | | | | | | |
| controls | 3 | 54.06± 4.04 | 8.88± 2.00 | 0.75±0.05 | 1.20±0.65 | 3.04±0.86 |
| light infection | 3 | 47.23±21.00 | 16.24±17.96 | 2.44±2.37 | 1.00±0.06 | 2.33±1.59 |
| heavy infection | 3 | 39.19±10.84* | 23.94±15.34* | 1.93±2.30 | 0.83±0.53 | 2.35±1.22 |
| Immune complexes (IC) | | | | | | |
| controls | 3 | 17.45± 1.33 | 47.10± 5.28 | 0.32±0.13 | 0.52±0.20 | 1.68±0.35 |
| light infection | 3 | 37.64±17.26 | 28.28±25.19* | 1.70±0.84 | 0.88±0.31 | 1.90±0.91 |
| heavy infection | 3 | 33.61±22.85 | 31.56±14.01* | 2.15±0.37 | 0.90±0.28 | 1.71±0.79 |

Relative tissue distribution of $^{125}I$-labeled $IgG_1$-anti-CAA (AB) and corresponding immune complexes (IC) prepared in vitro with AWA-TCA 30 min after injection in mice with a light (30 cercariae/animal) and in mice with a heavy (150 cercariae/animal) bisexual *S. mansoni* infection, and in noninfected control mice. Results are given as the mean ($x$) ±95% confidence intervals ($t_{95}.s_x$). $n$ = number of animals

* Value differs significantly from controls ($P<0.05$)

**Table 3.** Relative tissue distribution of antibody and immune complexes 96 h after injection in *S. mansoni*-infected mice and in noninfected controls

| | *n* | Blood $x \pm 95\%$ | Liver $x \pm 95\%$ | Spleen $x \pm 95\%$ | Lungs $x \pm 95\%$ | Kidneys $x \pm 95\%$ |
|---|---|---|---|---|---|---|
| Antibody (AB) | | | | | | |
| controls | 6 | ND | 2.22±0.29 | 0.15±0.03 | 0.52±0.10 | 0.65±0.17 |
| light infection | 3 | 3.96±8.68 | 0.82±1.09 | 0.56±0.82 | 0.12±0.27 | 0.22±0.46 |
| heavy infection | 3 | 0.81±0.50 | 0.35±0.21 | 0.18±0.46 | 0.02±0.02 | 0.04±0.05 |
| Immune complexes (IC) | | | | | | |
| controls | 3 | 3.70±1.02 | 0.57±0.08 | 0.04±0.03 | 0.10±0.04 | 0.16±0.05 |
| light infection | 3 | 3.02±1.60 | 0.83±0.58 | 0.24±0.33 | 0.09±0.05 | 0.14±0.09 |
| heavy infection | 3 | 0.58±0.64 | 0.50±1.31 | 0.08±0.22 | 0.05±0.14 | 0.07±0.20 |

Relative tissue distribution of $^{125}I$-labeled $IgG_1$-anti-CAA (AB) and corresponding immune complexes (IC) prepared in vitro with AWA-TCA 96 h after injection in mice with a light (30 cercariae/animal) and in mice with a heavy (150 cercariae/animal) bisexual *S. mansoni* infection, and in noninfected control mice. Results are given as the mean ($x$) ±95% confidence intervals ($t_{95}.s_x$). $n$ = number of animals; ND = not done

ance of the injected IC, but in lightly infected mice, IC were cleared at a significantly higher rate than antibody. These findings possibly indicate that in heavily infected mice, IC of a size comparable to that of the injected model IC were formed after injection of antibody. In lightly infected mice, fewer or smaller IC were produced than in heavily infected mice. Indeed, results from SDG analysis indicated that in heavily infected animals, heavier IC are produced with the injected antibody than in lightly infected mice. Nash (1982), who carried out clearance studies after injection of antigen (GASP), has observed that the clearance of GASP occurred at a lower rate in heavily infected mice than in lightly infected mice. These results were explained by the findings that in lightly infected mice, large IC were produced with the injected GASP, whereas in heavily infected animals, small IC were formed (Nash 1984). Our findings are not necessarily in conflict with the results from these studies. The IC produced after the injection of free antibody (our study) or free antigen (Nash) could result in a shift of the antigen: antibody ratio in such a way that small IC are produced after the injection of antigen (antigen excess), or large IC, after the injection of antibody (smaller antigen excess or antibody excess). However, both studies lead to the conclusion that clearance of CAA IC (or GASP IC; Nash 1984) is determined by their molecular size.

The liver accumulated the largest part of the injected IC, which is in agreement with other studies using heterologous (Haakenstad and Mannik 1974) and homologous IC (Nash 1982). The uptake of IC by the liver, however, was significantly reduced in infected mice, suggesting that the liver's uptake is impaired in infected animals. Similar results have previously been reported using heterologous HSA-anti-HSA IC (Kestens et al. 1983). The mechanism still remains to be solved, but a reduction of the number of immune complex receptors might be possible. Clearance can be depressed after the blockade of the Fc receptors on phagocytes (Kurlander et al. 1984). Circulating IC can also induce a loss of Fc- and C3-receptor function in Kupffer cells in vivo, resulting in diminished clearance of IC (Nishi et al. 1981). Large-latticed IC in particular seem to be important, as they have been found to affect the phagocyte system function, in contrast to small-latticed IC (Jimenez et al. 1983; Finbloom and Plotz 1979).

In conclusion, our study has demonstrated that the clearance of 11S IC prepared with CAA and monoclonal $IgG_1$ is impaired in *S. mansoni*-infected mice. Further studies using other schistosome antigens may, together with the present results, shed more light on the possible pathogenicity of circulating IC in schistosomiasis.

*Acknowledgements.* This investigation received financial support from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases and from the Belgian Fund for Medical Scientific Research (FGWO 3.0021.78).

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Accepted November 23, 1987