

Effect of locally induced intestinal anaphylaxis on the uptake of a bystander antigen

Kurt J. Bloch, M.D., and W. Allan Walker, M.D. *Boston, Mass.*

Adult Sprague-Dawley rats weighing 250 gm were immunized with 100 µg egg albumin (EA) and alum. Fourteen days later, intestinal anaphylaxis was induced by intraduodenal or intragastric challenge with EA. To test for the changes in vascular and mucosal permeability that accompany anaphylaxis, the rats were injected intravenously with ¹²⁵I-rat serum albumin (RSA) at the time of challenge and the amount of radioactivity retained in the wall of gut segments, as well as the trichloroacetic acid (TCA)-precipitable radioactivity present in the secretions obtained from these segments, was determined. Enhanced retention of ¹²⁵I-RSA was found in the wall of the duodenum and first and second halves of the small intestine, and increased amounts of TCA-precipitable radioactivity was found in gut segments obtained from antigen-challenged compared with buffer-challenged rats. In other EA-immunized rats, bovine serum albumin (BSA) was administered by gavage 1 hr before challenge with EA. Increased amounts of immunoreactive BSA were detected by radioimmunoassay in the serum of rats subjected to local intestinal anaphylaxis. Possible consequences of the enhanced systemic uptake of protein present during local intestinal anaphylaxis were considered. It was suggested that such proteins might induce an IgE antibody response, thereby broadening the anaphylactic sensitivity of the animal to additional antigens.

Considerable indirect¹⁻⁵ and limited direct⁶⁻⁸ evidence has suggested that following their ingestion, antigenically intact proteins may gain access to the systemic circulation. We have recently shown that nanogram amounts of immunoreactive bovine serum albumin (iBSA) may be detected in serum of normal adult rats for several hours after feeding by gavage with large amounts of this protein.⁹ During intestinal inflammation provoked by infection with the nematode parasite *Nippostrongylus brasiliensis* or during mild systemic anaphylaxis, enhanced uptake of BSA was observed. The present study tested the effect of local

intestinal anaphylaxis on the uptake from the gut of a protein other than the antigen utilized to induce anaphylaxis.

MATERIALS AND METHODS

Animals

Adult Sprague-Dawley rats, both male and female, weighing ~250 gm, were obtained from Charles River Breeding Laboratories (Wilmington, Mass.). Rats were maintained on rat Chow (Ralston Purina, St. Louis, Mo.) devoid of cow's-milk protein for 6 wk before use in experiments.

Immunization

Rats received a single intraperitoneal injection of 100 µg crystalline (5×) egg albumin (EA) (Nutritional Biochemical, Cleveland, Ohio) in 1 ml aluminum hydroxide gel containing 10 mg potassium alum.¹⁰ Rats were tested 14 days later. At this time, rats had 24-hr passive cutaneous anaphylaxis (PCA) titers of 1:5 to 1:20.

Preparation of radiolabeled proteins

Rat serum albumin (RSA) was prepared by starch block electrophoresis.¹¹ Solutions of RSA and crystalline BSA (Sigma Chemical Co., St. Louis, Mo.) were labeled with ¹²⁵I.¹² The amount of iBSA present in serum was detected

From the Departments of Medicine and Pediatrics, Harvard Medical School, and the Clinical Immunology and Allergy Units of the Medical Services and the Pediatric Gastrointestinal and Nutrition Unit, Massachusetts General Hospital.

Supported by National Institutes of Health grants AM-23099, AM-21505, AM-16269, and T32-AI-10704 and T32-AM-07258-3, the Arthritis Foundation, and L. H. Bendit Foundation.

Received for publication Oct. 6, 1980.

Accepted for publication Dec. 18, 1980.

Reprint requests to: Dr. Kurt J. Bloch, Clinical Immunology and Allergy Units, Massachusetts General Hospital, Boston, MA 02114.

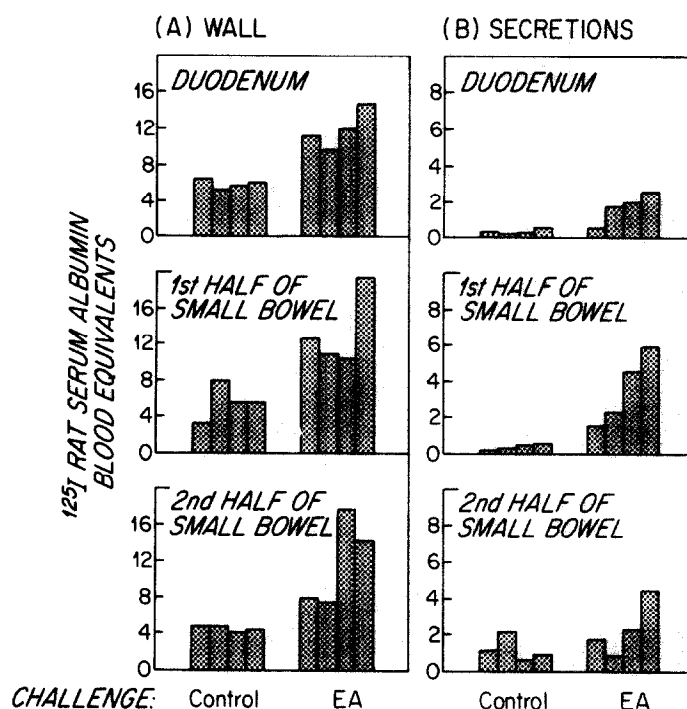


FIG. 1. Radioactive counts present in the duodenal wall or first and second halves of the small intestine (A) or in TCA precipitates of intestinal secretions obtained from these gut segments (B) from rats immunized with EA and alum and challenged *intraduodenally* with buffer or buffer containing 100 mg EA. Each bar represents the values obtained with gut segments or secretions of a single rat. ("Blood equivalents" is defined in Methods.)

by radioimmunoassay performed as described previously.⁹ The anti-BSA antiserum used in these assays was absorbed with an EA immunoabsorbent¹³; the absorbed antiserum did not react with EA added to the test system.

Absorption studies

Rats fasted overnight were subjected to light ether anesthesia, a plastic cannula was passed into the stomach, and 2 ml of saline solution containing 1,000 mg BSA (Fraction V, Sigma) and 25 mg NaHCO_3 were introduced. Blood was obtained from a cut made in the tail under light ether anesthesia at intervals after feeding. These blood samples consist of a mixture of arterial and venous blood.

Induction of intestinal anaphylaxis

Local intestinal anaphylaxis was elicited by intraduodenal or intragastric challenge with EA solution 1 hr after feeding with BSA. Rats were anesthetized with ether and a small abdominal incision was made. The pylorus was occluded with forceps and 2 ml of solution (either 0.1 M K_2HPO_4 , pH 7.0, or 100 mg EA in this buffer) were injected via a 25-gauge needle into the duodenum. The incision was closed and the animals allowed to recover from anesthesia. Blood was obtained from the tail 1, 3, and 6 hr after challenge. Intragastric challenge was performed by administering 2 ml buffer or 2 ml buffer containing 500 mg EA by gavage 1 hr after the initial feeding with BSA. Serum

samples were tested for iBSA as cited above. Rats challenged by gavage with 100 mg EA showed less consistent differences compared with controls; for this reason a challenge dose of 500 mg EA was used in the following experiments.

Changes in vascular and intestinal permeability were assessed by the method of Byars and Ferraresi.¹⁴ These studies were conducted separately from the above in immunized rats challenged *intraduodenally* or *intragastrically* with EA. Immediately after challenge, the rats were injected intravenously with 0.2 μCi ^{125}I -labeled RSA ($\sim 1 \mu\text{g}$ protein) in 1 ml phosphate-buffered (0.01 M, pH 7.5) saline solution. One hour after the injection of ^{125}I -RSA, rats were exsanguinated under anesthesia. The entire small intestine was removed, divided into duodenum and first and second halves of the remaining small bowel. The segments of intestine were opened separately and their contents rinsed in 5 ml iced phosphate-buffered saline (PBS). The radioactivity remaining in the intestinal wall and the TCA-precipitable (final concentration of TCA = 10%) radioactivity present in the luminal rinse fluid were determined. In addition, TCA-precipitable radioactivity in 100 μl of serum and the weight of the gut segments were determined. The radioactivity in wall segments and secretions was expressed as "blood equivalents" (counts per minute recovered in a segment/weight of the organ in grams/counts per minute in 1 ml serum $\times 100$). Statistical comparisons were made with Student's *t* test.

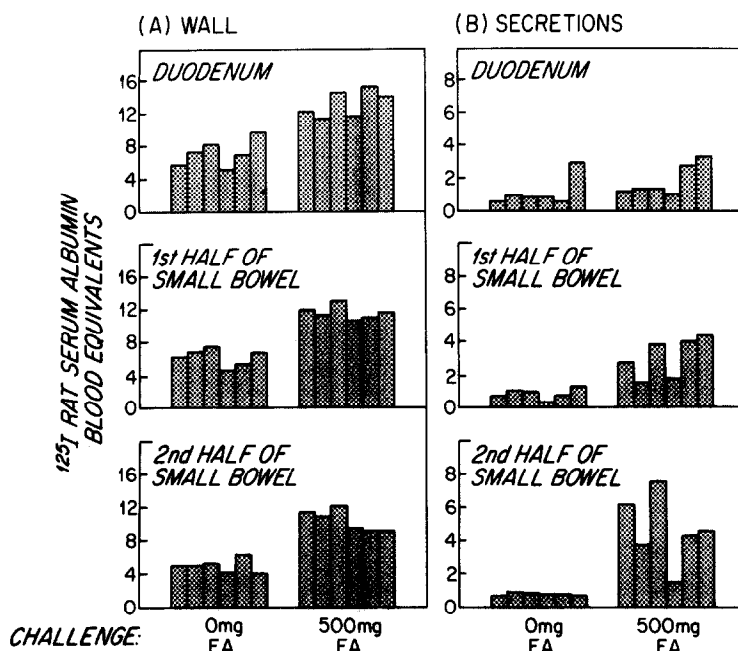


FIG. 2. Radioactive counts present in the duodenal wall or first and second halves of the small intestine (A) or in TCA precipitates of intestinal secretions obtained from these gut segments (B) from immunized rats challenged *intra*gastrically with buffer or buffer containing 500 mg EA. Each bar represents the values obtained with gut segments or secretions of a single rat. ("Blood equivalents" is defined in Methods.)

RESULTS

Intestinal anaphylaxis induced in immunized rats by intraduodenal or intragastric administration of antigen: Evidence for altered vascular and intestinal permeability

Rats immunized with EA and alum 14 days earlier were injected with ^{125}I -RSA immediately after the intraduodenal instillation of either buffer or 100 mg EA in buffer. One hour later, the retention of radioactive counts in segments of the intestine and the amount of protein-bound radioactivity present in the luminal fluids were determined. The amount of radioactivity retained in gut segments was greater in the duodenum and first and second halves of the small bowel of rats exposed to 100 mg EA compared with such segments from rats exposed to buffer alone ($p < 0.001$, $p < 0.02$, and $p < 0.05$, respectively) (Fig. 1). Similarly, the amount of TCA-precipitable radioactivity was greater in the secretions from the duodenum (in three of four rats) and the first half of the small intestine of antigen-challenged compared with control rats ($p < 0.05$ and $p < 0.02$, respectively) (Fig. 1).

In other experiments, 2 ml of buffer alone or of buffer containing 500 mg EA were administered to immunized rats by gavage under light ether anesthesia. Rats were immediately injected with ^{125}I -RSA and 1 hr later, the retention of radioactive counts in segments of the intestine and the amount of protein-

bound radioactivity present in the luminal fluids was determined. Again, the amount of radioactivity retained in gut segment was greater in the duodenum and first and second halves of the small intestine of antigen-challenged compared with buffer-fed rats ($p < 0.001$ in each instance) (Fig. 2). The amount of TCA-precipitable radioactivity was greater in the luminal secretions from the two segments of small intestine of antigen-challenged compared with control rats ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 2).

Influence of intestinal anaphylaxis induced by intraduodenal or intragastric administration of antigen on the uptake of an unrelated protein

Rats immunized with EA and alum 14 days earlier received 1,000 mg BSA by gavage. In some experiments, the BSA was "marked" by the addition of a small amount of Evans blue dye. At laparotomy 1 hr later, the blue BSA solution was seen to fill the entire small intestine to the cecum. Buffer or EA in buffer was injected into the duodenum and the laparotomy incision was closed. Table I lists the highest value of iBSA achieved in serum 1, 3, or 6 hr later. In all instances, the highest levels attained in antigen-challenged rats exceeded the highest levels of the controls ($p < 0.01$).

In other experiments, intestinal anaphylaxis was

elicited in immunized, BSA-fed rats by gavage with 500 mg EA. The highest serum values of iBSA obtained in these animals are shown in Table I. Five of the six rats challenged with EA showed larger amounts of iBSA in serum than did control rats challenged with buffer alone ($p < 0.05$).

DISCUSSION

In the rat, systemic anaphylaxis has been shown to be accompanied by changes in vascular¹⁵ and mucosal¹⁶ permeability. These changes are reflected in an enhanced retention of labeled serum albumin in intestinal tissues and by the increased concentration of such proteins in intestinal fluids.^{9, 14} For the present experiments rats were immunized with small amounts of protein antigen (EA) and alum so as to favor the production of IgE anti-EA antibodies. Local intestinal anaphylaxis was elicited by either intraduodenal or intragastric infusion of EA. Animals challenged by these routes did not show signs of systemic anaphylaxis. Mild clinical anaphylaxis might, however, be blunted by, or be difficult to distinguish from, the effects of recent ether anesthesia. Intestinal anaphylaxis was accompanied by enhanced retention of ¹²⁵I-RSA in the wall of the duodenum and first and second halves of the intestine of rats challenged with antigen by either intraduodenal or intragastric challenge (Figs. 1 and 2). We did not attempt to distinguish between retention of ¹²⁵I-RSA which had extravasated into tissues and that which was retained in dilated blood vessels. In the intestine, evidence for extravasation and for increased mucosal permeability is provided by the detection of TCA-precipitable radioactivity in the intestinal fluids. The latter findings indicated that ¹²⁵I-RSA, or large fragments thereof, had passed from the lamina propria into the intestinal lumen. The present experiments suggest that during intestinal anaphylaxis there is also an increased movement of protein from the lumen into the systemic circulation (Table I).

In our experiments, intestinal anaphylaxis is presumably triggered by protein antigen that is initially delivered into the lumen but that must subsequently gain access to IgE antibody-coated mast cells in the lamina propria of the gut. The precise route by which antigen traverses the gut has not been traced. The reverse movement of labeled proteins from the interstitial fluids of the lamina propria into the lumen occurs, at least in part, via the interepithelial spaces.¹⁶ It seems likely that during local intestinal anaphylaxis, the enhanced uptake of protein from the lumen into the systemic circulation occurs via the same channels.

The experiments of Byars and Ferraresi¹⁴ have shown that after intragastric challenge with antigen,

TABLE I. Effect of local intestinal anaphylaxis on uptake of an unrelated protein, BSA

Immunized rats* challenged with:	Highest concentration of iBSA detected in serum ($\mu\text{g/ml}$)
Buffer†	0.3, 0.3, 0.5, 0.9, 0.9, 1.0
EA (100 mg) administered into duodenum at laparotomy	1.8, 8, 9, 11, 14, 18
EA (500 mg) administered by gavage	0.6, 1.2, 2.0, 11, 14, 18

*All rats were immunized with 100 μg EA and alum 14 days earlier. One hour before challenge 1,000 mg BSA and 25 mg NaHCO_3 were administered by gavage in 2 ml buffer; blood was obtained for iBSA assay at 1, 3, and 6 hr after challenge.

†Administered either by injection into the duodenum or by gavage.

tissue permeability is altered only briefly, returning to prechallenge levels by 45 to 60 min. It might therefore be expected that peak blood levels of BSA would be reached promptly and uniformly shortly after challenge. While the highest blood levels of iBSA were observed at 1 hr (data not shown), in other cases, peak levels were observed at 3 or 6 hr. The delay in achieving peak serum levels may reflect prolonged retention in the lamina propria in some animals, by the requirement for saturating certain extravascular sites, such as the liver, or other unknown variables.

Is the enhanced uptake of a protein that happens to be present in the lumen when intestinal anaphylaxis occurs of biologic significance? Deliberate administration of protein antigens by the oral route may result in local and systemic immunization to the protein^{6, 17} or to the induction of systemic tolerance.^{7, 18} Primary and secondary IgE antibody responses following oral administration of protein have been described.^{19, 20} Thus the enhanced uptake of bovine serum albumin from the gut observed in the present experiments might promote the induction of IgE antibody to BSA, thereby eventually broadening the anaphylactic sensitivity of the animal to a new antigen. The simultaneous uptake of endotoxin might provide the adjuvant effect required for the induction of an antiprotein antibody response. To the extent that these observations in the rat can be extended to man, the present findings may explain in part the development of sensitivity to multiple food antigens in certain patients.

REFERENCES

1. Walzer M: Absorption of allergens. *J ALLERGY* 13:554, 1942.
2. Bernstein ID, Ovary Z: Absorption of antigens from the gastrointestinal tract. *Int Arch Allergy Appl Immunol* 33:521, 1968.

3. Taylor KB, Truelove SC, Wright R: Serologic reactions to gluten and cow's milk protein in gastrointestinal disease. *Gastroenterology* **46**:99, 1964.
4. Buckley RH, Dees SC: Correlation of milk precipitins with IgA deficiency. *N Engl J Med* **281**:464, 1969.
5. Falchuk KR, Isselbacher KJ: Circulating antibodies to bovine albumin in ulcerative colitis and Crohn's disease. Characterization of the antibody response. *Gastroenterology* **70**:5, 1976.
6. Rothberg RM, Kraft SC, Farr RS: Similarities between rabbit antibodies produced following ingestion of bovine serum albumin and following parenteral immunization. *J Immunol* **98**:386, 1967.
7. Thomas HC, Parrott DMV: The induction of tolerance to a soluble protein antigen by oral administration. *Immunology* **27**:631, 1974.
8. Cunningham-Rundles C, Brandeis WE, Good RA, et al: Milk precipitins, circulating immune complexes, and IgA deficiency. *Proc Natl Acad Sci USA* **75**:3387, 1978.
9. Bloch KJ, Bloch DB, Stearns M, Walker WA: Intestinal uptake of macromolecules. VI. Uptake of protein antigen in vivo in normal rats and in rats infected with *Nippostrongylus brasiliensis* or subjected to mild systemic anaphylaxis. *Gastroenterology* **77**:1039, 1979.
10. Williams CA, Chase MW: *Methods in immunology and immunochemistry*. New York, 1967, Academic Press, Inc. vol. I, p. 201.
11. Kunkel HG, Trautman R: Zone electrophoresis in various types of supporting media, in Bier M, editor: *Electrophoresis: Theory, methods and applications*. New York, 1959, Academic Press, Inc., p. 225.
12. Greenwood FC, Hunter WM, Glover JS: The preparation of ¹³¹I-labelled growth hormone of high specific radioactivity. *Biochem J* **89**:114, 1963.
13. Cuatrecasas P, Anfinsen CB: Affinity chromatography, in Jakoby WB, editor: *Methods in enzymology*. New York, 1971, Academic Press, Inc. p. 345.
14. Byars NE, Ferraresi RW: Intestinal anaphylaxis in the rat as a model of food allergy. *Clin Exp Immunol* **25**:352, 1976.
15. Urquhart GM, Mulligan W, Eadie RM, et al: Immunological studies on *Nippostrongylus brasiliensis* infection in the rat: The role of local anaphylaxis. *Exp Parasitol* **17**:210, 1965.
16. Murray M, Jarrett WFH, Jennings FW: Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with *Nippostrongylus brasiliensis*. *Immunology* **21**:17, 1971.
17. Pierce NF, Gowans JL: Cellular kinetics of the intestinal immune response to cholera toxoid in rats. *J Exp Med* **142**:1550, 1975.
18. Tomasi TB Jr: Oral tolerance. *Transplantation* **29**:353, 1980.
19. Jarrett EEE, Haig DM, McDougall W, McNutty E: Rat IgE production II. Primary and booster reaginic antibody responses following intradermal or oral immunization. *Immunology* **30**:671, 1976.
20. Bazin H, Platteau B: Production of circulating reaginic (IgE) antibodies by oral administration of ovalbumin to rats. *Immunology* **30**:679, 1976.