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IMMUNE RESPONSE IN DIFFERENT CLINICAL GROUPS OF SCHISTOSOMIASIS PATIENTS

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In endemic area, the human host acquires schistosomiasis slowly over a long period of time. The initial phase of the infection is generally asymptomatic and clinical manifestations of acute phase are unusual, and occurs almost eclusively in visitors to endemic area primary exposure to schistosome infection. Most of the infected patients gradually develop a chronic phase usually not associated with clinical disease. Severe manifestation of the disease occur in a relatively small percentage of the infected population and is characterized by liver enlargement which after a variable period of 5 to 15 years may be associated with splenomegaly (Prata & Bina, 1968) due to congestion and reticulo-endothelial hyperplasia of the spleen (Magalhães-Filho & Coutinho-Abath, 1960; Cheever & Andrade, 1967). This organomegaly is most likely established as a consequence of granulomatous reaction to schistosome eggs lodged in the liver and intestinal tissues with development of fibrous and subsequent portal hypertension (Warren, 1979). It should be stressed that not every patient with organomegaly has established portal hypertension. Enlargement of the liver and spleen has been frequently observed in acute schistosomiasis (Striver, 1984).

The determining factors leading to the development of hepatosplenic schistosomiasis are not completely known. While the intensity of patient's infection is an important contributing factor (Cheever, 1968; Kloetzel, 1962; Cook et al., 1974) it remains unclear why the vast majority of those living in endemic areas and infected with *Schistosoma mansoni* develop only intestinal symptomatology while some patients develop hepatosplenomegaly, portal hypertension and esophageal varices. Genetic factors probably are relevant to the development of the disease. Heaptosplenic schistosomiasis is less frequent in black patients than in while or mullato patients with similar levels of infection (Prata & Schroeder, 1967) and it has been more often observed in patients of blood group A (Camus et al., 1977). There is, however, conflicting reports in relation to HLA type and hepatosplenic schistosomiasis.

The regulation of immune responses to schistosome infection must certainly play a role in the development of the disease as well as in the partial resistence to infection believed to exist in certain persons. Several regulatory mechanisms have been described in human schistosomiasis (Colley et al., 1977; 1978; Todd et al., 1979; Ottesen, 1979; Ottesen & Poindexter, 1980; Rocklin et al., 1980; 1981; Barsoum et al., 1983; 1984) and the absence of such mechanisms has been correlated with the hepatosplenic disease (Ellner et al., 1981; Colley et al., 1986). A brief report of the recent progress in our understanding of the immune response in relation to different clinical groups of schistosome infected patients are presented herein.

IMMUNEREGULATION OF PERIPHERAL BLOOD MONONUCLEAR (PBMN) RESPONSE TO SCHISTOSOME ANTIGENS

Early in the acute phase most of the patient's lymphocytes express high reactivity to schistosome antigens (Ottesen et al., 1978; Gazzinelli et al., 1985). This reactivity is higher to soluble egg antigen (SEA) than to adult worm antigen (AW) in contrast to chronically infected patients when the response to AW was higher than that observed to SEA (Fig. 1). Furthermore, parallel study of PBMN proliferation responses in groups of children and adults chronically

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infected indicated that this loss of reactivity observed in the chronic phase was significantly greater in adults than in children (Fig. 2) suggesting that there is a gradual decline of reactivity as the infection progresses. The loss of reactivity to schistosome antigens is correlated with several suupressor mechanisms developing during the chronic phase which limit or modulate the responsiveness to these specific antigens. These mechanisms include serum factors (Colley et al., 1977; Ottesen & Poindexter, 1980) adherent mononuclear cells (Todd et al., 1979; Ottesen, 1979) and suppressor T cells (Colley et al., 1978; Rocklin et al., 1981).

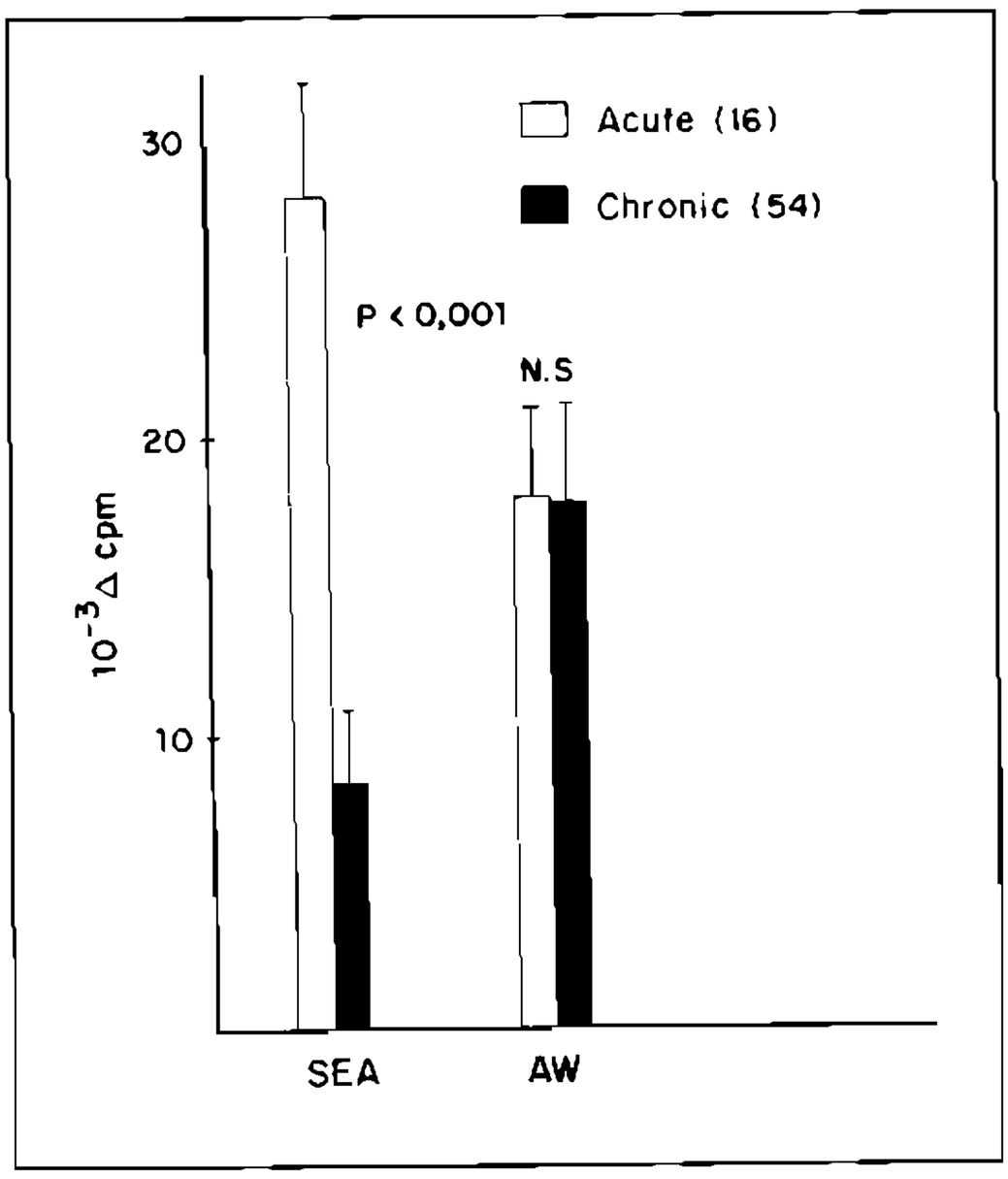

Fig. 1: mean tritiated thymidine incorporation by peripheral blood mononuclear cells (PBMN) from patients with acute or chronic schistosomiasis. Cultures were exposed to medium alone (control) or either 25 μg of soluble egg antigens (SEA) or 20 μg of adult worm antigens (AW) as described in Gazzinelli et al. (1985). In parenthesis the number of patients studied.

Cellular immune responses to schistosome antigens evaluated by the level of ^{3}H-TdR incorporation vary widely among individual patients and clinical groups. Based on the degree of PBMN responsiveness to SEA we designated groups of patients studied as non-responder to moderate (Δcpm $<$ 8000) and high responder (Δcpm $>$ 8000) (Colley et al., 1986). The percentage of high responders in each group to SEA antigen is presented in Fig. 3. It is clear that levels of SEA-induced responsiveness differed among the clinical groups studied. Most patients (94%) from the acute group express high response to SEA. The majority of acute patients then progress to the intestinal stage of chronic schistosomiasis infection during which only 25% retaining high responsiveness. A high percentage of chronically infected individuals who can be identified clinically as being hepatointestinal and compensated (ambulatory) hepatosplenic expressed, however, higher levels of SEA-stimulated response than the intestinal group. Eventually hepatosplenism becomes severe and decompensated and in these cases half of the population exhibits unresponsiveness to SEA possibly due to additional pathogenic mechanisms. In fact in these patients imbalances in their general lymphocyte phenotypes (low T4:T8) were observed (Colley et al., 1983; Gastl et al., 1984).

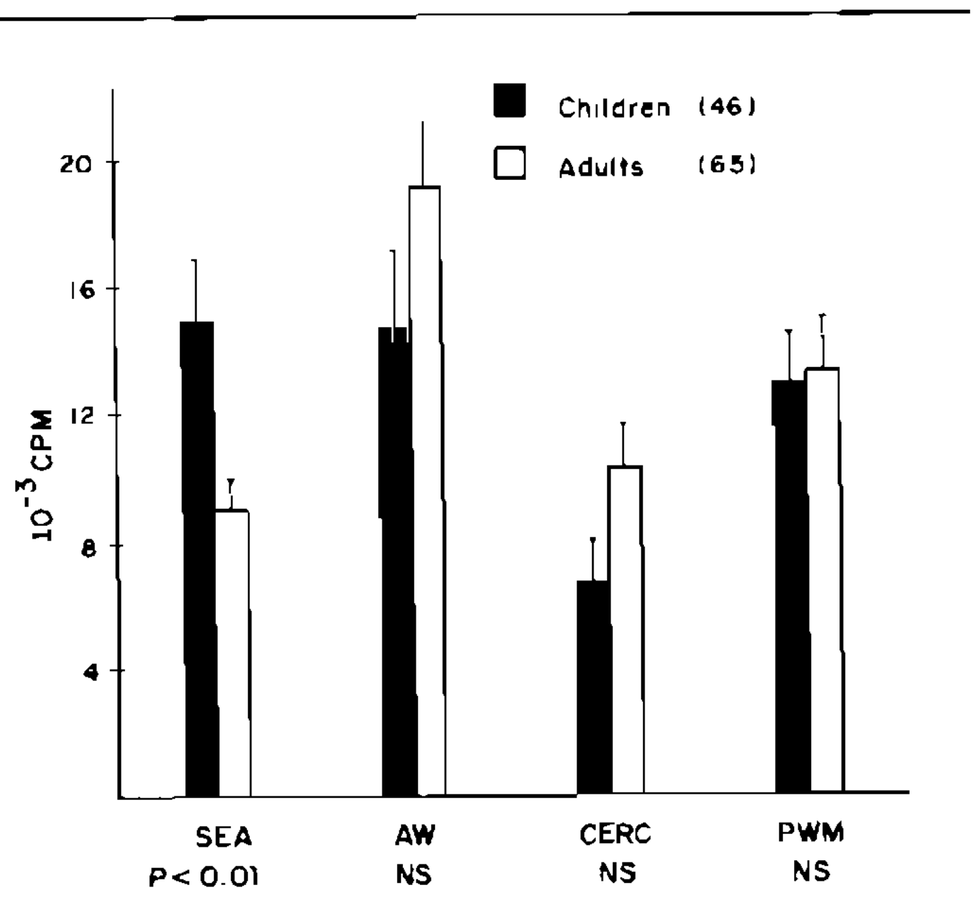

Fig. 2: mean tritiated thymidine incorporation by PBMN from children and adults with chronic (Intestinal) schistosomiasis mansoni. Cultures were exposed to medium alone (control), the mitogen pokeweed (PWM) and the antigens SEA, AW and Cerc (Cercaria). In parenthesis the number of patients studied. Experimental conditions as in Fig. 1.

Several studies in endemic areas indicate that chronic intestinal infection can evolve to hepatointestinal or hepatosplenic disease and the percentage of the population that presents with hepatointestinal disease is always higher than that with early compensated hepatosplenic disease and that is again higher than the proportion that appear with decompensated hepatosplenic (Brener & Mourão, 1956; Katz &

Brener, 1966; Sleigh et al., 1985). Because of the progressive decrease in these percentages, we propose that some patients in the early, clinically-involved groups eventually do regulate their responses and spontaneously proceed to the chronic intestinal form of the infection, rather than on to the decompensated hepatosplenism (Colley et al., 1986). We further suggested that the high percentages of SEA-induced response in groups of former patients and uninfected individuals in endemic areas (Fig. 3) is due to their loss of immunoregulatory constrains upon adequate treatment or perhaps spontaneous cure.

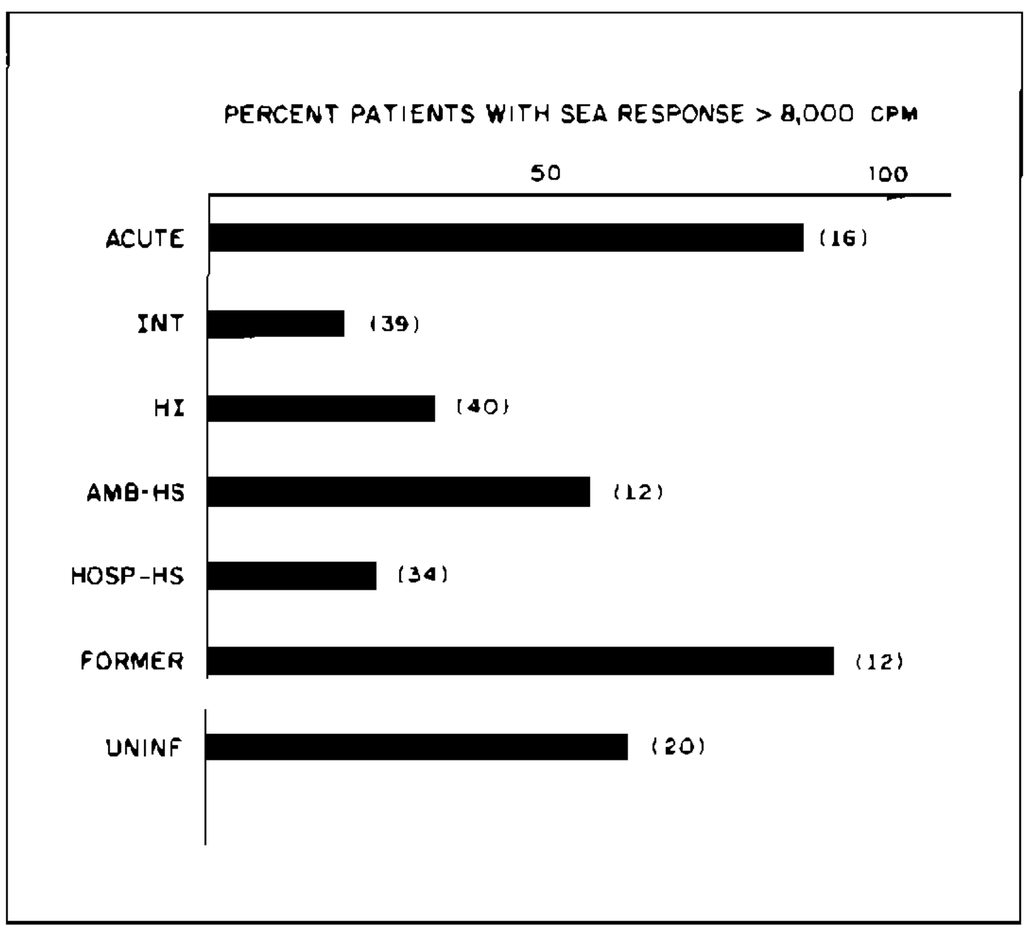

Fig. 3: percentage of patients who were high responder (measured by thymidine incorporation) in the following clinical groups: Acute; Intestinal (I); Hepato-intestinal (HI); Hepatosplenic compensated (HS_{Amb}); Hepatosplenic decompensated (HS_{Hosp}); Former (patients treated and cured from 7-40 years previously); Uninf (Uninfected individuals from endemic area during the period of observation 2,5 years). In parenthesis the number of patients studied.

Based on the above considerations we proposed that a more severe pathology is expected in those patients who were not able to modulate their own immune response to SEA antigens. Many studies have demonstrated that the intensity of the infection is a key determinant in the evolution, persistence and regression of hepatic and splenic enlargements (Cheever, 1968; Kloetzel, 1962; Cook et al., 1974). According to our hypothesis, however, this determinant might be considered in relation to the potential ability of each patient to modulate its own anti-SEA responsiveness.

Further evidences for the relationships between immunoregulation and morbidity is presented in the next section.

IMMUNOREGULATION OF HUMORAL RESPONSE AGAINST SCHISTOSOME ANTIGENS

Modulation of the host response to schistosome antigens is not restricted to cell mediated immunity (Ottesen, 1981). Further aspects on the modulation of specific antibody response is considered in this section. Specific antibodies levels in sera from patients with acute schistosomiasis mansoni differred considerably from those with chronic infection, but was unrelated to the intensity of infection (Colley et al., 1985). In addition, the SEA antigen used in our study was not able to discriminate between sera from individuals with or without hepato splenomegaly by ELISA technique (Table I). A significantly higher sero-reactivity in persons with hepatosplenomegaly, however, has been observed with other antigens (Mott & Dixon, 1982). Also, higher levels of lethal antibody were detected in sera of hepatosplenic patients as compared to those from hepatointestinal and intestinal groups (Dusse, 1986). Western blot analysis against SWAP antigens revealed that a large number of antigens are recognized by sera from *S. mansoni* infected patients and no clear pattern was observed between individuals of the same clinical group. Nevertheless in contrast to intestinal and hepatointestinal groups most sera of patients from the hepatosplenic group recognized 66 and 14 kDa antigens (Corrêa-Oliveira, manuscript in preparation) suggesting that the anti-soluble adult worm antigens (SWAP) specificities changed during the development of hepatosplenic disease.

The anti-schistosome antibodies were, further analysed by studying their idiotypic expression in regard to clinical groups. Two approaches have been used on this study: 1) patients PBMN cellular reactivity against potential idiotypes expressed by antibodies against SEA as previously described (Lima et al., 1986); 2) search for "cross-reactive" dominant idiotypes in an idiotype/anti-idiotype competitive ELISA assay. The anti-idiotypic sera were prepared by immunizing rabbits with affinity purified anti-SEA antibodies from pooled patient sera. These rabbit anti-idiotypic sera were made id-specific by multiple passages over normal human Ig columns, until they reacted only with patients anti-SEA antibodies and not with normal human Ig (Montesano et al., manuscript in preparation).

TABLE I

ELISA against SEA by using sera of different clinical groups of *Schistosoma mansoni* infected patients

Patients	I	HI	HS_{Amb}	C
		A_{490} at 1:100 serum dilution		
Children	.290 ± .134 (48)	.252 ± .138 (13)	.269 ± .156 (10)	.100 ± .049 (8)
Adults	.296 ± .124 (69)	.250 ± .072 (4)	.235 ± .121 (7)	

(I) As described in Gazzinelli et al. (1985); C = control.

TABLE II

PBMN proliferation upon exposure to anti-SEA idiotype obtained from pooled sera of different clinical groups

Anti-SEA* preparations from:	PBMN from:			
	Intestinal (Adults)	Intestinal (Children)	Hepato intestinal	Hepato splenic
Intestinal (Adults)	8/9	4/4	4/5	2/3
Intestinal (Children)	0/9	0/4	0/5	0/3
Hepatointestinal	9/9	4/4	5/5	3/3
Hepatosplenic				
Compensated	0/9	0/4	0/5	0/3
Decompensated	0/9	0/4	0/5	0/3
Acute	0/9	0/4	0/5	0/3

* Immunoaffinity purified anti-SEA from pooled sera.
Figures indicate no. of patients stimulated/tested.
Experimental conditions as described by Lima et al. (1986).

PBMN stimulation by anti-SEA idiotypes: anti-SEA antibodies from pooled sera of acute or from pooled sera of children with chronic infection (intestinal) were not able to stimulate PBMN from patients of any clinical group, suggesting that the stimulatory idiotypes seen in chronic adult sera, which do stimulate, require a long time to develop. Most of the PBMN responses tested, irrespective of the clinical group, were strongly stimulated by pooled anti-SEA idiotype prepared from chronic intestinal or hepatointestinal. Two preparations of anti-SEA idiotype from HS did not stimulate any of the nine patient's PBMN tested (Table II). These preliminary results indicate that dominant stimulatory idiotypes were expressed on anti-SEA antibodies derived from intestinal (I) and hepatointestinal (HI) patient's sera. Coincidently, a higher percentage of individuals able to modulate their own immune response belongs to these clinical groups (Fig. 1) establishing a possible relationship between id/anti-id interaction and immunoregulation in schistosomiasis patients.

Search for "cross-reactive" dominant idiotype: Many anti-SEA idiotypes, both stimulatory and non-stimulatory in the proliferative assay and prepared from pooled sera of defined clinical group of patients were tested in the competitive ELISA system mentioned above. The anti-idiotypic ELISA system was set up as follows: the immunizing anti-SEA antibodies from chronic adult patients (pooled sera) were bound to the wells; and rabbit anti-idiotypic antibodies, biotin-conjugated goat anti-rabbit Ig, streptavidin conjugated peroxidase and finally substrates added in this order observing the required incubation time. This anti-anti-id ELISA was then used as competitive assay by adding various dilutions of the competing idiotype preparations in combination with a fixed concentration of rabbit anti-id against idiotypes from intestinal group (Montesano et al., manuscript in preparation). Addition of the original (immunizing) idiotype preparation (AM1) competed with itself (Fig. 4). Preparations from HI (AM 7) and HS (AM8 and AM3) pooled sera also competed but to a

lesser extent. Idiotypes prepared from acute (AM9) or chronically infected children (AM4) did not compete at all. These data suggested that during the intestinal and hepatointestinal chronic phases there was a gradual change of idiotypes expressed by the anti-SEA antibodies leading to the development of stimulatory cross- reactive idiotype which are apparently not expressed in the hepatosplenic disease. Suppression of B-cells that express cross-reactive, regulatory idiotype without altering the overall magnitude of the response has been shown to be mediated by suppressor T cell in several experimental systems (Bona & Paul, 1979; Dohi & Nisonoff, 1979; Hirai & Nisonoff, 1980). In addition regulation of granulomatous inflammation around parasite eggs has been reported in experimental schistosomiasis (Olds & Kresina, 1985; Powell & Colley, 1987).

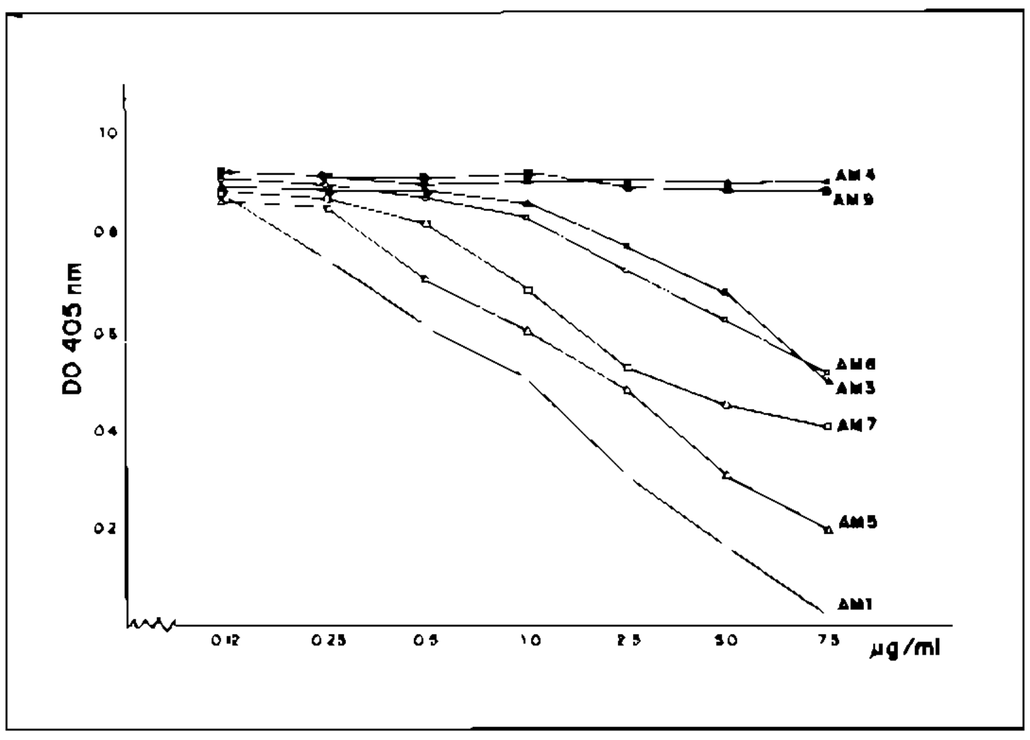

Fig. 4: inhibition of idiotype (AM1)/anti-idiotype (anti-AM1) binding by: itself (AM1), AM5 (anti-SEA from intestinal patients), AM7 (anti-SEA from hepato-intestinal patients), AM3 and AM8 (anti-SEA from hepatosplenic patients), AM4 (anti-SEA from chronic infected children) and AM9 (anti-SEA from acute patients). To anti-SEA (AM1) bound to ELISA plate was added 100 µl of a mixture of anti-AM1 (sera from rabbit immunized against AM1 at 1:250 dilution) plus the anti-SEA (AM) above specified at concentrations indicated in the abcissa. Color was developed by the system described in the text.

In conclusion, our current study suggests that the expression of a certain stimulatory cross-reactive idiotypes on anti-SEA antibodies are correlated with the immunoregulation of chronic human schistosomiasis, and the degree or extention of this immuneregulation phenomena is possibly an additional determining factor in the development of hepatosplenic disease.

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REFERENCES

BARSOUM, I. S.; TODD, C. W.; HABIB, M.; EL ALAMY, M. A. & COLLEY, D. G., 1983. The effects of indomethacin on *in vitro* peripheral blood mononuclear cell reactivity in human schistosomiasis. *Parasite Immunol., 5:* 441-447.

BARSOUM, I. S.; DAHAWI, H. S. S.; GAMIL, F. A.; HABIB, M.; EL ALAMY, M. A. & COLLEY, D. G., 1984. Immune responses and immuno-regulation during human schistosomiasis in Egypt. II. Cimetidine reversal os histamine-mediated suppression of antigen-induced blastogenesis. *J. Immunol., 133:* 1576-1580.

BONA, C. & PAUL, W. E., 1979. Cellular basis of regulation of expression of idiotype. T-suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med., 149:* 592-603.

BRENER, Z. & MOURÃO, O. G., 1956. Inquéritos clínico-epidemiológicos em focos endêmicos da esquistossomose mansoni em Minas Gerais. *Rev. Bras. Malariol. D. trop., 8:* 519-526.

CAMUS, D.; BINA, J. C.; CARLIER, Y. et al., 1977. Grupos sanguíneos A, B, O e formas clínicas da esquistossomose mansônica. *Rev. Inst. Med. trop. São Paulo, 19:* 77-79.

CHEEVER, A. W., 1968. A quantitative post-mortem study of schistosomiasis mansoni in man. *Am. J. Trop. Med. Hyg., 17:* 38-64.

CHEEVER, A. W. & ANDRADE, Z. A., 1967. Pathological lesions associated with *Schistosoma mansoni* infection in man. *Trans. R. Soc. Trop. Med. Hyg., 61:* 29-39.

COLLEY, D. G.; LEWIS, F. A. & GOODGAME, R. W., 1978. Immune responses during human schistosomiasis mansoni. IV. Induction of suppressor cell activity by schistosome antigen preparations and concanavalin A. *J. Immunol., 120:* 1225-1232.

COLLEY, D. G.; HIENY, S. E.; BARTHOLOMEW, R. K. & COOK, J. A., 1977. Immune responses during human schistosomiasis. Regulatory effect of patient sera on human lymphocyte blastogenic responses to schistosome antigen preparations. *Am. J. Trop. Med. Hyg., 26:* 917-925.

COLLEY, D. G.; KATZ, N.; ROCHA, R. S.; ABRANDES, W.; DA SILVA, A. L. & GAZZINELLI, G., 1983. Immune responses during human schistosomiasis mansoni. IX. T-lymphocyte subset analysis by monoclonal antibodies in hepatosplenic disease. *Scand. J. Immunol., 17:* 197-302.

COLLEY, D. G.; GARCIA, A. A.; LAMBERTUCCI, J. R.; PARRA, J. C.; KATZ, N.; ROCHA, R. S. & GAZZINELLI, G., 1986. Immune responses during human schistosomiasis. XII. Differential responsiveness in patients with hepatosplenic disease. *Am. J. Trop. Med. Hyg., 35:* 793-802.

COOK, J. A.; BAKER, S. T.; WARREN, K. S. & JORDAN, P., 1974. A controlled study of morbidity of schistosomiasis in St. Lucian school

children, based on quantitative egg excretion. *Am. J. Trop. Med. Hyg., 23:* 625-633.

DOHI, Y. & NISONOFF, A., 1979. Suppression of idiotype and generation of suppressor T cells with idiotype conjugated thymocytes. *J. Exp. Med., 150:* 909-919.

DUSSE, L. M. S., 1986. *Citotoxicidade de soros de pacientes infectados com* Schistosoma mansoni *contra esquistossômulos* in vitro. Ms. Thesis (Instituto Oswaldo Cruz), Rio de Janeiro.

ELLNER, J. J.; OLDS, G. R.; OSMAN, G. S.; EL KHOLY, A. & MAHMOUD, A. A. F., 1981. Dichotomies in the reactivity to worm antigen in human schistosomiasis mansoni. *J. Immunol., 126:* 300-312.

GASTL, C. H.; FELDMEIER, H.; DOEHRING, E.; KORTMANN, C.; DAFFALLA, A. A. & PETER, H. H., 1984. Numerical and functional alterations of lymphocytes in human schisto chistosomiasis. *Scand. J. Immunol., 19:* 469-479.

GAZZINELLI, G.; LAMBERTUCCI, J. R.; KATZ, N.; ROCHA, R. S.; LIMA, M. S. & COLLEY, D. G., 1985. Immune responses during human schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. *J. Immunol., 135:* 2121-2127.

HIRAI, Y. & NISONOFF, A., 1980. Selective suppression of the major idiotypic component of an anti-hapten response by soluble T cell-derived factors with idiotypic or anti-idiotypic receptors. *J. Exp. Med., 151:* 1213-1221.

KATZ, N. & BRENER, Z., 1966. Evolução clínica de 112 casos de esquistossomose mansoni observados após 10 anos de permanência em focos endêmicos de Minas Gerais. *Rev. Inst. Med. trop. São Paulo, 8:* 139-142.

KLOETZEL, K., 1962. Splenomegaly in schistosomiasis mansoni. *Am. J. Trop. Med. Hyg., 11:* 472-476.

LIMA, M. S.; GAZZINELLI, G.; NASCIMENTO, E.; PARRA, J. C.; MONTESANO, M. A. & COLLEY, D. G., 1986. Immune responses during human schistosomiasis mansoni. Evidence for anti-idiotypic T lymphocyte responsiveness. *J. Clin. Invest., 78:* 983-988.

MAGALHÃES FILHO, A. & COUTINHO-ABATH, E., 1960. Lesões esplênicas iniciais na esquistossomose mansônica humana. *Rev. Inst. Med. trop. São Paulo, 2:* 251-259.

MOTT, K. E. & DIXON, H., 1982. Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bull. WHO, 60:* 729-753.

OLDS, R. G. & KRESINA, T. F., 1985. Network interactions in *Schistosoma japonicum* infection. Identification and characterization of a sorologically distinct immunoregulatory auto anti-idiotypic antibody population. *J. Clin. Invest., 76:* 2338-2347.

OTTESEN, E. A., 1979. Modulation of the host response in human schistosomiasis. I. Adherent suppressor cells that inhibit lymphocyte proliferative responses to parasite antigens. *J. Immunol., 123:* 1639-1644.

OTTESEN, E. A. & POINDEXTER, R. W., 1980. Modulation of the host response in human schistosomiasis. II. Humoral factors which inhibit lymphocyte proliferative responses to parasite antigen. *Am. J. Trop. Med. Hyg., 29:* 592-597.

OTTESEN, E. A.; POINDEXTER, R. W. & HUSSAIN, R., 1981. Detection, quantitation and specificity of antiparasite IgE antibodies in human schistosomiasis mansoni. *Am. J. Trop. Med. Hyg., 30:* 1228-1237.

OTTESEN, E. A.; HIATT, R. A.; CHEEVER, A. W.; SOTOMAYOR, Z. R. & NEVA, F. A., 1978. The acquisition and loss of antigen-specific cellular immune responsiveness in acute and chronic schistosomiasis in man. *Clin. Exp. Immunol., 33:* 38-47.

POWELL, M. R. & COLLEY, D. G., 1987. Anti-idiotypic T-lymphocyte responsiveness in murine schistosomiasis mansoni. *Cell. Immunol., 104:* 377-386.

PRATA, A. & SCHROEDER, S., 1967. A comparison of Whites and Negroes infected with *Schistosoma mansoni* in a hyperendemic area. *Gaz. Med. Bahia, 67:* 93-98.

PRATA, A. & BINA, J. C., 1968. Development of the hepatosplenic form of schistosomiasis. *Gaz. Med. Bahia, 68:* 49-60.

ROCKLIN, R. W.; TRACY, J. W. & EL KHOLY, A., 1981. Activation of antigen-specific suppressor cells in human schistosomiasis mansoni by fractions of soluble egg antigens nonadherent to Con A Sepharose. *J. Immunol., 125:* 1916-1923.

ROCKLIN, R. W.; BROWN, A. P.; WARREN, K. S.; PELLEY, R. P.; HOUBA, V.; SIONGOK, T. K. A.; OUMA, J.; STURROCK, R. F. & BUTTERWORTH, A. E., 1980. Factors that modify the cellular-immune response in patients infected by *Schistosoma manson mansoni. J. Immunol., 125:* 1916-1923.

SLEIGH, A. C.; HOFF, R.; MOTA, E. A.; SHERLOCK, I.; MOTT, K. E.; BARRETO, M. L.; MAGUIRE, J. H. & WELLER, T. H., 1985. Three-year prospective study of the evolution of Manson's Schistosomiasis in northeast Brazil. *The Lancet, July 13:* 63-66.

STRIVER, P. C., 1984. Acute schistosomiasis (Katayama fever). *British Med. J., 288:* 221-222.

TODD, C. W.; GOODGAME, R. W. & COLLEY, D. G., 1979. Immune responses during human schistosomiasis mansoni. V. Suppression of schistosome antigen-specific lymphocyte blastogenesis by adherent/phagocytic cells. *J. Immunol., 122:* 1440-1446.

WARREN, K. S., 1979. The pathogenesis of hepatosplenic schistosomiasis: from man to monkey to mouse to molecule, p. 439-455. In Popper, H. & Schaffner, F., *Progress in liver disease*, vol. VI. New York, Grune and Stratton.