

AN ATTEMPT TO INDUCE RESISTANCE IN MICE TO *SCHISTOSOMA MANSONI* INFECTION USING MILLIPORE DIFFUSION CHAMBERS

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SUMMARY

The Authors have demonstrated that implantation into mice of Millipore diffusion chambers of 0.45 μ pore size containing live *S. mansoni* adult worms results in the production of specific antibodies to the life cycle stages of this parasite. This was determined by the cercarial slide flocculation test and by the Ouchterlony double diffusion method using adult worm and egg antigenic extracts. Challenge of these mice with *S. mansoni* cercariae resulted in an infection rate similar to that of normal controls, in spite of the former group having measurable levels of antibodies. Thus, induction of resistance to *S. mansoni* infection in mice was not attained.

INTRODUCTION

LEVINE & KAGAN⁶ were able to induce partial protection in mice to *Schistosoma mansoni* infection by inoculating metabolic products antigens of this parasite. In order to test this approach further we entertained the possibility that by introducing live worms into the peritoneal cavities of mice using Millipore diffusion chambers of 0.45 μ size we could expose these mice to large amount of excretory and secretory antigens, thereby inducing production of high titers of specific antibody. Preliminary experiments supported our assumption. Anti-adult worm and egg precipitins were demonstrated by the Ouchterlony double diffusion method in sera from mice that had contained these live worms for one week in their peritoneal cavities. The serum had been collected one week after removal of the capsules. It was then desirable to observe if this induction of antibody for-

mation had any relation to resistance to a challenge infection.

MATERIAL AND METHODS

The experimental design was as follows: sufficient mice harboring adult *S. mansoni* worms were perfused to collect 150 pairs of adult worms. Five pairs of worms were introduced into each of 25 sterile Millipore diffusion chambers of 0.45 μ pore size (Millipore Filter Corp., Bedford, Massachusetts), and these chambers introduced into the peritoneal cavities of each of 25 mice (Group I). The procedure was accomplished using sterile equipment and under ultra-violet light. Seven days later the capsules were removed and the liquid present (presumably antigenic) was collected and pooled in sterile tubes and

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diluted 20-fold with physiological saline. These animals were allowed to recover prior to challenge infection. The centrifuged liquid was found to contain 6.7 mgm protein per ml. Group II contained an equal number of mice inoculated with 0.5 ml of this protein liquid and Freund's adjuvant (1:1) twice over a period of two weeks. Group III consisted of 25 mice which were bled before challenge. The following week the mice were bled and exposed one day later, along with 25 untreated mice (Group III) as controls, to 100 *S. mansoni* cercariae (Puerto Rican strain). One week after challenge, Group II received a third inoculation of the liquid protein and adjuvant. Seven weeks later all the mice were bled and perfused for the collection of schistosomes.

Pooled sera from each group were tested for antibody activity by the slide flocculation test of ANDERSON¹. Ouchterlony double diffusion tests using adult worm and egg extracts were performed as described by HILLYER & FRICK³. Serum proteins were observed by immunoelectrophoresis using anti-mouse goat serum (Behring Diagnostics, Woodbury, New York)³.

RESULTS AND DISCUSSION

Preliminary experiments established the following: adult worms could survive in the capsules for 8 days in the peritoneal cavities of mice but not for 15 days; a minimum of two adult worm and egg precipitins were observed in the serum of mice exposed to these adult worms for 7 days, but were not observed in the sera of mice exposed to empty capsules; eosinophiles and large lymphoid cells with basophil granulations penetrated the capsules, indicating exposure of the worms to host cells (This is not unusual and has been reported when using Millipore diffusion chambers of 0.45 μ pore size^{2,7}); and finally, mice receiving empty capsules and subsequently exposed to *S. mansoni* cercariae developed the same number of worms as normal controls.

Worm counts revealed that there was no induction of resistance to *S. mansoni* infection in either of the two experimental groups. The average worm burdens per mouse were

as follows: Group I: 32 ± 15 adult worms; Group II: 28 ± 13 worms; Group III: 29 ± 8 worms. These differences are not statistically significant.

The slide flocculation test revealed that prior to exposure to normal cercariae the groups had the following titers (4^+ maximum): Group I: 4^+ ; Group II: 1^+ ; Group III: Negative. At the time of sacrifice the titers of all groups was 2 to 3^+ . Serum protein levels were likewise elevated in Groups I and II as demonstrated by immunoelectrophoresis. This was particularly evident when the sera were diluted 1:4 and 1:8 and compared with the normal control (Group III). These increases were observed in the α C and in an unidentified α 1 protein fractions.

This experiment provides evidence that production of antibodies as determined by slide flocculation and double diffusion methods can be produced in mice when exposed to live *S. mansoni* adult worms in Millipore diffusion chambers. However, in spite of the presence of circulating antibodies at the time of challenge infection no evidence of resistance to this parasite could be demonstrated. Similar results were obtained in rhesus and green monkeys where precipitating antibodies and protection against schistosomiasis could not be correlated^{4,5}. If specific antibodies do confer resistance to schistosomiasis, be it humoral or cellular, or a combination of both, one must determine the type in order to use it as an index of resistance. We are still confronted with the need to demonstrate this relationship.

RESUMO

Tentativa de produzir resistência à infecção pelo Schistosoma mansoni em camundongos, usando cápsulas Millipore de difusão

Cápsulas de Millipore (porosidade 0,45 μ), contendo esquistossomos adultos (*S. mansoni*), foram implantadas na cavidade peritoneal de camundongos. A formação de anticorpos específicos, nos animais implantados, foi demonstrada pela reação de floculação e pelo método de dupla difusão em ágar usando, como antígenos, extratos homólogos de esquistossomos e de ovos. A exposição des-

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tes animais a cercárias do *S. mansoni* resultou em infecção semelhante à verificada no grupo controle. Portanto, com o método empregado, não foi possível produzir resistência ao *S. mansoni*, a despeito da formação de anticorpos específicos.

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