SYSTEMIC CELLULAR HYPERSENSITIVITY INDUCED BY AN INTESTINALLY ABSORBED ANTIGEN*

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The normal mammalian intestine is exposed to innumerable potential antigens. Neonatal and adult rodents are known to absorb small quantities of these materials sufficiently intact (1, 2) to induce a humoral immune response (3–6). No information is available, however, on the ability of intestinally administered antigens to induce systemic cellular immune responses (7, 8). Since it has been shown that soluble antigens secreted by *Schistosoma mansoni* eggs are capable of inducing delayed hypersensitivity reactions when injected parenterally in minute amounts without adjuvant, it was decided to test the capability of these substances to induce cellular immune responses when administered intragastrically or intraduodenally.

Materials and Methods

Schistosome eggs were isolated by the method of Coker and von Lichtenberg (9) from the livers of hamsters which had been infected 8 wk previously with a Puerto Rican strain of *S. mansoni*, and soluble egg antigen (SEA) was prepared from the eggs by the method of Boros and Warren (10). Intact eggs or SEA were administered to neonatal and adult CF1 mice (Carworth Farms, New City, N.Y.) by three different routes. At 12 h of age neonatal mice received intragastrically via polyethylene 50 tubing, 10,000 live schistosome eggs, or 10 μg SEA in phosphate-buffered saline (PBS) or PBS alone, other mice received 10 μg of SEA in PBS intraperitoneally. Adult mice weighing 18–22 g were injected intraperitoneally, intragastrically, or following laparotomy, intraduodenally (controls were sham operated) with 28,000 live schistosome eggs in PBS or 1.3% sodium bicarbonate ($NaHCO_3$); 80 μg of SEA in PBS or $NaHCO_3$; PBS; or $NaHCO_3$. At 8 wk of age for the neonatal mice and 2 wk after injection for the adult mice, the animals were challenged with 2,000 eggs injected via a tail vein into the microvasculature of the lungs. 8 days after injection of eggs, the mice were anesthetized and 1 ml of 10% buffered formalin solution was injected intratracheally into the lungs, the trachea was ligated, and the lungs removed and placed in 10% buffered formalin. Three sections from each lung 250 μ apart and 5 μ in thickness were mounted on microscope slides and stained with hematoxylin and eosin. Each section was examined for parasite eggs and the size of each egg, including the reaction around it, was determined by measuring two diameters at right angles to each other with a Cooke AEI image-splitting eyepiece (Cooke Engineering Co.,

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Alexandria, Va.) mounted on a Nikon microscope (Nikon Inc., Div. of EPOI, Garden City, N.Y.). The diameters of approximately 100 egg lesions were measured and the means and standard errors determined. Granuloma volumes were calculated from the mean diameters.

In order to determine the fate of eggs administered intragastrically to adult mice, the animals were fed a complete synthetic diet (without roughage) and were maintained on wire mesh. The feces were collected, comminuted, washed, and the eggs examined and counted.

RESULTS

Mice exposed neonatally to schistosome eggs or SEA by the intragastric route developed markedly augmented granulomatous reactions on subsequent intravenous challenge with intact eggs. These reactions were comparable to those in animals exposed to antigen by parenteral injection (Table I). Mean granuloma volumes in adult control mice injected parenterally (intraperitoneally) with PBS and $NaHCO_3$, schistosome eggs or SEA were respectively ($\pm$ SE): 5 $\pm$ 0.4, 63 $\pm$ 7.9 and 51 $\pm$ 9.7 $mm^3 \times 10^{-4}$. Mice given eggs intragastrically in PBS had a mean granuloma volume on egg challenge of 6 $\pm$ 0.6 $mm^3 \times 10^{-4}$; those receiving eggs in $NaHCO_3$ had a mean granuloma volume of 19 $\pm$ 1.9 $mm^3 \times 10^{-4}$ ($P < 0.001$). Sham operated mice injected intraduodenally with $NaHCO_3$ had a mean granuloma volume on egg challenge of 6 $\pm$ 1.2 $mm^3 \times 10^{-4}$; those given eggs in $NaHCO_3$ had a mean granuloma volume of 50 $\pm$ 4.6 $mm^3 \times 10^{-4}$ ($P < 0.001$). SEA given intragastrically or intraduodenally with PBS or $NaHCO_3$ did not sensitize adult mice.

Egg recovery from the stools of the adult mice given schistosome eggs intragastrically was approximately 80% of the administered dose over 3 days and 95% over 5 days. The eggs collected at 24 h after intragastric infusion showed no hatching or flame cell movement.

DISCUSSION

The *S. mansoni* egg granuloma has been shown to be a form of delayed hypersensitivity (11) which correlates with skin test reactivity, delayed footpad

TABLE I

Material	No. mice/no. lesions	Granuloma diameter $\pm$ S.E.	Granuloma volume
			$mm^3 \times 10^{-4} \pm S.E.$
Intragastric			
PBS	5/107	116.4 $\pm$ 3.8	8.2 $\pm$ 0.80
SEA (10 μg)	4/104	194.6 $\pm$ 4.9	38.2 $\pm$ 2.89
Schistosome eggs (10,000)	4/98	219.6 $\pm$ 5.7	55.0 $\pm$ 4.29
Intraperitoneal			
SEA (10 μg)	6/97	198.8 $\pm$ 5.2	40.5 $\pm$ 4.26

Granulomatous response around *S. mansoni* eggs in the pulmonary vasculature of neonatal mice which had been sensitized by the intragastric administration of schistosome eggs or SEA in the neonatal period. Control mice received PBS intragastrically or SEA intraperitoneally.

swelling, macrophage migration inhibition and lymphocyte transformation (10, 12). Using the induction of granulomatous hypersensitivity as an assay system, it has been demonstrated for the first time that neonatal and adult mice can be systemically sensitized by the intestinal administration of a soluble antigen (SEA). While previous studies have revealed that systemic cell-mediated reactions occur in intestinal helminth infections such as *Nippostrongylus brasiliensis* and *Trichostrongylus colubriformis*, antigens from both of these parasites enter the body parenterally. *N. brasiliensis* larvae penetrate the skin and migrate through the lungs before reaching their final habitat in the gut; *T. colubriformis* larvae burrow into the intestinal wall and the adults live with their heads embedded in the epithelium of the small intestine (15).

The present study also reveals that the mode of administration of SEA is important. Neonatal rodents, which do not have gastric acidity and little proteolytic enzyme activity, can be sensitized by the intragastric instillation of purified antigen or live eggs in PBS. In adult mice, which have gastric acidity, live eggs administered intragastrically in PBS did not sensitize while those given in bicarbonate caused partial sensitization. A maximal effect was seen, however, when the eggs were injected intraduodenally. SEA given intragastrically or intraduodenally to adult mice did not induce sensitization. The demonstration that one soluble antigen can induce cellular immune responsiveness via the intestinal route suggests that other antigens of dietary and bacterial as well as parasitic origin may also do so.

SUMMARY

Neonatal mice given intact living *Schistosoma mansoni* eggs or soluble schistosome egg antigens intragastrically developed systemic cellular hypersensitivity as shown by markedly accelerated, augmented granulomatous inflammation around *S. mansoni* eggs subsequently injected intravenously into the pulmonary microvasculature. To achieve partial sensitization in adult mice schistosome eggs had to be administered intragastrically with bicarbonate; full sensitization occurred when the eggs were injected intraduodenally. These data indicate that under appropriate conditions intestinal administration of antigen can result in systemic cellular immune sensitization.

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