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INDUCED TOLERANCE TO *SCHISTOSOMA MANSONI* ANTIGENS MODULATES PERIOVULAR GRANULOMA

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Immunological tolerance to Schistosoma mansoni *antigens induced by oral exposure of neonatal and adult mice to adult worm, soluble egg and polysacaride antigens conduced to modulated periovular granuloma of infected mice. However the tolerance do not interfere in the infection. The estimative population and subpopulation of lymphocytes in the spleen of tolerized (not infected) animals do not differ from normal animals but Lyt 2.2 reactive lymphocytes to Schistosoma antigens was demonstrated in the tolerized animals.*

The *Schistosoma mansoni* egg induces a granulomatous reaction which evolve to fibrosis and is the primary factor responsible to hepatosplenic disease (Warren, 1961; Andrade & Warren, 1964). It has been shown that in this inflammatory granulomatous host response participate a mecanism of cellular hypersensitivity (Warren et al., 1970). Diminution of the granulomatous reaction has been achieved with immunosupressive drugs (Domingos et al., 1967), neonatal thymectomy (Domingo & Warren, 1967) and heterologous antisera to lymphocyte and macrophage (Domingo & Warren, 1968; Boros & Warren, 1971). Experimental work in mice has shown that offspring of heavily infected female mice presented granuloma around the *Schistosoma* eggs smaller than those formed in offspring of non infected female mice suggesting an induction of tolerance by the *Schistosoma* antigens passed by the milk or across the placenta (Lewert & Mandlowitz, 1969).

Experimental induction of tolerance in mice by intravenous injection of soluble egg antigen was reported reduce granuloma diameter (Hang et al., 1974). The present work describes the effect of tolerance induced by oral exposure of mice to adult worm, soluble egg and polysacaride antigens in the *S. mansoni* infection.

MATERIAL AND METHODS

To Swiss mice, SJL were given orally 20 μg of total adult worm antigen (WA), soluble egg antigen (SEA) or Polysscaride antigen (PA) (Nash et al., 1974) in alternating days during four weeks.

To each antigen were used two groups of mice: one group constituted of young adult mice and other group constituted of new-born mice. One week after completed the treatment with antigens all the animals were infected with 60 cercarias of *S. mansoni*. As control were used young adult mice which were infected with 60 cercarias of *S. mansoni* but were not treated with antigens. All the animals were sacrificed 60 days after infection. Sera collected before infection and before sacrifice were used for immunofluorescence test (Andrade & Sadigursky, 1978). Three days before the sacrifice the animals were submitted to skin test for delayed type hipersensitivity with soluble egg antigen. All the infected mice were perfused to collect the worms. Sections of the organs were fixed in 10% formalin and processed for histologic study. The diameter of the granulomas were measured in a Olympus microscope equiped with a micrometric ocular OSM. In another experiment groups of animals treated oraly with antigens but not infected were used to evaluate lymphocyte subpopulation. Clones of Lyt 2.2 lymphocytes specifics to *S. mansoni* antigens were evaluate by panning technique (Taniguchi & Miller, 1977) and immunofluorescence test.

RESULTS AND DISCUSSION

After oral treatment the immunofluorescence test to evaluate antibodies against worm and egg was positive in 20% of animals

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in the group adult treated with worm antigen and in 33% of animals in the group adult treated with soluble egg antigen.

In both two groups the titer was 1:5. All the other groups were negative. After infection, the sera collected at the sacrifice had the following titers: 1:20 in the control group; 1:10 in the group adult treated with WA; 1:5 in the group new-born treated with WA; 1:10 in the group adult treated with SEA and 1:5 in the group new-born treated with SEA. Both the two groups of animals treated with Polissacaride antigen had titer 1:20 equal to the group control. The skin test for delayed type hipersensitivity was negative in all the groups just after the oral treatment. Before the sacrifice the skin test was slight positive but the foot pad reaction occured one hour after injection of antigen and decreased slowly. After 36 hour the foot pad had the same thickness as before the injection of antigen. That reaction was caracterized as a humoral type reaction.

The number of worms collected after perfusion was similar in all the groups. Were recuperated 14 worms (23%) in the control group and 11 (18%); 13 (22%); 17 (28%); 13 (22%); 12 (20%); 11 (18%) respectively in the following groups: adult/WA, newborn/WA, adult/PA, newborn/PA, adult/SEA and newborn/SEA.

There was not statisticaly significant difference between the subpopulation of spleen lymphocytes in the different groups. (B cells: 15-25%; Lyt 1.2:41-51% and Lyt 2.2:11-15%). Nevertheless the clones of Lyt 2.2 lymphocytes specifically reactive to Schistosome antigens were expanded in tolerized mice (5.1-8.7% in tolerized mice, versus 0,47% in normal mice). The effect of induced tolerance was better seen by measurement of periovular granuloma. The Table shows the mean and standard error of the diameters of the periovular granuloma in different groups. As can be seen the granulomas were smaller in the groups of mice treated since the birth and particularly smaller in the mice treated with soluble egg antigen. There was not a statistically significant difference (t. student) between the groups of mice treated with Polissacaride antigen and the control group. Histologicaly the granulomas in the tolerized mice were predominantly of the proliferative type.

TABLE

Effect of Induced Tolerance with *S. mansoni* antigens on periovular granuloma size

Group	Inductor antigen	Mean diameter of granuloma ± S. E. (μm)
Control	–	392 ± 2.87
Newborn	Total worm	325 ± 3.98
Adult	Total worm	309 ± 2.36
Newborn	Polyssacaride	341 ± 2.25
Adult	Polyssacaride	342 ± 1.45
Newborn	Soluble egg	256 ± 3.61
Adult	Soluble egg	274 ± 3.27

The data shown in the present paper permit conclude that *S. mansoni* antigens given oraly results in a hyporesponsiveness with diminished periovular granulomas in the liver and low titer of antibodies to *S. mansoni* antigens. The induced hyporesponsiveness do not interfere with the infection. The number of recuperated worms was the same expected for not treated animals. The induced hyporesponsiveness alone do not alter the relative number of the subpopulations of lymphocytes but induces the expansion of clones of lymphocytes Lyt 2.2 reactives to *S. mansoni* antigens.

Hyporesponsiveness was reported first by Lewert & Mandlowitz in 1969 in offspring of mice heavily infected with *S. mansoni* in a phenomenon that they ascribed to acquired tolerance. Hang et al. in 1974 confirmed those observations but both the two reports do not clarify the possibility of tolerance be induced by antigens passed by the placenta or in the milk. In this present investigation the tolerance was induced by antigens given oraly what suggest that the *S. mansoni* antigens passed in the milk of infected mother can also induces tolerance in the offspring.

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