

# Anti-Embryonation Immunity in Murine Schistosomiasis Japonica (Philippines)

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*The hypothesis that granuloma modulation and disease abatement in chronic infection with Schistosoma japonicum could be ascribed to antibody-mediated effects on egg maturation and egg viability, arose from studies performed with mice in the Philippines. This novel hypothesis has not yet been integrated into the schistosomiasis literature despite being formulated more than a decade ago. One reason for this is that the phenomenon might be confined to S. japonicum, even S. japonicum (Philippines).*

Key words: schistosomiasis japonica - granuloma modulation - COP test - egg antigens - egg embryonation - egg viability

Chronic schistosomiasis mansoni and chronic schistosomiasis japonica are immunopathologic diseases that develop following T cell-dependent immune responses to antigens produced by eggs entrapped in organs and tissues, principally the liver and intestinal wall. Manifestations of chronic disease result from obstructive granuloma formation and fibrosis with consequent impedance of blood flow through the liver (Warren 1982). Supposed differences between the inductive events of granuloma formation between *Schistosoma mansoni* and *S. japonicum* that have been highlighted in the past are now de-emphasized. However, there may well be very significant differences in mechanisms of granuloma modulation (see below) between *S. mansoni* and *S. japonicum* infections and even between the different "geographical" *S. japonicum* infections - eg *S. japonicum* (Philippines) versus *S. japonicum* (Chinese). In a previous publication (Mitchell et al. 1991) we have emphasized the features of *S. japonicum* (Philippines) infections that appear to differentiate this parasite from other schistosomes causing hepato-intestinal schistosomiasis (Table, see also Cheever 1987).

A significant advance in the understanding of the immunology of disease in schistosome infections came with the observation that granuloma formation in chronically-infected hosts was modulated; the intensity of granulomatous hypersensitivity and its consequences were decreased in chronic infections (Andrade & Warren 1964, Domingo & Warren 1968). This phenomenon, referred to initially as "endogenous desensitization", has sparked considerable research activity and speculation on the grounds that it opens up a pos-

TABLE

*Schistosoma japonicum* (Philippines): parasite and murine infection characteristics

1. High infectivity of cercariae (>50% infectivity the norm)
2. Highly pathogenic parasite (mice usually exposed to no more than 20-25 cercariae in challenge experiments)
3. Obvious lung petechiae (on day 6 of infection; number correlates with number of adult worms at later time point)
4. Schistosomules detected rarely in lung tissue (rapid transit through lungs)
5. No demonstration of homologous or heterologous protection against infection with *S. japonicum* (Philippines) using irradiated cercariae
6. Early appearance of many young worms in liver (< 1 week of infection)
7. Rapid maturation of worms (egg laying at 24 days; 12-14 day maturation time [embryonation] in tissues; faecal eggs >35 days of infection)
8. Multiple uterine eggs and high egg production (eggs often found as clusters in intestinal wall)
9. No hepatotoxic antigen described in eggs (c.f. *S. mansoni* eggs)
10. Granuloma modulation early in infection (? entirely antibody-mediated)
11. Wide range of definitive (vertebrate) hosts (but faecal egg counts often very low relative to worm burden - e.g. rats).

"a highly infective, potentially highly pathogenic, rapidly-maturing parasite that does not linger in the lungs and in which, during infection, anti-egg immune responses have transmission blocking as well as disease modulating consequences".

sible approach to vaccination against *disease*. The extent of modulation and a modulation-inducing effect of serum factors (presumably antibodies) can be dramatic in the mouse model of *S. japonicum* (Philippines) infection as demonstrated by Olds and colleagues (1982).

In the early 80's we proposed that a key event in granuloma modulation and disease abatement in chronic infection with *S. japonicum* (Philippines) was inhibition of maturation (i.e. inhibition of embryonation) of the egg, and its destruction at the pre-miracidial stage of development. This anti-egg response inhibits production of immunopathologic antigens by the maturing egg and thereby inhibits the formation of T cell-dependent granulomas and subsequent disease. Effector molecules are likely to include anti-egg antibodies but the antigenic specificities of putative anti-embryonation antibodies and/or cells, and the effector mechanisms involved in destruction of maturing eggs, remain unknown (Garcia & Mitchell 1982, 1985, 1987, Garcia et al. 1981, 1983, 1985, 1987, 1989, 1992, Mitchell 1990, Mitchell et al. 1984, Tiu 1988). The essential features of the anti-embryonation hypothesis and supporting data are provided below.

During the course of studies designed to optimize the circumoval precipitin test (COPT) for diagnosis (Garcia et al. 1981), it was noted that eggs harvested at > 70 days of infection in donor rabbits (these eggs to be incubated with human sera in the COPT), performed poorly in the test. Newly laid eggs, as distinct from those containing a miracidium, also perform poorly in the COPT and a possible explanation of the rapid decline in suitability of eggs harvested from infected rabbits is that the eggs are not embryonating because of induced embryonation-inhibiting immune responses (Garcia & Mitchell 1982).

We have been able to demonstrate that fewer uterine eggs matured in the lungs after intravenous injection into egg-sensitized mice compared with unsensitized mouse recipients in a von Lichtenberg - type assay (Garcia et al. 1983). Using this same assay, granuloma formation in lungs of egg-sensitized mice could be inhibited by human sera that produce large segmented precipitates in the COPT (Garcia et al. 1985). Human sera could also inhibit egg maturation in livers of infected mice (Garcia et al. 1985). [Since antibodies in human sera could not be expected to engage efficiently in many immune effector mechanisms in mice, simple binding to antigen and occlusion of pores in the egg shell may reduce metabolite export or nutrient uptake with subsequent maturation arrest and premature death of the maturing miracidium.]

Mice were infected with a low number of cercariae during a five week course of injections of live eggs enriched for immature eggs. When killed at about six weeks of infection, many egg-sensitized infected mice contained a much lower proportion of mature eggs and a higher proportion of dead eggs in livers and intestines relative to mice not immunized with live immature eggs. Fewer granulomas were present around eggs in the liver, splenomegaly was absent and portal pressures were normal (Garcia et al. 1987, 1989). Thus, egg sensitization around the time of infection (with small numbers of cercariae and thus low resultant worm burdens) results in reduced egg maturation, reduced granuloma formation and reduced disease. Antibodies (of unknown specificity) are likely to be the principal effector molecules. Comprehensive reviews on these phenomena are available (Garcia et al. 1992, Mitchell et al. 1994).

The phenomenon of granuloma modulation involving maturation arrest and early death of eggs in chronically-infected vertebrate hosts may be related to another phenomenon - that of skewed sex ratios in schistosomiasis. Male schistosomes predominate over females in many instances (Liberatos 1987, Mitchell et al. 1990). On the basis of some supporting evidence, we have proposed that host immune responses may preferentially eliminate eggs containing female miracidia with the inference that anti-embryonation immune responses (? antibodies) may be directed preferentially to W chromosome-encoded antigens (that, in turn, may be major immunopathologic antigens) (Mitchell et al. 1990, 1991). Moreover, exposure to male-only cercariae in endemic areas prior to exposure to mixed sex cercariae, may result in a degree of resistance to infection (Vogel & Minning 1953). All this will contribute to reduced morbidity in schistosomiasis japonica endemic areas of the Philippines despite the organism being innately highly pathogenic and highly infective.

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