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CIRCULATING ANTIGENS, ANTIBODIES AND GLOMERULAR IMMUNE COMPLEXES IN MICE WITH UNISEXUAL SCHISTOSOMA MANSONI INFECTION (*)

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SUMMARY

Circulating antigens, antibodies and glomerular deposits of immune complexes were investigated in mice infected with unisexual **Schistosoma mansoni.** Circulating antigens were detected by the ELISA double-antibody technique in 35% of infected mice after 3 weeks, in 54% after 8 weeks and in 71% after 24 weeks of infection. Antibody titers against worm antigens, as detected by indirect immunofluorescence, raised steadily after the third week reaching the highest levels (IgG) after 24 weeks of infection. Glomerular deposits of antibody (IgG and IgM), complement and schistosome antigen were investigated by direct immunofluorescence in frozen kidney sections. After 3 weeks, 55% of infected mice had immune complexes. Proportions of mice with immune complexes after 8 and 24 weeks of infection were 82% and 86% respectively. Unisexual infection excludes schistosome eggs and egg-derived substances as sources of antigens. Therefore, the positive results herein reported show that antigens specifically originated from **S. mansoni** worms can be frequently found as immune complexes in kidneys from infected animals. The role played by such antigens on the appearance of glomerular lesions in still unclear.

INTRODUCTION

Present knowledge on circulating antibodies [20,25,26,27], antigens [20,24,27,29,30], immune complexes [10,11,32,33,34,35] and on the deposition of immune complexes in the kidney [3,4,21,28,31,38], in schistosomiasis, derives mainly from studies of human infections [2,13,26,38] and of experimental bisexual infections of rodents [4,6,7,15,19] and primates [12,22]. Although these reports strongly suggest the participation of immune complexes in the pathogenesis of renal lesions, a correlation with worm burden, circulating antigens and antibodies as well as with the time course of the infection is still controversial. Also debatable is the participation of worm or egg-derived antigens in the pathogenesis of schistosomiasis.

The demonstration that antibodies eluted from immune complexes located at human kidneys react with worm gut and tegument [21,28] has led to the assumption that worm antigens constitute an important part of glomerular deposits. Unisexual schistosome infections of mice may constitute a suitable model to study immunological aspects of schistosomiasis unrelated to the presence of parasite eggs [1]. Such infections have been used to investigate the induction of resistance to challenge infection by the adult worm [8].

Unisexual **S. mansoni** infections of mice (2.5 months of age) did not produce significant lesions in the glomeruli (NATALI & CIOLI [31]).

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FERREIRA et al.[18] showed the presence of circulating worm antigens also in unisexual infections. To further investigate the immunopathological role of these antigens in glomerular lesions, unisexual infections were produced in mice and studied at different stages of the disease.

MATERIALS AND METHODS

Mice: duplicate experiments were performed using in each 60 female albino mice, 4 weeks old. These mice were divided in three groups of 20, to be sacrificed 3 weeks, 8 weeks and 24 weeks after infection. On each group, 15 were infected and 5 maintained as controls.

Infections: snails of **Biomphalaria glabrata** were infected in the laboratory with a single miracidium of **S. mansoni**, and 100 cercariae, obtained from one of these snails were injected percutaneously in each mouse. Controls were injected with saline.

Sera: mice were bled from the retro-orbital plexus and sera were separated by centrifugation and stored at -70C.

Worm recovery: after ether anesthesia, mice were submitted to liver perfusion[17] and the recovered worms were counted and identified as to their sex.

Preparation of specimens: 1. **Histology:** pieces of liver, lung and kidney obtained from mice were fixed in 10% formaldehyde (v/v) and embedded in paraffin. After standard procedure, they were stainded with Hematoxylin and Eosin and examined under light microscopy.

2. **Immunofluorescence:** half of each kidney was wrapped in aluminum foil, labeled and frozen in liquid nitrogen. Cryostat sections, 4μ thick, were laid on microscopical slides and used for immunofluorescence reactions. The same procedure was employed with livers from bisexually infected mice used for detecting circulating antibodies[14]. Sections of whole worms were prepared according to WILSON et al.[40].

Serological tests: 1. **Circulating antibodies:** these were titrated by indirect immunofluorescence against frozen liver sections of bisexually infected mice or whole adult worms as described by KANAMURA et al.[26]. Fluorescein-conjugated rabbit anti-mouse IgM and IgG were obtained from Meloy Labs (*). Reactions were read with a Zeiss microscope equipped with an OSRAM HBO-200W mercury lamp.

2. **Circulating antigens:** the total protein containing worm antigen was prepared from homogenates of fresh adult worms[18]. Soluble ("metabolic") worm antigen was obtained from the supernatant of suspensions of living worms incubated in saline at 37°C for 2 hours[9]. Both were used as standards in the ELISA double antibody technique for detection of circulating antigens as described by FERREIRA et al.[18].

3. **Glomerular deposits:** the presence of antigen, antibody and complement in the kidney sections was investigated by direct immunofluorescence. Fluorescein-conjugated rabbit anti-mouse C'_3 was obtained from Cappel Labs (**). Fluorescein-conjugated goat anti-**S. mansoni** hyperimmune serum was prepared as described elsewhere[21].

RESULTS

Worm burden, as measured by liver perfusion in 66 mice, ranged from 1 to 53 worms per animal. In all cases, the infection was exclusively unisexual, either male or female. No worms were found in 15 mice, probably due to a failure in recognising schistosomula; however, antibodies to **S. mansoni** were detected in their sera, in contrast to the controls which were completely negative.

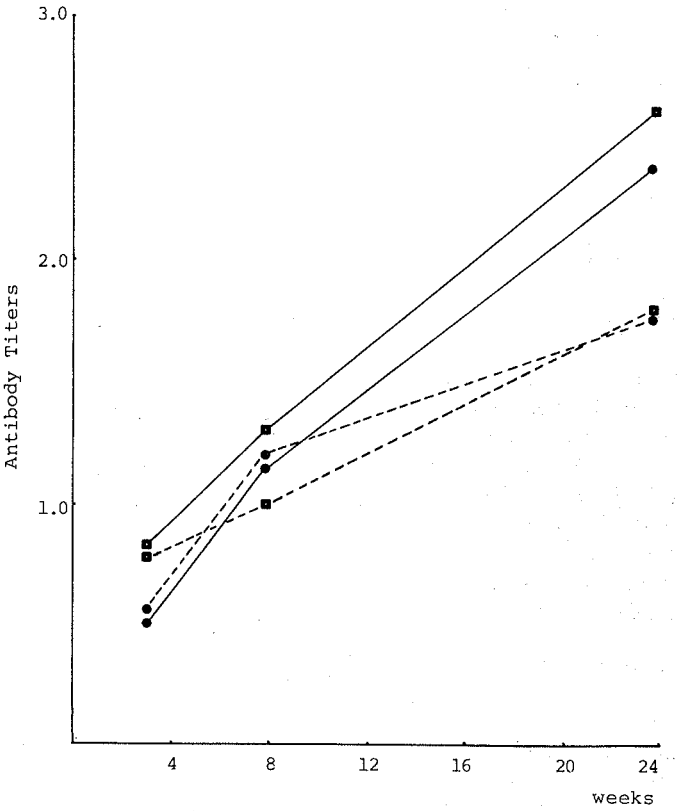
No eggs were found in histologic examination of liver tissue, even after 24 weeks of infection, thus confirming the unisexual infection. In some liver specimens, mild inflammation and schistosomotic pigment in Kupfer cells were observed. Lung specimens were normal. Some kidney showed mild mesangial proliferation but no relationship to worm burden was established.

Figure 1 shows the mean geometric titers of IgG and IgM antibodies to **S. mansoni** at different stages of infection. Serum antibodies were detected in all infected mice and were negative in the controls.

(*) Meloy Laboratories Inc., Biological Products Division, Springfield, Virginia, U.S.A.

(**) Cappel Laboratories Inc., Downington, U.S.A.

Titers of fluorescent IgG and IgM antibodies* anti-*S. mansoni* after 3, 8 and 24 weeks of unisexual infections.



* Plotted as the mean of the logarithm of the reciprocal of titers plus 1, as follows: (■—■) IgG against worm sections; (●—●) IgG against liver sections; (■--■) IgM against worm sections; (●--●) IgM against liver sections.

Fig. 1

The overall estimation of worm bound and soluble antigens, and glomerular deposits containing antibody, antigen and complement is presented in Table I. Positivity for glomerular immune complexes was considered only when all three components were present. It was observed that 3 weeks after infection 65% (13/20) of the animals had no worm antigen either in the serum or in the kidney glomeruli, whereas 2 and 6 months after infection these antigens were found in almost all animals. No circulating antigens were detected in the serum samples from 20 normal mice, not included in the Table.

DISCUSSION

Serum immunological abnormalities [1,6,7,10,13,18] and renal involvement [5,12,21,38] after experimental **S. mansoni** infections are well established. Some controversy remains, however, concerning the source of antigens, whether from eggs or worms, which participate in the formation and deposition of immune complexes in glomeruli in schistosomiasis [31]. As for the egg-derived antigens, no direct participation could be determined by using a soluble egg antigen (SEA) [5]. It has been conjectured [39] that eggs could only indirectly enhance the deposition of

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TABLE I

Circulating antigens and glomerular immune complexes in mice harbouring unisexual **S. mansoni** infections

Time of infection	Circulating antigens			Glomerular immune complexes *		
	Number of mice studied	Number of positives	%	Number of mice studied	Number of positives	%
3 weeks	20	7	35	31	17	55
8 weeks	26	14	54	28	23	82
6 months	17	12	71	22	19	86

* Containing antibody + complement + **S. mansoni** antigen.

immune complexes in the kidney after the development of liver granulomas, and a concomitant bypass of such antigens and complexes through portal collaterals into the general circulation. The participation of worm antigens, if any, could be determined through unisexual infections, in the absence of worm's eggs. It has been argued, however, that in unisexual infections the levels of circulating antigens and antibodies are lower than in bisexual infections, 6 months being needed for antibody levels to become comparable to those reached in bisexual infections after 2 months [1,18].

Our results show otherwise: unisexual schistosome infections of mice can induce an early production of antibodies against **S. mansoni**, with increasing titers along the infection. Difficulties in detecting worm antigens in the sera or in kidney immune complex deposits have been reported [16,22] and could be due to the test antisera used. An antiserum anti-**S. mansoni**, which previously revealed parasite antigens in the sera of mice with bisexual infections [12] and the renal lesions of human schistosomiasis [21], enabled us to demonstrate both circulating worm antigens and glomerular immune complexes bearing the schistosome antigen in mice with unisexual infections of **S. mansoni.** In effect, some mice showed circulating worm antigens by the third week of infection and most of them were positive after 6 months. Glomerular deposits were detected in half of the infected mice after 3 weeks and in almost all of them after 6 months. These findings demonstrate that the worm is a powerful source of antigens which might participate in immune complex formation. Furthermore, the presence of worm antigens in mouse sera after 3 weeks of infection suggests that young schistosomula may also be a source of antigens.

Circulating antigens and antibodies, and renal immune complexes increased proportionally during the course of infection. No correlation, however, was found between the worm burden and the serological or renal findings. Technical failure in recognising schistosomula, variability of individual host responses or differences between strains of **S. mansoni** could explain such discrepancy, which is also obtained in bisexual infections. The more frequent detection of parasite antigens in the kidney rather than in the respective serum could be due to the lower sensitivity of the ELISA test as compared to the direct immunofluorescence employed for detecting kidney deposits. The possibility of modulation of antigen release by worms in the course of infection cannot be ruled out.

The present results may have implication on the mechanisms of protective immunity in mice. It has been established that in mice there is a delay in the schistosomula migration on the third week of infection, mediated by an IgG_2 antibody [36]. By this time, however, no protective immunity has yet developed. Mice will become partially protected against reinfection only after the seventh week [37]. Our findings show that the formation of immune complexes, with possible immunopathological consequences occurs before immune protection. This implyes therefore that, considering the apparent risks involved, vaccination procedures using worm-derived antigens should be done with great care.

RESUMO

Antígenos e anticorpos circulantes e imuno-complexos glomerulares em camundongos com infecção unissexual por Schistosoma mansoni

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Com o fim de investigar antígenos circulantes, anticorpos séricos e depósitos glomerulares de imunocomplexos, camundongos foram submetidos a infecções unissexuadas por **Schistosoma mansoni** e sacrificados após 3, 8 e 24 semanas. Antígenos circulantes foram pesquisados pelo método ELISA de duplo anticorpo e encontrados no soro de 35% dos camundongos infectados após 3 semanas, em 54% após 8 semanas e em 71% após 24 semanas de infecção. Os títulos de anticorpos contra antígenos de verme, medidos por imunofluorescência indireta, aumentaram progressivamente desde a terceira semana, os mais altos sendo da classe IgG e detectados após 24 semanas de infecção. Depósitos glomerulares contendo anticorpos, tanto IgG como IgM, complemento e antígeno de verme foram pesquisados por imunofluorescência direta em cortes não fixados de rim. Após 3 semanas, 55% dos camundongos infectados apresentavam imuno-complexos, o mesmo ocorrendo com 82% após 8 semanas; com 24 semanas de infecção, esses depósitos foram encontrados em 86% deles.

A infecção unissexuada exclui os ovos e as substâncias deles derivadas como fonte de antígenos. Portanto, os resultados aqui relatados mostram que antígenos oriundos dos vermes são frequentemente encontrados no rim de animais infectados. O papel desses antígenos no desencadeamento de lesões glomerulares não pode ser completamente definido.

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