Rev. Inst. Med. trop. S. Paulo
39 (5): 271-277, September/October, 1997

DECAY OF ANTIBODY ISOTYPES AGAINST EARLY DEVELOPMENTAL STAGES OF *Schistosoma mansoni* AFTER TREATMENT OF SCHISTOSOMIASIS PATIENTS

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SUMMARY

Antibodies to a number of parasite antigens are found in schistosomiasis patients, and antibodies to early developmental stages were demonstrated to be efficient immunologic markers for the diagnosis of schistosomiasis. In the present study, decay patterns of IgM and IgG antibodies against cercariae and schistosomula were investigated, in comparison to antibodies against worms and eggs in schistosomiasis patients after chemotherapy, for an investigation of seroepidemiologic aspects. Data obtained in the study of 359 serum samples from patients with *Schistosoma mansoni* infection, noninfected individuals, and patients followed-up for a period of 12 to 15 months after treatment provided the basis to postulate a general pattern for the kinetics of antibody decay. Before treatment, the antibody pattern was represented by a unimodal curve, which shifted to a bimodal curve after treatment, and ended with a unimodal curve similar to that for the noninfected group. Different types of antibodies were classified into four categories according to their decay features, and anti-schistosomulum IgM was classified into the moderate-decay category, whereas other antibodies to early parasite stages were classified into the slow-decay category. The present methodology permits the identification of the most suitable antibodies to be detected in field control programs for schistosomiasis or other parasitoses.

KEYWORDS: Schistosomiasis mansoni; Antibody decay; Chemotherapy; Anti-cercaria; Anti-schistosomulum.

INTRODUCTION

Schistosoma mansoni infection represents a serious public health problem in tropical countries because of its high morbidity. In Brazil, a large-scale epidemiologic survey was conducted by the Ministry of Health and about 10% of the population studied was found to be excreting parasite eggs[23].

Schistosomiasis mansoni patients present antibody isotypes to a spectrum of antigen epitopes from different developmental stages of the parasite[4, 9, 10, 12, 25], as well as to a repertoire of circulating parasite antigens[5, 24]. Circulating antigens and antibodies, however, tend to disappear after chemotherapy. In general circulating antigens disappear within a shorter period of time as compared to most antibodies to *S. mansoni*. Nevertheless, immunologic tests based on circulating antigen capture by monoclonal antibodies are not yet available for routine use.

It has long been known[21] that shortly after chemotherapy the levels of specific antibodies increase, boosted by dead parasite antigens, and decay thereafter. According to the initial antibody levels, type of immunoassay, antigen used, the antibodies may disappear within 6 to 12 months or over 5 years after chemotherapy[2, 3, 9]. Some antibodies against egg antigens detected by the circumoval precipiting test (COPT)[2] and by the immunoenzymatic assay (ELISA)[3, 19], and also agglutinating antibodies against worm antigens revealed by the indirect hemagglutination test (IHAT)[9] have been found to be more suitable than other antibodies to monitor patients after chemotherapy.

Antibodies against early developmental stages of *S. mansoni* have been identified but there are few studies concerning the seroepidemiologic aspects of schistosomiasis. In our previous work[12], IgM and IgG antibodies against cercariae and schistosomula were observed to be as efficient immunologic markers for the diagnosis of schistosomiasis in the population as antibodies against adult stages of the parasite.

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Thus, the decay of same antibody isotypes against cercariae and schistosomula were investigated here by following up patients submitted to specific chemotherapy, in comparison to antibodies against adult stages, in order to obtain data that might provide a framework for schistosomiasis control programs.

MATERIALS AND METHODS

Subjects and serum samples

Serum samples were collected from 245 individuals, 108 of them excreting *S. mansoni* eggs and 137 noninfected subjects with negative stool examination. All subjects infected with *S. mansoni* were treated with Oxamniquine (20 mg/kg weight) or Praziquantel (50 mg/kg weight). Serum samples were also collected from 57 patients who agreed to participate in the follow-up study, 3 to 6 months and 12 to 15 months after chemotherapy. These patients comprised males and females ranging in age from 4 to 56 years old, migrants from Brazilian areas endemic for schistosomiasis and living in the City of São Paulo for many years. During the present study they did not return to these endemic areas, and therefore the possibility of reinfection was ruled out. The intestinal form[16] of the disease was found in 37 patients, the hepatointestinal form in 10 and the hepatosplenic form in 10. Noninfected individuals (137) included in the study as a control group comprised males and females ranging in age from 5 to 52 years who were born in nonendemic areas for schistosomiasis of the State of São Paulo with no history of travel to endemic areas.

Parasitological diagnosis

The Kato-Katz quantitative diagnostic procedure[13], with three slides, was applied to all individuals and to follow-up patients, with fecal examinations repeated 3 to 4 times over a period of 3 to 15 months after chemotherapy. Patients with consistently negative results for fecal examination from the 3rd month onwards was considered to be cured.

Immunological tests

Antibody isotypes (IgM and IgG) were detected by the immunofluorescence test using cryostat sections of cercariae, *in vitro* cultivated schistosomula, adult worms and hamster liver with egg granulomata as previously described[12]. Serum samples were also assayed by the indirect hemagglutination test utilizing fresh worm antigen[9]. The antibody titers were transformed to a code using the equation: coded titer = $[\log_2 (\text{titer} \times 0.2)]$ in order to calculate the geometric mean titers (GMT) and the standard deviations, as well as to compare titers before and after treatment using *t*-test statistic, in paired samples[17]. An approximate rate of antibody decay was estimated in terms of titer difference between antibody levels before and about one year after treatment. The antibody decay was also estimated in terms of an arbitrary unit (u) as follows: u = coded antibody titer after treatment/coded antibody titer before treatment.

RESULTS

The Kato-Katz parasitological diagnostic method showed that 108 individuals had an egg excretion rate ranging from 24 to 7,000 eggs per gram feces (epg), and the 57 patients who were followed up had rates ranging from 96 to 1,132 epg, with the median rate being 208 epg. The stools of these patients became negative 3 to 6 months after treatment and remained unchanged even when the examination was repeated 3 to 4 times up to 15 months after treatment, when the number of patients was reduced to 27. Thus we only considered the data obtained for these 27 patients, excluding those for 30 patients who abandoned the study before the end.

The control group of 137 individuals were all negative by the Kato-Katz method.

In the study of antibody decay, serum samples from the 27 patients were divided into two groups: 14 (group A) were assayed by 8 types of immunofluorescence (IF) tests and the remaining 13 (group B) by the indirect hemagglutination (IHA) test because of the low volume of serum available.

The overall serologic data showed that all 14 patients in group A presented antibody decay for at least one of the 8 tests, 4 (29%) of them presented antibody decay in one or two tests, 7 (50%) in 4 to 6 tests, and 3 (21%) in 7 or 8 tests. Also all 13 (100%) patients in group B showed antibody decay by the IHA test.

The decay of different types of antibodies, expressed in terms of coded geometric mean titers (GMT), are shown in Table 1. In general the titers of IgM antibodies were lower than the titers of IgG antibodies. The *t*-test statistic, however, showed that the GMT of the anti-schistosomulum IgM, along with anti-worm IgM, anti-egg IgM, anti-egg IgG and agglutinating antibodies against worms corresponding to periods of 3 to 6 months and 12 to 15 months after treatment differed significantly from the GMT before treatment. Significant *t* values were found for the four former antibody isotypes ranging from 1.808 to 4.693 (d.f. = 13), whereas the agglutinating antibodies presented *t* values of 4.326 and 7.747 (d.f. = 12), respectively for 3 to 6 months and 12 to 15 months after treatment.

The proportions of patients presenting decay of antibodies to different *S. mansoni* antigens, including those who presented seroconversion to negative at 3 to 6 months and 12 to 15 months after treatment are illustrated in Figs. 1 and 2. Decay of agglutinating antibodies was observed in all patients (100%), with seroconversion to negative in 38% and 54% of patients, respectively, at 3 to 6 months and 12 to 15 months after treatment.

Antibody levels were also estimated in terms of units (u), with the level corresponding to that before treatment being 1.00 u and after treatment values higher than 1.00 u indicating increased antibody levels and values lower than 1.00 u indicating a decrease of antibody levels (Table 2). An approximate decay rate (per year) was estimated for different antibodies, and data presented in the last column (0.49 to 0.0 units) indicate short or long lasting antibodies. The IgM antibodies against cercariae 12 to 15 months

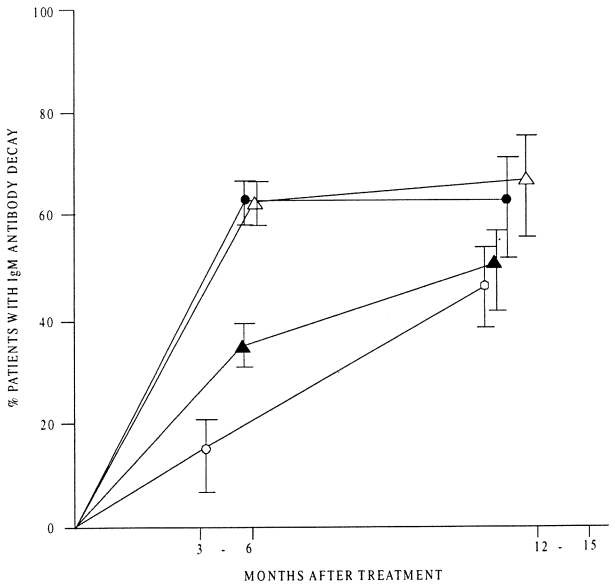

Fig. 1 – Frequency of schistosomiasis patients showing decay of IgM antibody to cercaria (O–O), schistosomulum (●–●), worm (Δ – Δ) and egg (▲–▲) antigens, 3 to 6 and 12 to 15 months after treatment.

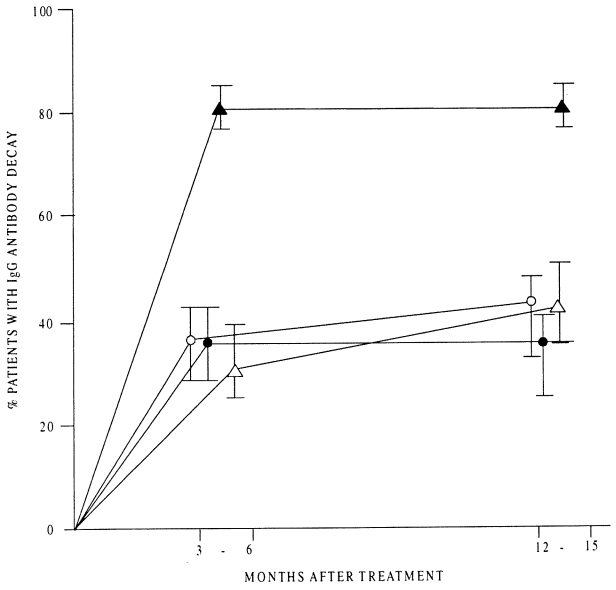

Fig. 2 – Frequency of schistosomiais patients showing decay of IgG antibodies to cercaria (O–O), schistosomulum (●–●), worm (Δ – Δ) and egg (▲–▲) antigens, 3 to 6 and 12 to 15 months after treatment.

after treament ranged from 1.50 u to 0.00 u, those against schistosomula from 3.00 u to 0.00 u, those against worms from 1.00 u to 0.50 u, and those against eggs from 2.00 u to 0.33 u. The IgG antibodies against the same antigens ranged from 1.60 u to 0.67 u, 1.25 u to 0.50 u, 1.50 u to 0.75 u, and 1.25 u to 0.00 u, respectively. In some patients the antibodies increased steeply after treatment and their levels were still higher one year after treatment in comparison to the levels before treatment. The agglutinating antibodies, on the other hand, were present at low levels ranging from 0.67 u to 0.00 u one year after treatment.

The curves in Fig. 3 were obtained by plotting the frequency of antibody titers detected in 108 individuals infected with *S. mansoni* (curve I) and in 137 noninfected individuals (curve II). Since they presented similar trends, single representative curves were constructed for the infected and noninfected groups, based mostly on agglutinating antibodies and anti-schistosomulum IgM antibodies. Theoretically, it was assumed that when chemotherapy is effective in a community, the curve I corresponding to the group of infected subjects will shift to the curve II of the noninfected subjects within a certain period of time.

The frequency of antibody titers from patients of group A 3 to 5 months and 12 to 15 months after treatment, detected by IF tests, as well as antibody titers from patients of group B detected by the IHA test, initially disclosed similar types of curves but the curves showed some differences one year after treatment. These data permitted us to postulate the kinetic patterns of the antibody decay also by constructing representative curves (Fig. 4). The antibodies found before treatment in 14 infected patients consisted of a unimodal curve (P0) which resembled that of the infected group of 108 patients (curve I) in Fig. 3, and because of antibody decays after treatment, the P0 pattern shifted to P1 towards the coordinate axis. The P1 pattern subsequently changes to P2, a bimodal curve, as the seroconversion to negative started to occur among patients. Afterwards the P3 pattern appeared, which was a more pronounced bimodal curve than P2, as the seroconversion to negative continued to occur. Finally it is expected that the P3 curve will become a theoretical unimodal P4 curve, approaching that of the 137 noninfected individuals (curve II) in Fig. 3.

Anti-cercariae IgM and anti-schistosomula IgM showed patterns of antibody decay which changed from the initial P0 to

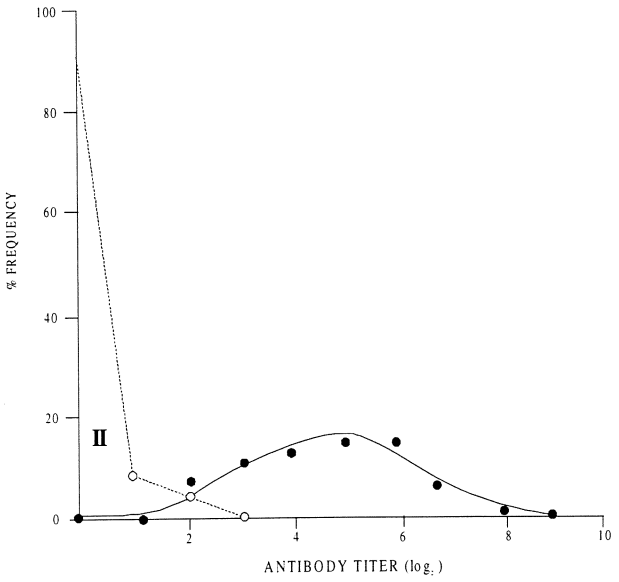

Fig. 3 – Frequency of antibody titers of 108 sera from schistosomiais patients (I) and 137 sera from noninfected individuals (II).

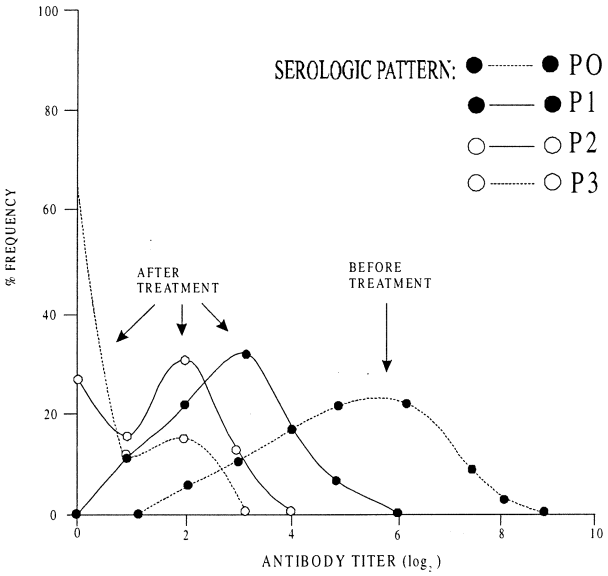

Fig. 4 – Patterns of antibody decays in schistosomiais patients from P0 before treatment and successive shifts to P1, P2 and P3 after treatment.

P1 and reached P2, with 14% and 21% seroconversion to negative occurring within a period of 12 to 15 months after treatment, in a fashion similar to that for anti-egg IgG, although in this isotype the negative results occurred less frequently (7%). Anti-cercariae IgG and anti-schistosomula IgG, on the other hand, presented patterns which shifted from P0 to P1, resembling those of anti-egg IgM, and anti-worm IgM and IgG. In the present study, IgM antibodies to early developmental stages showed changes in pattern up to P2.

Taking together the data obtained in this study, it was possible to rank antibodies into four categories, i.e., I (slow-decay), II (moderate-decay), III (substantial-decay) and IV (fast-decay) (Table 3). Most antibodies against early developmental stages of *S. mansoni* fell into the slow-decay category. But, anti-schistosomulum IgM antibodies were included in the moderate-decay category, similar to the anti-egg IgM and anti-worm IgM, because of significant GMT decay 12 to 15 months after treatment.

DISCUSSION

Antibodies against early *S. mansoni* developmental stages were traced in schistosomiasis patients after chemotherapy. The antibody decays provide signals for successive shutdowns occurring in the specific immune responses, mainly polarized towards a T helper 2-type response[7], in those patients whose immunoregulatory mechanisms were fully operative before treatment. In children with schistosomiasis infection these mechanisms, particularly in the B cell compartment, are suggested to differ significantly from those of adults[8], probably because their immune system is not yet completely mature. In this study, however, the follow-up patients were all considered to be adults since their ages ranged from 17 to 49 years.

All patients followed up 12 to 15 months after treatment were considered cured by a parasitological criterion, and the decay of antibodies revealed by 8 serologic tests also suggested that "immunologic recovery" occurred in most patients. However, 29% patients had decay in only one or two types of antibodies, in contrast to the remaining 71% who exhibited decays in 4 to 8 types of antibodies. It is possible that the former group of patients had an incomplete cure, since chemotherapy has been reported to fail in about 10% to 40% of schistosomiasis patients[1, 18]. Thus, chemotherapy seems to affect parasites to a different extent, still leaving a few crippled parasites or possibly leaving a few parasites of the same sex. Under these circumstances, parasite egg outputs are not seen and although the majority of antibodies will persist, some types of antibodies may decay to some extent. If the treatment is repeated in these patients, significant decays of different antibody types are observed[22]. Thus, it is difficult to determine the patients who were completely cured and those who were not, but the 29% patients showing poor antibody decay were considered to be low-responders to chemotherapy, whereas the 50% patients who had decay in 4 to 6 types of antibodies were considered to be medium-responders, and the remaining 21% patients with decay in 7 to 8 types of antibodies were considered to be high-responders. An influence of the type of drug, Oxamniquine or Praziquantel, on the immune response was not observed, since these three types of immune responders comprised patients who were treated either with the former or latter drug.

In this study, serological data were interpreted based on the findings of anti-egg IgG and agglutinating antibodies because both antibody types have been found to be sensitive for chemotherapy control[2, 3, 9, 19]. The agglutinating antibodies were detected here using fresh worm antigen because these antigens yielded sensitive results in the follow-up studies of patients after chemotherapy, in comparison to other types of worm preparations[11, 15] mostly obtained from freeze-dried worms.

Generally speaking, based on standard deviations, the proportion of patients showing anti-cercaria IgM was similar to the proportion of patients showing anti-egg IgM antibody decay, whereas the proportion of patients showing anti-schistosomulum IgM antibody decay was similar to that of patients showing anti-worm IgM antibody decay. However, 12 to 15 months after treatment all four types of antibodies showed similar features (Fig. 1).

The IgG antibodies (Fig. 2) against these early developmental stages of *S. mansoni* in turn showed features similar to those of IgG against worms, but differed significantly from anti-egg IgG. On the other hand, the anti-egg IgG presented features similar to those of the anti-schistosomulum IgM and anti-worm IgM antibodies.

The geometric mean titers (GMT) showed that IgM and IgG antibodies against early developmental parasite stages decreased slowly compared with the reference antibodies, anti-egg IgG and agglutinating antibodies (Table 1). The anti-schistosomulum IgM antibodies, however, demonstrated more significant decay than the anti-cercariae IgM, though both

TABLE 1

Decay of antibodies against different developmental stages of *Schistosoma mansoni*, expressed as coded geometric mean titers (GMT), before and after treatment of schistosomiasis patients.

Type of antibodies	Before treatment GMT (sd)	After treatment 3-6 months GMT (sd)[a]	After treatment 12-15 months GMT (sd)
IgM[b]			
Anti-Cercaria	2.1 (2.0)	1.8 (1.1)	1.6 (1.2)
Anti-Schistosomulum	2.0 (1.4)	1.5 (1.0)*[d]	1.4 (1.3)*
Anti-Worm	3.0 (1.5)	2.2 (1.3)*	1.9 (1.2)*
Anti-Egg	1.5 (1.1)	1.1 (0.8)*	1.0 (0.6)*
IgG[b]			
Anti-Cercaria	4.1 (0.5)	3.9 (0.7)	3.7 (0.4)
Anti-Schistosomulum	4.9 (0.4)	4.9 (0.8)	4.8 (1.2)
Anti-Worm	5.5 (1.2)	5.5 (1.1)	5.3 (1.7)
Anti-Egg	5.8 (0.4)	4.0 (1.2)*	3.7 (1.7)*
Agglutinating[c]			
Anti-Worm	5.3 (0.9)	1.6 (1.5)*	0.9 (0.9)*

a (sd): Standard deviation; *b* (IgM and IgG): Antibody isotypes detected by immunofluorescence tests; *c* (Agglutinating): Antibodies detected by the indirect hemagglutination test; and d (*) $p < 0.05$ (with before treatment).

antibodies presented P2 decay pattern at the end of this study (Table 2). In some instances the GMT represents a valuable seroepidemiologic tool because of their correlation with prevalence of infection as observed with anti-worm antigens[20].

The decay rate of different types of antibodies was also better demonstrated when their levels were transformed into units, with a value of 1.00 attributed to any antibody titer before treatment. This criterion provided a fast quantitative evaluation of the increase or decrease of antibodies after chemotherapy. Thus the anti-schistosomulum IgM antibodies were observed to decay (< 0.79 u) in a higher proportion (64%) of patients than other antibodies to early developmental stages. This proportion, however, was lower compared to the reference anti-egg IgG (72%) and agglutinating anti-worm (100%) antibodies.

The antibody patterns (Fig. 3) of infected and noninfected groups of individuals were similar to those shown by other serological tests[6], presenting a small overlapping area because of the use of whole crude antigens. In a group of schistosomiasis patients submitted to successful chemotherapy, it was postulated that the kinetics of antibody decay disclosed by different quantitative tests underwent several pattern shifts, among which three were considered basic. A typical unimodal curve for untreated patients observed before treatment shifted thereafter to a bimodal curve and then became a different unimodal curve typical of noninfected individuals (Fig. 4). The period of time required to change from the first to the last pattern depends on the type of antibody concerned, which may last from 3 to 6 months or 5 years after treatment as reported for antibodies against adult stages of the parasite[2, 3, 9].

In the present study antibodies were ranked into four categories, and antibodies against early developmental stages of *S. mansoni* were observed to belong to the slow-decay (I) category, except the anti-schistosomulum IgM classified as moderate-decay (Table 3). The slow-decay and moderate-decay antibodies are suitable for the diagnosis of schistosomiasis since they are not influenced much by worm burden. In our previous investigation[14] several different types of antibodies to *S. mansoni* were found to be adequate for the hypoendemic areas for schistosomiasis because their sensitivities were not affected by the intensity of infection. Among these types of antibodies included the anti-worm IgG and anti-egg IgM, belonging to slow-decay and moderate-decay categories, respectively. Conversely, several other types of antibodies, such as those referred to here as agglutinating antibodies, although being sensitive in the presence of a high intensity of infection, show decreased sensitivity as the intensity of infection diminished. Therefore the latter type of antibodies were suggested to be appropriate to trace antibody decay after treatment.

In schistosomiasis control programs, the type of antibody to be detected is a relevant issue because some of them, although suitable for serodiagnosis, are not appropriate for the follow-up evaluation of treated individuals.

The anti-schistosomulum IgM antibodies were included in the moderate-decay (II) category, and these may give some helpful information since their decays are significant at 3 to 6 months after treatment in 64% patients, showing seroconversion to negative in one patient, and a year after treatment an evident P2 pattern of antibody decay as several patients (21%) presented seroconversion to negative.

The antibodies against cercaria and schistosomulum antigens are under investigation showing IgG subclasses combined with

TABLE 2

Antibody levels to different developmental stages of *Schistosoma mansoni* in schistosomiasis patients 12 to 15 months after treatment.

Type of antibody	% Patients (sd)[a], according to Antibody Units[b]		
	≥ 1.00 to 0.80	0.79 to 0.50	0.49 to 0.0
IgM[c]			
Anti-Cercaria	57 (13.7)	0	43 (13.7)
Anti-Schistosomulum	36 (13.3)	7 (7.0)	57 (13.7)
Anti-Worm	28 (12.5)	36 (13.3)	36 (13.3)
Anti-Egg	50 (13.9)	7 (7.0)	43 (13.7)
IgG[c]			
Anti-Cercaria	64 (13.3)	36 (13.3)	0
Anti-Schistosomulum	79 (11.3)	14 (9.6)	7 (7.0)
Anti-Worm	93 (7.0)	7 (7.0)	0
Anti-Egg	28 (12.4)	43 (13.7)	29 (12.6)
Agglutinating[d]	0	8.0 (7.0)	92 (7.0)

a (sd): Standard deviation, *b* (Unit): Antibody unit equal to 1.00 before treatment, *c* (IgM and IgG): Antibody isotypes detected by immunofluorescence tests, *d* (Agglutinating): Antibody detected by the indirect hemagglutination test.

TABLE 3

Antibodies against different developmental stages of *Schistosoma mansoni* ranked on the basis of their decay features 12 to 15 months after treatment of schistosomiasis patients.

Category	Type of antibody	% Sera with Ab[a] decay	Ab decay rate	Type of profile
I	IgM[b] Anti-Cercaria	43	0.5	P2
(Slow-decay)	IgG[b] Anti-Cercaria	50	0.4	P1
	IgG Anti-Schistosomulum	36	0.1	P1
	IgG Anti-Worm	50	0.2	P1
II	IgM Anti-Egg	50	0.5	P1
(Moderate-decay)	IgM Anti-Schistosomulum	64	0.6	P2
	IgM Anti-Worm	71	1.1	P1
III (Substantial-decay)	IgG Anti-Egg	79	2.1	P2
IV (Fast-Decay)	Agglutinating[c] Anti-Worm	100	3.6	P3

a (Ab): Antibody; *b* (IgM and IgG): Antibody isotypes detected by immunofluorescence tests; and *c* (Agglutinating): Antibodies detected by the indirect hemagglutination test.

antigenic bands, so as to determine further their decay features, since the IgG_4 antibodies to cercariae are thought to decrease shortly after treatment[8].

RESUMO

Queda de isotipos de anticorpos contra formas evolutivas jovens do *S. mansoni* após tratamento de pacientes com esquistossomose

Em pacientes com esquistossomose, são encontrados anticorpos contra grande número de antígenos parasitários, e aqueles contra formas evolutivas jovens do parasita demonstraram que eram eficientes marcadores imunológicos para o diagnóstico da esquistossomose. Padrões de queda de anticorpos IgM e IgG contra cercária e esquistossômulo foram aqui estudados, comparativamente aos dos anticorpos contra verme e ovo, em pacientes esquistossomóticos após quimioterapia, abordando aspectos soroepidemiológicos. Dados obtidos no estudo de 359 amostras de soros, pertencentes a pacientes infectados por *Schistosoma mansoni*, indivíduos não infectados e pacientes acompanhados pós-tratamento por um período de 12 a 15 meses, forneceram base para postular padrão geral da cinética de queda de anticorpos. Antes do tratamento, o

padrão de anticorpos é representado por curva unimodal, que se modifica para curva bimodal após tratamento, e finaliza com curva unimodal semelhante à do grupo de indivíduos não infectados. Diferentes tipos de anticorpos foram classificados em quatro categorias, conforme características apresentadas na queda, e o anticorpo IgM anti-esquistossômulo foi considerado como pertencente à categoria de anticorpos com queda moderada, enquanto que os demais anticorpos contra formas evolutivas jovens do parasita, à categoria daqueles com queda lenta. A metodologia aqui empregada permite identificar anticorpos potencialmente mais adequados a serem detectados em programas para controle da esquistossomose ou de outras parasitoses no campo.

ACKNOWLEDGEMENTS

This work was supported by grants from the Fundação Nacional de Saúde (FUNASA), Hospital das Clínicas da Faculdade de Medicina USP – LIM 06, Fundação E. J. Zerbini, Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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Recebido para publicação em 18/07/1997
Aceito para publicação em 31/10/1997