The Fate of Challenge Schistosome Cercariae in a Monkey Immunized by Cercariae Exposed to High Doses of X-Irradiation (35351)

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In a previous publication (1), we have pointed out that a strong acquired resistance against schistosome infection in rhesus monkeys can be induced by previous immunization with cercariae exposed to a large amount of X-irradiation, *i.e.*, 24,000 or 48,000 R. Immunizing cercariae exposed to such high doses of X-irradiation have been found to perish in the skin (2), and their use can avoid the inflammatory reactions which occur in the lungs and liver during the course of immunization (1). Furthermore, under this scheme of immunization, the functional antigen is believed to be entirely derived from the very early stage of schistosomula. The functional antibody which prevents the establishment of the challenge cercariae in immunized monkeys should be antischistosomular antibody. This finding will strengthen the theory of stage specificity of schistosome immunity (3).

The question has been raised that to verify the advantage of immunization by cercariae exposed to high doses of X-irradiation and to support the theory of stage specificity of schistosome immunity, the death of the schistosomula derived from the challenge cercariae should be demonstrated histopathologically mainly in the skin of the challenge monkey (4). Results of a further investigation of this problem are given below.

Materials and Methods. The size of monkey, the source of the cercariae of the Japanese strain of *Schistosoma japonicum*, the method of exposing cercariae to X-irradiation, and the method of exposing monkeys to the immunizing and challenge cercariae were those as described previously (1). A rhesus monkey was immunized with a total of 53,000 X-irradiated cercariae which were divided in 11 doses in a period of 871 days. Cercariae exposed to 48,000 R were given during the 1st, 2nd, 3rd, 4th, 5th, 6th, and 9th immunizations (total 32,000 cercariae); and cercariae exposed to 24,000 R, during the 7th, 8th, and 11th immunizations (total 21,000 cercariae). The number of immunizing cercariae in each immunization varied from 1000 to 9000 and the time intervals between two successive immunizations, from 26 to 281 days. The challenge cercariae (without exposure to X-irradiation) were given 80 days after the last immunization. Altogether 8000 cercariae shed from 60 positive snails were applied on the abdominal wall during the challenge. Among them, 2100 cercariae were applied on seven circumscribed areas of about 15 mm in diameter. Each of these areas was exposed to about 300 cercariae and the skin was biopsied, one area each day, from days 1 to 7. The incised skin was fixed in 10% formalin. The monkey was killed on day 7 and tissues from lungs and liver were fixed in 10% formalin. Paraffin sections 6–8μ thick were made from the fixed tissues and then stained with hematoxylin and eosin.

As the lesion-control, an unimmunized monkey was challenged with the same number of cercariae and in the same manner of application of cercariae as in the process of challenging the immunized monkey. Skin was also biopsied each day from days 1 to 7 and the monkey was killed on day 7. Tissues were fixed and sectioned as for the experimental monkey.

As a control for the infectivity of the challenge cercariae, two albino mice were exposed to 50 cercariae each. The cercariae were from the same source as the cercariae for challenging the above-mentioned monkeys. They were killed at day 34 and perfused for

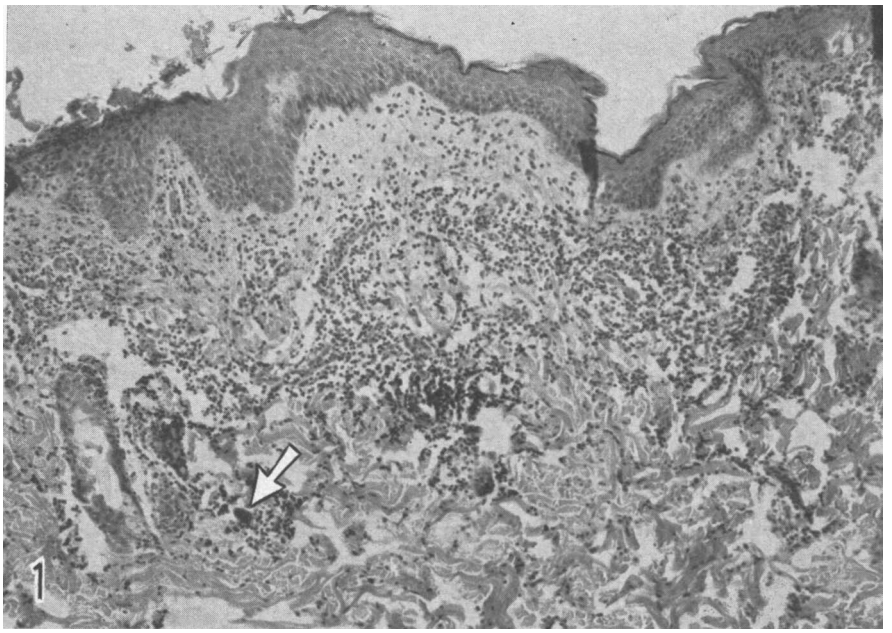

FIG. 1. Skin, immunized monkey, 2 days after challenge, showing the diffuse perivascular infiltration in the dermis. The infiltrates were composed mainly of eosinophils, (arrow) a smudged schistosomulum; ×90.

the number of worms developed in them.

Results; experimental monkey. (a) *Skin.* On day 1 many schistosomula were found in the epidermis and dermis, more in the former than the latter. The schistosomula appeared either intact or with signs of degeneration. In the epidermis, there were areas of superficial ulceration with a fibrinopurulent crust. There were also small intra-epidermal abscesses which were composed of an accumulation of neutrophils. In the dermis, there was a diffuse inflammatory response with margination of neutrophils and eosinophils about small arterioles and capillaries. About half of the number of schistosomula were associated with a mild infiltration of eosinophils, neutrophils, and histocytes. On day 2 the number of schistosomula was about the same as on day 1. However, the schistosomula were mainly found in the dermis and only a few of them in the epidermis. An intra-epidermal abscess was found composed almost entirely of eosinophils, with a partly deteriorated schistosomulum (Fig. 2). In the dermis, the diffuse perivascular inflammatory reaction, with endothelial swelling, was more severe than in the previous day (Fig. 1). Most of the schistosomula were found amid the infiltrate of eosinophils and showed signs of deterioration (Fig. 3). A few eosinophils were observed closely on the surface of the organisms (Fig. 4). On day 3, schistosomula were fewer in number and were present only in the dermis, and the diffuse perivascular inflammatory reaction reached a peak. There were edema and intensive perivascular infiltrates composed predominantly of eosinophils. Most schistosomula showed signs of degeneration. On day 4, only a few deteriorated schistosomula were found in dermis (Fig. 5). Although the diffuse perivascular inflammatory reaction was about the same as in the previous day, lymphocytes and histiocytes began to appear in greater number, along with the predominating eosinophils, in the perivascular inflammatory nodules. From days 5 to 7, no schistosomula were found. The intensity of the inflammatory reaction in the dermis gradually abated so that on day 7, although a few eosinophils remained, the perivascular inflammatory foci were composed mostly of lymphocytes and histiocytes.

(b) *Lungs.* Thirty-four sections obtained from various portions of the lungs of the

monkey killed on day 7 were examined. The average size of sections was 16 × 12 mm. No schistosomula were found although there were occasionally small interstitial linear and nodular infiltrates which ranged up to 0.8 mm in diameter. These infiltrates were composed of histiocytes and granulocytes with approximately an equal mixture of neutrophils and eosinophils. There was an associated mild vascular response with margination of granulocytes about small vessels, swelling of endothelial cells, and a trace of intersti-

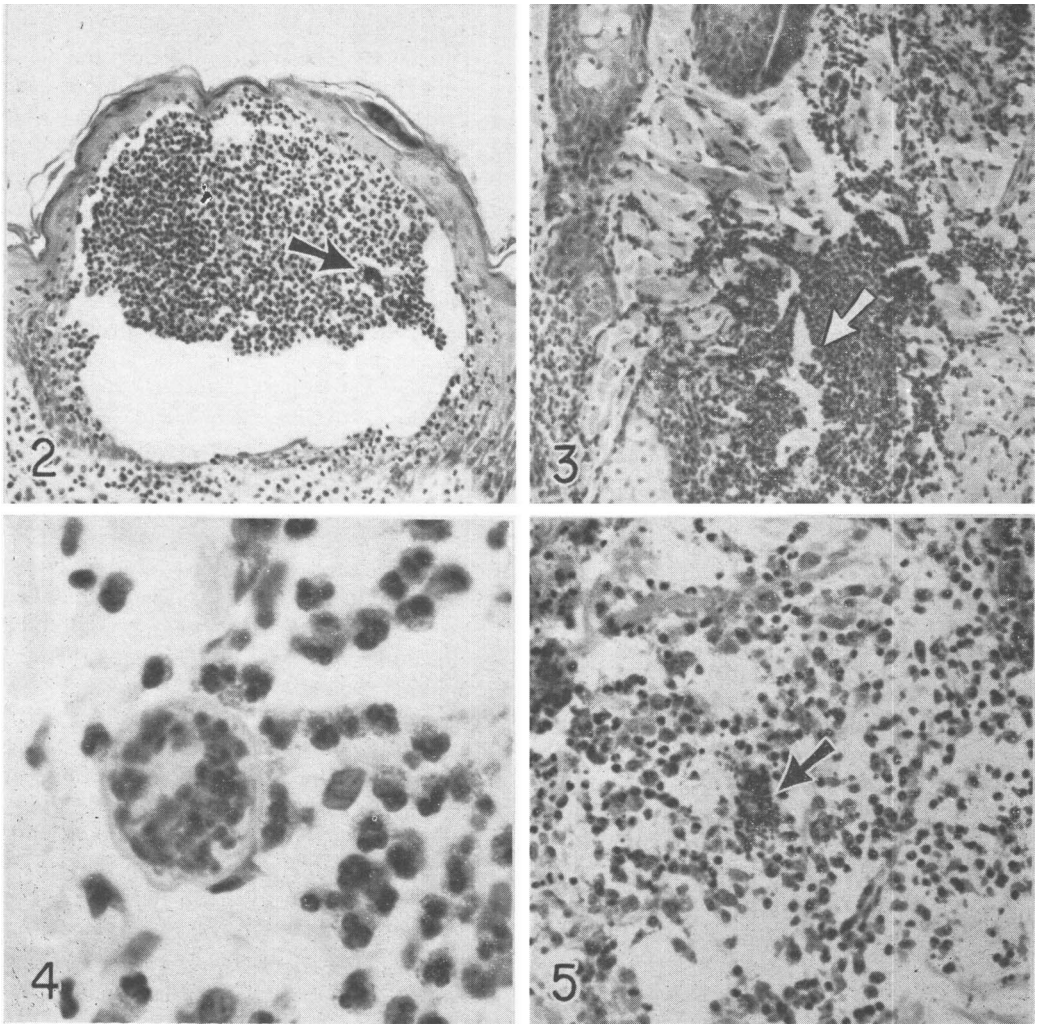

FIG. 2. Skin, immunized monkey, 2 days after challenge, showing an intra-epidermal abscess which was composed almost entirely of eosinophils, with a partly deteriorated schistosomulum (arrow); ×150.

FIG. 3. Skin, dermis, immunized monkey, 3 days after challenge, showing a deteriorated schistosomulum (arrow) amid a nodular infiltrate of eosinophils: The vertical cleft in the nodule is an artefact, ×125.

FIG. 4. Skin, dermis, immunized monkey, 2 days after challenge, showing a schistosomulum amid the infiltrate of eosinophils: Four eosinophils were found closely applied to the schistosomulum; ×625.

FIG. 5. Skin, dermis, immunized monkey, 4 days after challenge: A highly deteriorated schistosomulum (arrow) was found amid the infiltrate which consisted of eosinophils, lymphocytes, and histiocytes; ×275.

tial fibrinous exudate. Only in one section, two small giant cells were found in a nodular infiltrate but there was no accompanying schistosomular remnant.

(c) *Liver.* Neither schistosomula nor other relevant evidence elicited by the schistosomular migration were found.

Results; control monkey. (a) *Skin.* As in the experimental monkey, more schistosomula were found in the epidermis than in the dermis at day 1, and *vice versa* at day 2. Schistosomula were present only in the dermis at day 3, occasionally found in the dermis at day 4, and none were found thereafter. Except for the occurrence of occasional degenerate schistosomula in the stratum corneum of the epidermis, no instances of schistosomular degeneration were encountered in epidermis and dermis. All the schistosomula migrating through the skin were free from intimate inflammatory reaction. Although in the first 3 days there were endothelial swelling and perivascular distribution of the acute exudative reaction, the severity was very mild in degree. The scarcely recognizable infiltrate consisted of half neutrophils, half mononuclear cells, and rarely eosinophils.

(b) *Lungs.* There was a diffuse mild to moderate thickening of alveolar septa with interstitial aggregates of lymphocytes, histiocytes, and occasional neutrophils. There were occasional nodular infiltrates which ranged up to 3 mm in diameter and composed of lymphocytes, large histiocytes, clustered multinucleated giant cells, and neutrophils. Although no intact schistosomula could be found, a minute cuticular remnant was encountered within a giant cell aggregate.

(c) *Liver.* At day 7, a few schistosomula were encountered in the dilated branches of the portal venous tree. The schistosomula appeared normal. Although a mild degree of periportal cellular infiltration was encountered in certain areas, some portal venules containing schistosomula were found to be free from inflammatory change.

Results; control mice. When the two mice were killed at day 34, 18 adult male and 17 female worms were obtained in one mouse and 19 pairs of adult worms from the other mouse.

Discussion. These results indicate that the death of schistosomula originated from the challenge cercariae occurred mainly, if not exclusively, in the skin of the monkey which was immunized with cercariae exposed to high doses of X-irradiation, whereas in the non-immunized monkey, rarely were dead schistosomula found in the skin. The strong inflammatory reaction, consisting mainly of eosinophils, in the epidermis and dermis, may indicate that immunological action which caused the death of the migrating schistosomula occurred in the skin. Thus our previous statement that a strong acquired resistance against schistosome infection in rhesus monkeys can be induced by previous immunization with cercariae exposed to a large amount of X-irradiation is confirmed.

In rhesus monkeys, which acquire solid immunity by repeated exposures to non-irradiated cercariae, Vogel and Mining (5) showed that the death of schistosomula originating from challenge cercariae of *S. japonicum* (Vogel's Chinese/Chekiang/Kashing strain) occurred mainly in the lungs at day seven. The schistosomula were found in the nodular infiltrates which consisted mainly of eosinophils. In rhesus monkeys immunized by several exposures to the cercariae of the zoophilic strain of *S. japonicum* (the Chinese/Taiwan/Changhua strain), results of the study in this laboratory (6) indicate that only a few schistosomula originating from challenge cercariae of the Japanese strain perished in the skin, but many in the lungs, and another large proportion in the liver. In the skin, even at the peak of the inflammatory reaction at days three and four, the intensity of the reaction was much less than that occurring in the monkey which was challenged after it had been immunized by cercariae exposed to high doses of X-irradiation. The inflammatory infiltrates consisted chiefly of segmented granulocytes, approximately only a third of which were eosinophils. In the lungs which were obtained at day seven of the challenge, a number of deteriorated schistosomula were found; in the liver, intact schistosomula were also encountered. The fate of immunizing carcariae of the Japanese strain exposed to low doses of X-irradiation has been found to be similar to

that of the zoophilic strain (7) and the site of death of the challenge cercariae may be assumed to be the same in monkeys immunized with the cercariae of the zoophilic strain and with the cercariae of the Japanese strain exposed to low doses of X-irradiation. The fact that when the perishment of immunizing cercariae occured in the skin, as in the present case of using cercariae exposed to high doses of X-irradiation, the death of challenge cercariae also occurred in the skin (not mainly in the lungs and liver as when immunized by other methods) strongly suggests that a stage specific anti-schistosomular antibody may function in this monkey.

Summary. In a rhesus monkey immunized by cercariae of *S. japonicum* exposed to high doses of X-irradiation, the perishment of challenge cervariae was found mainly in the skin. Thus our former statement that a strong resistance against schistosome infection in rhesus monkeys can be induced by previous immunizations with cercariae exposed to a large amount of X-irradiation is confirmed. The fact that the perishment of the immunizing cercariae took place in the skin of the rhesus monkey, and the death of the challenge cercariae occurred also in the skin, strongly suggests that a stage specific anti-schistosomular antibody may function in this case.

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