SYSTEMIC CELLULAR HYPERSENSITIVITY INDUCED BY AN INTESTINALLY ABSORBED ANTIGEN

BY JOSEPH L. PERROTTO, LE MING HANG, K. J. ISSELBACHER, AND K. S. WARREN

(From the Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114 and the Departments of Medicine and Community Health, Case Western Reserve University and University Hospitals, Cleveland, Ohio 44106)

(Received for publication 8 April 1974)

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 (NaHCO3); 80 µg of SEA in PBS or NaHCO3; PBS; or NaHCO3. 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.,

* This research was supported by a grant from the National Colitis and Ileitis Foundation and by U.S. Public Health Service grant no. AI 08163.

296 THE JOURNAL OF EXPERIMENTAL MEDICINE • VOLUME 140, 1974
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 NaHCO3, schistosome eggs or SEA were respectively (± SE): 5 ± 0.4, 63 ± 7.9 and 51 ± 9.7 mm³ × 10⁻⁴. Mice given eggs intragastrically in PBS had a mean granuloma volume on egg challenge of 6 ± 0.6 mm³ × 10⁻⁴; those receiving eggs in NaHCO3 had a mean granuloma volume of 19 ± 1.9 mm³ × 10⁻⁴ (P < 0.001). Sham operated mice injected intraduodenally with NaHCO3 had a mean granuloma volume on egg challenge of 6 ± 1.2 mm³ × 10⁻⁴; those given eggs in NaHCO3 had a mean granuloma volume of 50 ± 4.6 mm³ × 10⁻⁴ (P < 0.001). SEA given intragastrically or intraduodenally with PBS or NaHCO3 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 ± S.E. (mm³ × 10⁻⁴) | Granuloma volume ± S.E. (mm³ × 10⁻⁴) |
|---------------------|----------------------|---------------------------------------|--------------------------------------|
| Intragastric        |                      |                                       |                                      |
| PBS                 | 5/107                | 116.4 ± 3.8                           | 8.2 ± 0.80                           |
| SEA (10 µg)         | 4/104                | 194.6 ± 4.9                           | 38.2 ± 2.89                          |
| Schistosome eggs (10,000) | 4/98              | 219.6 ± 5.7                           | 55.0 ± 4.29                          |
| Intraperitoneal     |                      |                                       |                                      |
| SEA (10 µg)         | 6/97                 | 198.8 ± 5.2                           | 40.5 ± 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-medi-
ated 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 pro-
teolytic 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 intragas-
trically or intraduodenally to adult mice did not induce sensitization. The
demonstration that one soluble antigen can induce cellular immune responsive-
ness via the intestinal route suggests that other antigens of dietary and bac-
terial as well as parasitic origin may also do so.

SUMMARY

Neonatal mice given intact living Schistosoma mansoni eggs or soluble schis-
tosome egg antigens intragastrically developed systemic cellular hypersensi-
tivity as shown by markedly accelerated, augmented granulomatous inflam-
mation 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.

REFERENCES

1. Walker, W. A., R. Cornell, L. M. Davenport, and K. J. Isselbacher. 1972. Mecha-
nism of horseradish peroxidase uptake and transport in adult and neonatal
rat intestine. J. Cell Biol. 54:195.
2. Cornell, R., W. A. Walker and K. J. Isselbacher. 1971. Small intestinal absorp-
tion of horseradish peroxidase. Lab. Invest. 25:42.
3. Bernstein, I. D., and Z. Ovary. 1968. Absorption of antigens from the gastro-
intestinal tract. Int. Arch. Allergy Appl. Immunol. 33:521.
4. Eddie, D. S., M. W. Schulterd, and J. B. Ross. 1971. The isolation and biologic
activities of purified secretory IgA and IgG anti-salmonella typhimurium “O”
antibodies from rabbit intestinal fluid and colostrum. *J. Immunol.* **106:** 181.

5. Ogra, P., and D. T. Karzon. 1969. Distribution of poliovirus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with poliovaccine. *J. Immunol.* **102:** 1423.

6. Rothberg, R. M., S. C. Kraft, and S. M. Michalek. 1973. Systemic immunity after local antigenic stimulation of the lymphoid tissue of the gastrointestinal tract. *J. Immunol.* **111:** 1906.

7. Uhr, J. W. 1966. Delayed hypersensitivity. *Physiol. Rev.* **46:** 359.

8. Mitchison, N. A. 1972. In Immunogenicity: Physico-chemical and Biological Aspects. Felix Borek, editor. North-Holland Publishing Co., Amsterdam. **25:** 103.

9. Coker, C. M., and F. von Lichtenberg. 1956. A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc. Soc. Exp. Biol. Med.* **92:** 780.

10. Boros, D. L., and K. S. Warren. 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. *J. Exp. Med.* **132:** 488.

11. Warren, K. S., E. O. Domingo, and R. B. T. Cowan. 1967. Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. *Am. J. Pathol.* **51:** 735.

12. Boros, D. L., H. J. Schwartz, A. E. Powell, and K. S. Warren. 1971. Delayed hypersensitivity, as manifested by granuloma formation, dermal reactivity, macrophage migration inhibition and lymphocyte transformation, induced and elicited in guinea pigs with soluble antigens of *Schistosoma mansoni* eggs. *J. Immunol.* **110:** 1118.

13. Dineen, J. K., and D. B. Adams. 1971. The role of recirculating thymus-dependent lymphocytes in resistance to *Trichostrongylus colubriformis* in the guinea pig. *Immunology.* **20:** 109.

14. Dineen, J. K., and J. D. Kelly. 1972. The suppression of rejection of *Nippostrongylus brasiliensis* in lactating rats: The nature of the immunologic defect. *Immunology.* **22:** 1.

15. Belding, D. L. 1965. Textbook of Parasitology. Appleton Century Crofts, New York. 3rd edition.