Intradermal Immune Responses to a Schistosomal Egg Antigen During Experimental Murine *Schistosoma mansoni* Infection[1] (36550)

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During the course of chronic hepatosplenic schistosomiasis, the mammalian host is exposed to penetrating cercariae, which transform into migrating schistosomules and develop into mature, egg laying adult worms. Presumably, each stage presents stage-specific, as well as shared, antigens, any of which may induce a variety of immune responses in the infected host. The pathogenesis of experimental murine *Schistosoma mansoni* infection, which is strikingly similar to the human infection in its development (1), has been attributed to an immune, cell-mediated granulomatous response (2). Other immune responses, such as reaginic antibodies (3) and a variety of circulating antibodies (4), have also been demonstrated during schistosomiasis. This array of antigenic complexities and immunologic responses may be simplified by identification of specific antigens and the immunologic response (or responses), to these antigens during the course of the infection. Accordingly, the importance of suitable immunological assay systems for testing possible antigenic materials is evident.

One such assay system, recently described by this laboratory (5), utilizes the blastogenic response of lymphocytes from experimentally infected mice following challenge with a soluble egg antigen (6) as an index of cell-mediated immunologic reactions. However, in light of evidence that previously infected schistosoma hosts have heightened dermal reactivities to cercariae (7, 8), the present study was undertaken to adopt a previously reported murine intradermal immune assay (9) to the schistosomal system.

Materials and Methods. Schistosoma mansoni infections were initiated and maintained in CBA/J mice (Jackson Memorial Laboratory, Bar Harbor, ME) as previously described (5). At various times during initial infection dermal reactivity was assayed by injection of a soluble schistosomal egg antigen. The antigen (SEA) was prepared, as previously described (5), using eggs obtained from the livers of 8 week infected mice. Challenge injections of 0.03 ml were made in the pinna of the mouse ears using disposable glass syringes (Becton, Dickinson and Co., Rutherford, NJ) and 27 gauge needles. The preinjection ear thickness of individual ears and all subsequent measurements were made with an engineer micrometer. Measurements were taken at 5, 14, 24, 48, and 72 hr intervals after challenge.

Lymph node (LN) cells for passive transfer experiments were obtained from accessible nodes of donor mice 6 and 8 weeks after infection. The nodes were gently teased in Hanks' balanced salt solution, and the nucleated cells were adjusted to a concentration of 80×10^6/ml. Sera for passive transfers were collected from donor mice 6 and 8 weeks after infection. Both cells and sera were injected intravenously in 0.25 ml volumes. Twenty hours after passive transfer of either cells or sera the recipient mice were challenged by ear injection of SEA and monitored as described above.

The data, recorded in units of 10^{-3} cm, were expressed as the specific ear thickening (SET) reactivity at each time interval. SET was defined as the mean increase in ear thickness of experimental mice (infected or

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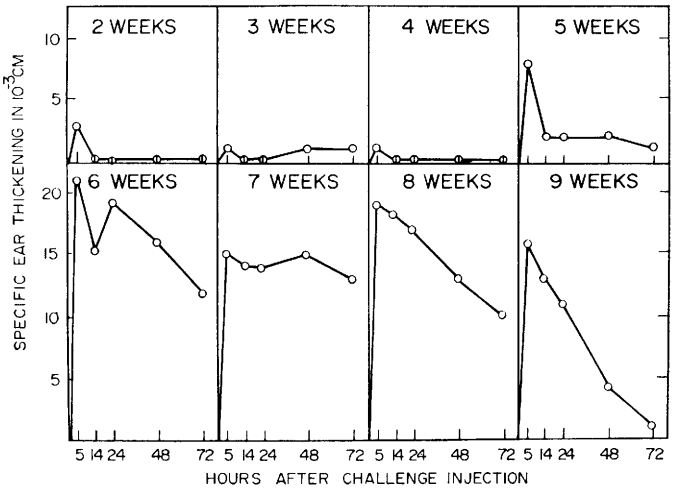

FIG. 1. Specific ear thickening in response to 0.03 ml SEA injected intradermally into the pinna of the ears of mice during primary infection with *S. mansoni*. The weeks after infection in which the assays were performed are indicated.

passively transferred) minus the mean increase in ear thickness of control (normal) mice.

Passive cutaneous anaphylaxis (PCA) studies for mouse reagin-like antibodies reactive with SEA were performed according to the method of Mota, Wong and Sadun (10) using sera from infected or normal mice. Lewis rats (Microbiological Associates Inc., Bethesda, MD) were shaved over the dosrum, rested 24 hr, and then injected intradermally with 0.05 ml of serum. After 72 hr the rats were challenged by an intracardiac injection of 1 ml of 0.25% Pontamine sky blue containing 0.22 mg of SEA protein. After 30 min the animals were killed, their dorsal skin was reflected, and the reaction sites measured.

Results. Figure 1 illustrates the development of intradermal reactivity in CBA/J mice during the course of a primary infection with *S. mansoni*. The first indication of any specific reactivity to SEA was noted as a low level, early (5 hr) response 5 weeks after infection. By the following week both an early and late (24–72 hr) response were obvious, but by 9 weeks the late response appears to have waned. Each data point was derived from 3 to 7 animals. It should be noted that with the infective dose of cercariae used in this study (100/mouse) a majority of the mice of this strain die between 8 and 10 weeks after infection.

Passive transfer to normal mice of either sera or lymphoid cells obtained from mice either 6 or 8 weeks after infection allowed dissection of the dermal response. Figure 2 demonstrates that the ability to produce the early (5 hr) anti-SEA response is only transferable by serum, while the capability to mount the later-developing (24 hr peak) anti-SEA response is only transferable by lymphoid cells. The kinetic developmental patterns of each response separate from the other could only be appreciated in these transfer experiments. It should be mentioned that neither sera nor cells from animals 4 weeks into infection conferred any anti-SEA dermal reactivity on normal recipients.

Preliminary PCA experiments to test for mouse heterologous (rat) skin sensitizing reagin-like antibodies, showed that sera from 8 week infected mice, but not 3 week infected mice, were capable of sensitizing rat skin so that a systemic challenge of SEA yielded a subsequent lesion site of 21 mm diameter, with intense leakage of dye. A 1:50 dilution of this serum contained sufficient activity to cause a lesion 12 mm in diameter. Heating the undiluted serum at 56° for 30 min destroyed its ability to induce the PCA reaction.

Discussion. The present studies demon-

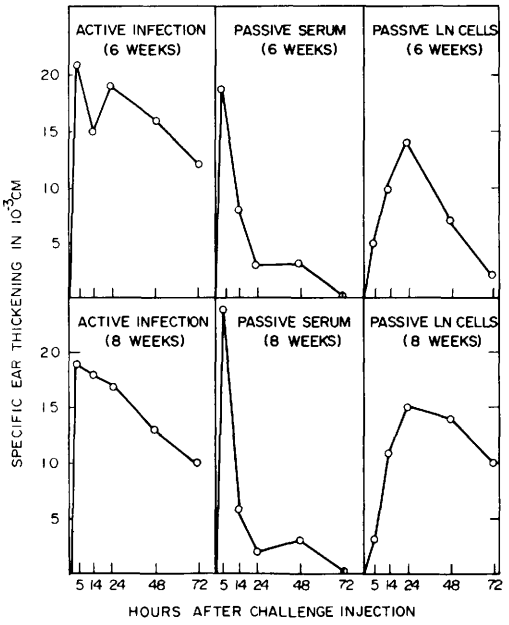

FIG. 2. Specific ear thickening in response to 0.03 ml SEA injected intradermally into the pinna of the ears of mice actively infected with *S. mansoni* for 6 or 8 weeks, or normal mice after they received passive transfers of either 0.25 ml serum or 20 × 10^6 lymph node (LN) cells from 6 or 8 week infected mice.

strate that intradermal ear thickening is a useful method to measure dermal reactivity of mice to schistosomal-derived materials. It is clear that dermal reactivity to SEA does not develop in infected mice prior to the maturation of the adult worms (4.5–5.5 weeks), and the initiation of egg production. The decreased late dermal response seen in 9 week infected animals is intriguing in light of presumed continued antigenic stimulation by newly produced eggs, and the persisting early (5 hr) responsiveness. It is also reminiscent of the previously reported decreased granuloma size around *S. mansoni* eggs in late stage infections (11). It is possible that the observation reflects induction of immunological tolerance with respect to cell-mediated responses to SEA but not to antibody-mediated responses. This formulation is consistent with the observation that it requires larger quantities of antigen to induce B-lymphocyte unresponsiveness than T-lymphocyte unresponsiveness (12). Alternatively, the dissociation of antibody-mediated and cell-mediated responses late in infection could reflect total immune unresponsiveness in the presence of continued circulation of previously synthesized antibody or the onset of cellular anergy as a preterminal event.

The data obtained from passive transfer experiments clearly demonstrated the early, presumably Arthus-type, response was antibody-mediated and the later, delayed hypersensitivity-type, response was dependent on sensitized lymphoid cells. The PCA reactivity demonstrates that an immediate, reagin-type response was also present. Thus, this simple method, in combination with the PCA reaction, can discern three separate types of anti-SEA immune responses.

Summary. Six weeks after infection with *Schistosoma mansoni* both an early (5 hr) and late (24–48 hr) intradermal response were elicited in mice challenged with a soluble schistosomal egg antigen (SEA). Passive transfer experiments showed that three anti-SEA immune reactivities exist in 8 week infected mice: passive cutaneous anaphylaxis due to reagin-like antibodies; an Arthus-type, 5 hr reaction due to circulating antibodies; a delayed (24-48 hr) hypersensitivity reaction dependent on lymphoid cells. The kinetics of immunization indicate that these anti-SEA responses are stage specific for the egg antigen preparation.

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