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VACCINATION AGAINST SCHISTOSOMIASIS IN MICE WITH KILLED SCHISTOSOMULA WITHOUT ADJUVANT

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Abstract. Attempts to develop a killed vaccine against schistosomiasis have generally resulted in failure. There are two recent reports, but unfortunately, harsh adjuvants were used in conjunction with the antigenic materials. In our laboratory, a killed vaccine was developed by freezing (-196°C) and thawing the schistosomula of S. mansoni. The use of such a preparation without adjuvant was effective in vaccinating mice. A worm reduction of 36.4-41.1% was achieved by one vaccinating injection, a 60.2% worm reduction by 3 injections, and a 63.7-66.0% reduction by 5 injections. The sequence of the development and the expression of the immune reactions were similar to those previously found in hosts immunized with highly X-irradiated schistosome organisms. Delayed hypersensitivity was demonstrated in histological sections of the skin in the challenged mice after one vaccination, showing that an adjuvant was not necessary to initiate the induction of cellular immunity.

Introduction. In the past 50 years, attempts to develop a killed vaccine against schistosomiasis have generally resulted in failure (1). Commencing with our first use of the highly irradiated schistosome larvae as a live vaccine against schistosomiasis, the method has been extensively studied in experimental animals with promising results (2-4). Although it has been tried in the field on bovines and a 65-76% worm reduction achieved, there are still some problems with its practical use (5). Therefore, investigators have re-intensified their research for a usable killed vaccine. Horowitz et al. reported that to enhance the level of IgE, a 34-90% worm reduction can be achieved in mice with intraperitoneal injections of a cercarial sonicate absorbed on alum (6). Using adjuvant BCG to initiate the induction of cellular immunity, James achieved a 42-54% worm reduction in mice immunized with a single intradermal/subcutaneous injection of frozen and thawed (FT) schistosomula (7). In both cases, a harsh adjuvant was used. The present paper is a report of our success in vaccinating against schistosomiasis mansoni in mice with FT schistosomular preparation without using adjuvant.

Materials and Methods. Schistosoma mansoni cercariae (Puerto Rican strain) shed from Biomphalaria glabrata snails were used as immunizing and challenge organisms and eight-week old CF1 female mice as experimental hosts. Cercariae were thoroughly washed with bacteria-free, millipore-filtered, dechlorinated tap water and concentrated with a millipore-vacuum apparatus. Schistosomula were transformed from cercariae by the vortex method (8). They were kept in Earle's balanced saline solution containing an additional 0.1% glu-

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Table 1. Percent of worm reduction in mice vaccinated with FT schistosomula and challenged with normal cercariae of *S. mansoni**

Exp. No.	Antigen preparation	No. vaccinations	Mean worm number ± SD recovered from control mice (no. mice)	Mean worm number ± SD recovered from vaccinated mice (no. mice)	% of worm reduction	p value
1	1,000 FT schistosomula	1	30.8 ± 1.6 (5)	19.6 ± 2.5 (5)	36.4	<0.001
2	1,000 FT schistosomula	1	28.2 ± 4.1 (6)	16.6 ± 3.1 (5)	41.1	<0.001
3	1,000 FT schistosomula	3	35.2 ± 5.0 (6)	14.0 ± 1.8 (6)	60.2	<0.001
4	500 FT schistosomula	5	43.4 ± 10.4 (9)	14.5 ± 3.9 (16)	66.6	<0.001
5	500 FT schistosomula	5	41.3 ± 6.4 (6)	15.0 ± 4.1 (6)	63.7	<0.001

* Interval between vaccination (the last vaccination in multiple vaccinations) and challenge: Experiment 1, 37 days; Experiment 2, 60 days; Experiment 3, 33 days; Experiment 4, 30 days; and Experiment 5, 30 days.

cose, 100 u/ml penicillin and 100 µg/ml streptomycin with either 5,000 or 10,000 schistosomula/ml. The preparation was then quickly frozen in liquid nitrogen to -196°C and thawed just prior to use. Immunization was done by intradermal injection of 0.1 ml of the FT schistosomular antigenic preparation. In case of the multiple vaccinations, the interval between two vaccinations was 34 days.

Mice were challenged with 100 normal cercariae 30 to 60 days after one, three, or five immunizations. The control mice were injected only with Earle's balanced saline solution and were challenged at the same time with the same number of normal cercariae from the same batch of snails. All the challenged mice were killed for worm perfusion 40 days after challenge. The non-vitality of the FT schistosomula was confirmed by the methylene blue test and by the absence of worms in lung recovery assays done at day 6 after the immunization (9). For study of dermal immuno-responses to the challenge cercariae, histological examination of skin lesions was made at 30 min, 2, 6, 12 hr, and 1, 2, 3, and 5 days after challenge.

The percent of worm reduction in the vaccinated and challenged mice was calculated from the percent of the recovery of worms with the following formula:

$$\% \text{ of worm reduction} = \frac{\bar{C}-\bar{E}}{\bar{C}} \times 100,$$

where $\bar{E}$ equals the mean recovery from experimental group and $\bar{C}$, the mean recovery from control group. Student's *t*-tests were used to compare mean number of worms recovered in control and vaccinated mice. Separate *t*-tests were computed for each experiment.

Results. Seventy mice were used for five experiments. In each experiment, mice were divided into a control group and a vaccinated group (Table 1). The mice in Experiments 1 and 2 were vaccinated once; in Experiment 3, three times; and in Experiments 4 and 5, five times. In all experiments, there were significant differences between the number of worms recovered from the control group compared to the vaccinated group ($p<0.001$). The worm reduction was 36.4 to 41.1% with one vaccination, 60.2 % with three vaccinations, and 63.7 to 66.6 % with five vaccinations.

Examination of histological skin sections of mice, challenged after one immunization with FT schistosomula, showed only slight tissue reactions before 12 hours. Degranulation of mast cells upon cercarial penetration and accumulation of neutrophils around the migrating schistosomula were seen occasionally. Delayed hypersensitivity was

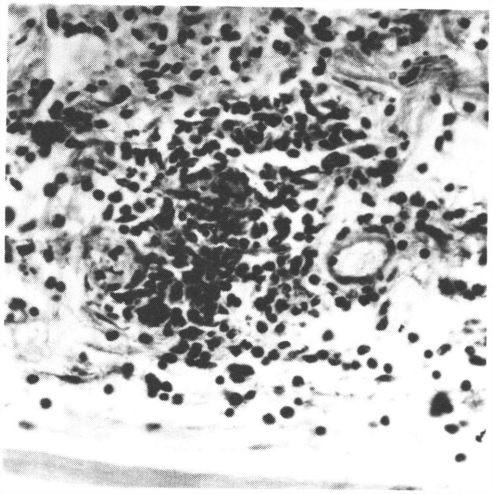

Fig. 1. Skin, areolar tissue below the dermis, 24 hr after challenge with normal cercariae in a mouse which had been immuized once with FT schistosomula, showing a patchy perivascular accumulation of mononuclear cells.

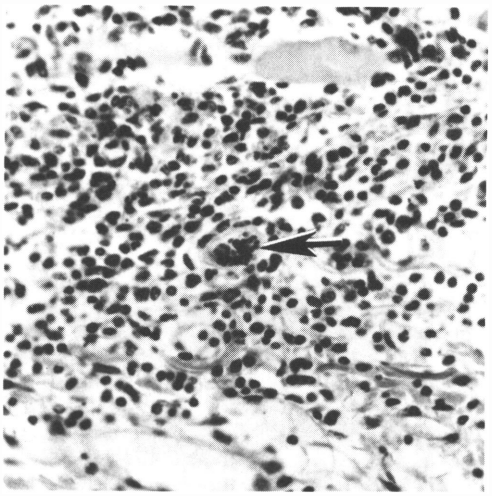

Fig. 2. Skin, subcutis, 48 hr after challenge with normal cercariae in a mouse which had been immunized once with FT schistosomula, showing a schistosomulum (arrow) amid an aggregation of mononuclear cells.

present at 24 hrs and persisted at least 5 days (end of the experiment). In this reactive period, there were moderate multifocal reactions with perivascular accumulation of mononuclear cells (Fig. 1) and mild diffuse infiltrations by lymphocytes. These patchy infiltrations were found mostly in the areolar tissue below the dermis. Schistosomula were sometimes found amid an accumulation of mononuclear cells (Fig. 2). Among them, only a few appeared to be disintegrated.

In the mice challenged after 3 to 5 repeated immunizations, three consecutive hypersensitive reactions--reagenic whealing, Arthus-like, and delayed hypersensitivity-occurred. Both live and disintegrated schistosomula could be found in the accumulation of granulocytes in the early dermal response, 12 hours or before (3) and in the mononuclear cell infiltrations in the later response, 24 hours or later. Most of the disintegrated schistosomula were found amid the granulocytic exudate in the epidermis, the granulocytic aggregates in the dermis and subcutis (Fig. 4), and in the granuloma-like foci in the subcutis.

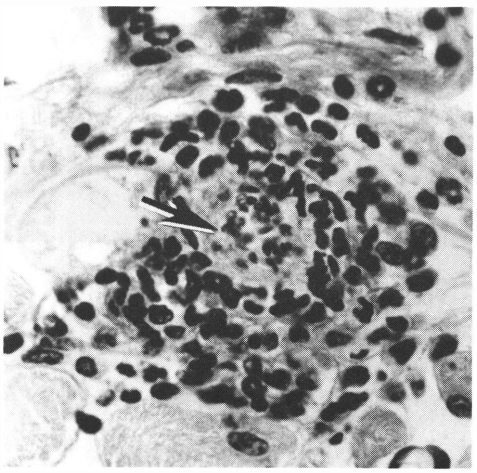

Fig. 3. Skin, subcutis, 12 hr after challenge with normal cercariae in a mouse which had been immunized three times with FT schistosomula, showing an aggregation of neutrophils surrounding partially deteriorated schistosomulum (arrow).

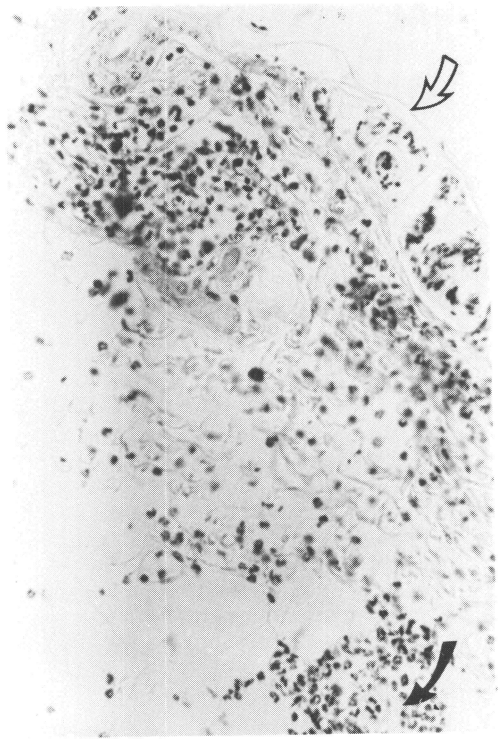

Fig. 4. Skin, 6 hr after challenge with normal cercariae in a mouse which had been immunized five times with FT schistosomula, showing a partially deteriorated schistosomulum (closed arrow) in aerolar tissue below the dermis.

Discussion. The results of this study clearly indicate the possibility of developing a non-living schistosome vaccine without using adjuvant and further support the concepts that schistosome protective immunity is initiated by the cell-mediated immunity and followed by the cooperation of the T and B-cells in the synthesis of the humoral antibodies to complete the effector mechanism (10-16). The present findings of the perivascular infiltration and accumulation of mononuclear cells surrounding the migrating schistosomula in the skin of the challenged mice which have been immunized once indicate that the T-cells necessary for the production of delayed hypersensitivity have been sensitized during the prime immunization of the FT preparation even without BCG. It shows also that upon stimulation by the antigens from the challenging organisms, the sensitized lymphocytes initiate reactions of delayed hypersensitivity against the target schistosomula at the site of infection. The occurrence of the sequential dermal immuno-reactions in the challenged mice immunized repeatedly reflects the involvement of the responses of both sensitized T and B lymphocytes in the effector mechanism, resulting in the death of more schistosomula at the time of exposure.

The variations in induction of cellular immunity noted between James' study and ours may be due to the different strains of the experimental mice or differences in the FT schistosomular preparation, recognizing such factors as time, medium, and especially temperature. The procedure used in our laboratory may minimize the antigenic denaturation resulting in a lesser degree of deviation from live organisms in the killed FT schistosomula so that their function in the induction and expression of immunity toward schistosome still remains similar to that of the live schistosome vaccine (10,16). The FT preparation may be considered a transitional stage between the living and non-living schistosomula, as was proposed by Smithers as he sought to develop a killed vaccine (17).

This is an important finding related to the use of the FT schistosomula for immunization. Further study relative to the mechanism of immunogenicity of schistosome antigens and the regulatory mechanisms of immune responses is needed to attain the final goal of developing an effective killed vaccine.

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