Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 82, Suppl. IV: 283-284, 1987
Intern. Symp. on Schistosomiasis

LEVELS OF LETHAL ANTIBODY DURING THE COURSE OF INFECTION WITH *SCHISTOSOMA MANSONI* IN RATS AND MICE

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Schistosoma mansoni *infected hosts produce an IgG that mediates the complement-dependent killing of schistosomula* in vitro. *In this study, we followed the levels of serum lethal antibody during infection of rats and mice. Rats presented detectable lethal activity early in the course of infection with a peak in the 6-8th week of infection. This activity declined to non-detectable levels within 2 weeks, remaining low up to the 20-26th week. In mice, lethal antibody was not detected before 7-12 weeks of infection, but raised to higher levels, as compared to non-infected animals, up to 20-24 weeks after infection. We correlate lethal antibody and protective immunity suggesting that the antibody-mediated complement-dependent cytotoxicity to schistosomula play a role in the immunity to reinfection.*

A number of reports have shown that, following an exposure with cercariae of *Schistosoma mansoni,* experimental hosts develop an immunity to further infection with the parasite. Extensive studies, particularly with rats and mice, showed that the acquired immunity to reinfection is directed towards young schistosomula (Perez, H. et al., 1974, *Parasitology, 69:* 349-359; Sher, A. *J. Infec. Dis., 130:* 626-633). Although several immune mechanisms have been shown to kill schistosomula *in vitro*, none has been conclusively demonstrated to participate in protective immunity *in vivo.* We have reported, however, that the same IgG isotype that kills young schistosomula in the presence of complement *in vitro* (lethal antibody) has the ability to confer protective immunity to normal rats (Horta, M. F. M. & Ramalho-Pinto, F. J., 1984, *J. Immunol., 133:* 3326-3332). To further investigate the relationship between *in vitro* lethal antibody activity and *in vivo* protective immunity, we have followed the levels of serum lethal antibody activity during infection in two well defined models of immunity to *S. mansoni* – the rat and the mouse.

Immune sera were weekly obtained from outbred Holtzman or inbred Lewis albino rats (IRS) and inbred C57B1/10J, CBA/J and C3H/HeJ mice (IMS) percutaneously exposed to 500 or 30 cercariae (L. E. strain, Belo Horizonte), respectively. *In vitro* lethal activity of immune sera was determined by using a complement-mediated cytotoxic assay (Horta, M. F. M. & Ramalho-Pinto, F. J., 1984, *J. Immunol., 133:* 3326-3332), slightly modified. Briefly, 250 mechanically prepared schistosomula (Ramalho-Pinto et al., 1974, *Exp. Parasitol., 36:* 360-372) were incubated for 30 min at room temperature with either individual rat sera or a pool of mouse sera from each week of infection at serial dilutions ranging from 1:4 to 1:128 in flat-bottomed 96-well plates. The excess serum proteins was washed out and antibody-coated larvae were cultured overnight with fresh guinea-pig serum, as source of complement, at a final dilution of 1:25. After 16-18 h at 37 ^{o}C and in 5% CO_2/95% air, the percentage of dead or damaged schistosomula were determined. The lethal activity of sera at each week of infection was evaluated by determining the Effective Dilution 50 (ED 50), the inverse of serum dilution that promoted death of 50% of the parasites. ED 50 was calculated by linear regression of curves obtained by plotting the percentage of dead schistosomula *versus* the serum dilution. The levels of lethal antibody were expressed as the mean of the ED 50 (± S. E.) obtained from the titration curves of 3-17 individual rat sera or 2-6 experiments with mice sera at each week.

The lethal antibody activity was followed during infection for 20-26 weeks in rats and 20-24 weeks in mice. Outbred Holtzman rats developed detectable levels of serum lethal antibody from the 4th week onward. The level of lethal antibody raised steadily to the 7th or 8th week, when it reached a peak with an ED 50 of 48 ± 13 and 51 ± 13, respectively. After the 8th week the lethal activity decreased

This research was supported in part by grants from CNPq, FINEP and FAPEMIG.

suddenly and reached an ED 50 of 3 ± 2 at 12 weeks. From 14 to 20 weeks after infection no detectable lethal activity was observed in IRS. In inbred Lewis rats lethal activity also became apparent 4 weeks after primary infection, but maximum activity was observed one week earlier than in Holtzman rats, when ED 50 of reached 151 ± 15. Lethal antibody titers decreased thereafter to very low levels up to the 26th week. Upon secondary infection with the same number of cercariae, when serum lethal activity was already negligeable, Lewis rats developed a typical anamnestic response. They presented high levels of serum lethal antibody with an ED 50 of 826 ± 392 in the 6th week after the reinfection. This activity, as in primary infection, did not last for the subsequent weeks.

Mice presented an entirely different kinetics of lethal antibody development, as compared to rats. Lethal antibody was not detected before 7-13 weeks of infection. This activity, unlike in rats, remained significantly higher than in non-infected animals up to 20 or 24 weeks after infection. By this time, levels of lethal activity reached ED 50 of 44 ± 18 in C57B1/10J, 26 ± 6 in C3H/HeJ and 15 ± 2 in CBA/J mice.

With regard to the development of acquired immunity, several studies showed that rats and mice also behave quite differently. Rats exposed to cercariae develop a resistance to reinfection which becomes evident early in the first few weeks following infection. This immunity reaches a peak around the 7-9th week after exposure to cercariae, decreasing to very low or non detectable levels by 12-17 weeks, depending on the strain of rat (Perez et al., 1974, *Parasitology, 69:* 349-359; Phillips et al., 1978, *Cell. Immunol., 38:* 225-238). Although there may occur slight variations in the kinetics of development of the acquired immunity in different strains of rats, the pattern of a transient strong immunity is common to all strains studied. Nevertheless, higher numbers of immunizing cercariae can induce an immunity that lasts for longer periods (Phillips et al., 1978, *Cell. Immunol., 38:* 225-238). After immunity had declined, reinfection produces an anamnestic response within 2 weeks (Perez et al., 1974, *Parasitology, 59:* 349-359; Phillips et al., 1977, *Cell. Immunol., 28:* 75-89).

Unlike rats, infected mice develop a chronic disease similar to that developed by man (Warren, K. S., 1972, *Trans. R. Soc. Trop. Med. Hyg., 66:* 417-434). The protective immunity induced in mice appears late in the course of infection, being detected only after the 8-12th week, but it lasts for as long as the infection is active (Dean, D. A., 1983, *Exp. Parasitol., 55:* 1-104).

If we compare the levels of lethal antibody in these hosts to the degree of immunity developed after infection with normal cercariae, we can notice that their patterns of evolution are essentially the same. Although we have not yet assessed protective immunity in the hosts described here, this correlation suggests that acquired immunity to reinfection might operate via an antibody-mediated complement-dependent cytotoxicity to schistosomula.

Our findings are compatible with previously reported results showing that IgG2a, the lethal antibody in rats (Horta, M. F. M. & Ramalho-Pinto, F. J., 1986, *Braz. J. Med. Biol. Res., 19* (4-5): 652A) is possibly the isotype responsible for immunity to reinfection, since a fraction of IRS containing only IgG2a and traces of IgG2b can also confer protective immunity when passively transferred to normal recipients (Horta, M. F. M. & Ramalho-Pinto, F. J., 1984, *J. Immunol., 133:* 3326-3332). Furthermore, previous results from our laboratory demonstrated that, in rats and mice, the immunity to reinfection mediated by antibody is dependent on an intact complement system and not on radiosensitive cells (Góes, A. M. & Ramalho-Pinto, F. J., 1980, *Ciência e Cultura, 32:* 613), which also suggest the antibody and complement mediated cytotoxicity as a mechanism of protective immunity to reinfection.

Taken together, these findings indicate that the protective immunity induced by an infection with *S. mansoni* is mediated, at least in part, by an IgG-dependent activation of complement on parasite surface.