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The Effect of Immediate-Type Gastrointestinal Allergic Reactions on Brush Border Enzymes and Gut Morphology in the Rat

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ABSTRACT. The aim of the present study was to create clearly documented immediate-type allergy to food protein in the intestine of rats and to study some pathophysiological phenomena induced by challenge with the allergen. To achieve this, rats were sensitized with ovalbumin. A passive cutaneous anaphylaxis reaction to ovalbumin was negative in all controls and positive in all test animals when *Bordetella pertussis* was used as adjuvant. Sixty minutes after an intravenous injection of ^{125}I-human serum albumin and 45 min after an ovalbumin challenge, given by gavage, the rats were sacrificed. The intestine was removed and sections taken for morphologic studies. The remainder was rinsed, opened, cut into measured segments, weighed, and the radioactivity was measured. Disaccharidases, alkaline phosphatase, and protein were estimated in homogenates of epithelium. Results in both control and test animals showed that radioactivity decreased as one moved distally along the intestine. However, radioactivity was significantly higher ($p < 0.01$) in the intestine of test animals than in controls. Radioactivity in liver, kidney, spleen, and lungs was identical in test and control animals. There was significant reduction in levels of alkaline phosphatase (p varied from <0.05 to <0.001), maltase ($p < 0.05$), and sucrase ($p < 0.05$ to < 0.01). Lactase activity in contrast was significantly raised ($p < 0.05$). There was no change in intestinal morphology or in the intestinal mast cell count. (*Pediatr Res* 19: 456–459, 1985)

Abbreviations

CMPH, cow's milk protein hypersensitivity
PCA, passive cutaneous anaphylaxis

The gastrointestinal manifestations of cow's milk protein hypersensitivity (CMPH) are frequently associated with morphologic damage to the intestinal mucosa (1), as well as with malabsorption (2). It is likely that these phenomena have an immunologic basis. Many of these infants have immediate type hypersensitivity to milk protein (3) and defects in immune regulation (4), as well as in cellular immune mechanisms. The aim of our study was to create clearly documented immediate type intestinal hypersensitivity to food protein in rats and to study some associated pathophysiologic phenomena. To do this, hypersensitivity to ovalbumin was induced. Following challenge with this allergen, changes in the content of the disaccharidases—maltase, sucrase, and lactase—as well as of alkaline phosphatase, were studied. The changes observed may explain some of the phenomena associated with gastrointestinal food hypersensitivity.

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MATERIALS AND METHODS

Sensitization and challenge. Rats of Hooded Lister and Charles River strains were used (see Tables 1–6). Their weight ranged between 150 and 180 g. The rats were sensitized by an injection of ovalbumin 250 μg/0.5 ml, one half subcutaneously, and one half intraperitoneally. At the same time adjuvant consisting of *Bordetella pertussis* (1.6×10^3/0.5 ml) was administered intraperitoneally. On the 14th day, a booster injection consisting of ovalbumin (2.5 μg/0.5 ml) was given subcutaneously. The sensitization protocol was identical for all groups except that in group 4, concanavalin A 100 μg was used as adjuvant. The challenge procedure was based on that described by Byars and Ferraresi (5). On the 18th day the rats were challenged with ovalbumin (Sigma Chemical Co, St Louis, MO) after an overnight fast. At 0 h, 0.5 μCi of radioiodinated human serum albumin (Amersham International, Amersham, England) was injected into the jugular vein. Fifteen minutes later, 25 mg of ovalbumin was introduced into the stomach through a tube. Forty-five minutes after the ovalbumin challenge, the rats were anesthesized with ether and 1.5 ml of blood was removed from the jugular vein for subsequent determination of PCA. The rats were then sacrificed. The first 15 cm of intestine immediately distal to the pylorus were removed, rinsed, opened, blotted, and weighed. In some experiments the 15-cm segment was subdivided into three 5-cm segments, while in others, a second 15-cm segment was removed for comparison. Sections were taken and placed in Bouin's fixative for histologic examination. Blocks were made and sections stained with hematoxylin and eosin for light microscopy and with astra-blau (6) as a specific stain for mast cells. The remainder was placed in ice-cold saline. The liver, spleen, lungs, and kidneys also were removed for measurement of radioactivity.

Assessment of immune response and changes in disaccharidase activity. Radioactivity was measured in a Packard auto-gamma scintillation spectrometer model 5110 (Packard Instruments Co, Downer's Grove, IL). The difference of radioactivity between control and test groups was taken to be an expression of the local immune response. Thereafter, the epithelium of the intestine was scraped off, homogenized in a model K-43 TRI-R homogenizer (TRI-R Instruments, Rockville Center, NY), centrifuged in a refrigerated centrifuge at 2000 rpm for 10 min, and the supernatant was stored at −20° C pending the estimation of enzymatic activity. Disaccharidases were estimated by the method of

Dahlquist (7), alkaline phosphatase as previously described (8), and protein by the Ponceau reaction (9).

Reaginic activity. Specific IgE titers were determined by PCA. Undiluted serum and dilutions up to and including 1:32 were injected intradermally into recipient rats. Twenty-four hours later, 1 mg of ovalbumin and 0.5 ml of 1% Evans blue in saline were injected intravenously; skin reactions were read after 20 min. Titers were recorded as the greatest dilution of serum producing a colored reaction measuring 5 mm or more in diameter.

Definition of controls. As not all parameters were investigated in all groups, nine groups of rats were investigated. The sensitization and challenge protocols were identical except that group 4 received concanavalin A as adjuvant. The parameters measured and the strain of rat are indicated in Tables 1–6. Each experiment included control rats of the same strain and age. These animals received adjuvant but were not sensitized with ovalbumin. They were challenged at the same time and in the same manner as the test animals. These are referred to as controls in the tables. In one experiment the control group was designed to study the effect of our sensitization schedule. In this group, sensitized unchallenged animals were compared with sensitized and challenged animals. The results of this group are referred to in the text, but not in the tables. Student's *t* test was used for assessing the statistical significance between groups.

RESULTS

The results of the PCA reaction are depicted in Table 1. The reaction was negative in all unsensitized control animals. The reaction was positive in all test animals when *B. pertussis* was used as adjuvant. When concanavalin A was used as adjuvant, less consistent PCA reactions were obtained.

In order to evaluate whether the local allergic reaction in the intestine varies at different levels, the first 15 cm were divided into three 5-cm segments (Table 2). It will be seen that both in the test animals and the control animals more radioactive albumin seeped into the proximal bowel than distally when measured as cpm/5 cm. The first 5-cm segment took up about 30% more radioactivity than the second 5-cm segment. Further but less striking reductions took place between the second and third segments. When radioactivity was expressed as cpm/g tissue, the decrease in radioactivity was present only when the first and second segments were compared. Table 3 presents the significance of differences among the three 5-cm segments. When we compared the radioactivity obtained in the first 15 cm with that in the subsequent 15 cm, significant differences again were observed in both control and test groups (Table 4).

Table 5 presents the radioactivity in the intestine in all groups expressed as cpm/g in the first 15-cm segment of intestine. There was a significantly higher radioactivity in the test animals. The difference was slightly more significant in Hooded Lister ($p < 0.01$) than in Charles River rats ($p < 0.05$). These differences were still significant in all groups but group 1, when the results were expressed as cpm/15 cm. Controls that were sensitized but not challenged showed results similar to those observed in unsensitized controls that were challenged.

The radioactivity levels in liver, lung, kidney, and spleen were similar in control and test animals.

Levels of brush border enzymes are shown in Table 5. There was a significant reduction in alkaline phosphatase, maltase, and sucrase in the test groups. However, in the same groups there was a significant rise of lactase activity. In rats that were sensitized but not challenged, no change in any of the disaccharidase levels was noted.

There were similar numbers of mast cells in the walls of the intestines of test and control animals. On light microscopy, no pathologic findings were noted in the test animals.

Table 1. *Results of passive cutaneous anaphylaxis (% positive)**

Adjuvant	*n*	Undiluted	Dilution of serum 1:2	1:4	1:8	1:16	1:32
B. pertussis	25	100	97	70	80	87	57
Concanavalin A	7	71	71	71	57	57	43

* PCA was negative in all unsensitized controls.

Table 2. *Radioactivity in three consecutive 5-cm segments of intestine (group 1) (mean ± SD)*

Segment	Control group ($n = 8$) (cpm/5 cm)	(cpm/g)	Test group ($n = 10$) (cpm/5 cm)	(cpm/g)
1	2369 ± 399	3335 ± 678	2205 ± 375	4149 ± 765
2	1457 ± 222	2729 ± 457	1645 ± 364	3846 ± 970
3	1287 ± 222	2617 ± 364	1436 ± 364	3825 ± 1200

Table 3. *Difference in cpm/5 cm in the three successive 5-cm segments of intestine starting at the pylorus [values are significance of difference (p)]**

Group	Strain†	Control group Segments 1 *vs* 2	2 *vs* 3	1 *vs* 3	Test group Segments 1 *vs* 2	2 *vs* 3	1 *vs* 3
1	CR	<0.01	NS	<0.001	<0.01	NS	<0.001
2	CR	<0.001	NS	<0.001	<0.01	NS	<0.001
6	HL	<0.01	NS	<0.01	<0.001	NS	<0.001

* When calculated as cpm/gm no significant differences could be detected between 1st, 2nd, and 3rd, 5-cm segments.

† CR, Charles River strain; HL, Hooded Lister strain.

Table 4. *Difference in radioactivity between first and second 15-cm segments of intestine when measured as cpm/15 cm [values are significance of difference (p)]**

Group	Control animals	Test animals
5	<0.01	NS
7	<0.05	<0.001
8	<0.01	<0.05
9	NS	<0.05

* No significant differences were found between the first and second 15-cm segments when radioactivity was expressed as cpm/g.

DISCUSSION

In our attempt to obtain an immediate type allergic response to ovalbumin, we first used rats of the Hooded Lister strain; these rats are known for their high IgE levels and propensity to develop immediate type allergy (8). Subsequently, we discovered that the more prolific Charles River strain also was suitable and more economical. Our model of sensitization and challenge was based on that described by Byars and Ferraresi (5). We modified the dosage schedule for optimal PCA reactions and local intestinal anaphylaxis. The doses used were considerably higher than described by Jarrett (10). We used ovalbumin as allergen.

The immune response was probably limited to the gastrointestinal tract, as no changes were observed in liver, spleen, kidneys, or lungs. It was probably an IgE-induced immediate allergic response for the following reasons: all animals in the test groups in which *B. pertussis* was used as adjuvant, but none of the control groups, had positive PCA reactions; the highest dilution of PCA which we used was 1:32. More than 50% of the animals tested still had a positive titer at this dilution showing the

Table 5. *Changes in intestinal brush border enzymes following challenge with ovalbumin in first 15 cm of intestine**

Group	Alkaline phosphatase					Maltase					Sucrase					Lactase				
	n	Control	*n*	Test	*p*	n	Control	*n*	Test	*p*	n	Control	*n*	Test	*p*	n	Control	*n*	Test	*p*
1	7	1.05 ± 0.38	9	0.64 ± 0.39	<0.05	8	300.6 ± 118	10	162 ± 128	<0.05	8	29.8 ± 10.9	10	23.1 ± 19.9	NS	8	0.261 ± 0.46	10	0.85 ± 0.56	<0.05
2	9	1.05 ± 0.4	10	0.045 ± 0.308	<0.001	9	97.7 ± 29.3	10	63.8 ± 27.9	<0.05	9	15.4 ± 5.9	10	7.52 ± 2.87	<0.01	9	0.779 ± 0.77	10	1.38 ± 0.33	<0.05
3	9	2.78 ± 1.29	7	1.64 ± 0.516	<0.05	3	152.7 ± 67.9	5	153 ± 33.9	NS										
4						10	384.6 ± 11.7	6	268.4 ± 48	<0.05	10	38.0 ± 10.7	7	27.6 ± 8.8	<0.05					

* Results are mean ± SD μmol/min/g protein.

presence of considerable reaginic antibody levels. Recently we reported using a similar experimental model that challenge with ovalbumin results in the release of considerable amounts of ovalbumin specific rat IgE antibodies (Freier S, Eran M, unpublished data). The group in which concanavalin A was the adjuvant was an exception in that only 71% of animals had a positive PCA reaction. Probably this is due to the fact that concanavalin A at the dose used is a less effective adjuvant than *B. pertussis.*

The mean level of radioactivity was significantly higher ($p < 0.01$) in the test animals than in controls that were challenged but not sensitized, or those that were sensitized but not challenged. It has been estimated that in 30% of infants with CMPH, specific IgE milk antibodies are present (3). Furthermore, it is known that IgE levels in the duodenal fluid are higher in patients with food allergy (11). Our model may, therefore, be relevant to that population of patients with CMPH in whom immediate type allergy can be demonstrated.

Both in the control and test animals the first 5 cm of intestine distal to the pylorus accumulated more radioactivity than did more distal segments. For group 1 this was highly significant ($p < 0.001$) when calculated as cpm/5 cm, but not when calculated as cpm/g. In other words, the greater bulk of tissue in the first 5 cm was largely responsible for this greater accumulation of radioactivity. There was no difference between the second and third 5-cm segments even when measured as cpm/5 cm. Comparison of the first and second 15-cm segments again showed greater radioactivity in the proximal segment.

The increased radioactivity in the wall of the intestines of test animals was probably due to greater vascular permeability after challenge, with greater movement of albumin from the intravascular to the extravascular compartment. The cause of this increased seepage of albumin was not obvious. Presumably a local vasodilator mediator was released. Although the intestinal mast cells are the obvious candidates for producing such a mediator, no change in the number of granulated mast cells in the walls of the intestines could be demonstrated. In infants with CMPH, granulated mast cells also remain apparently unaffected after a single challenge with cow's milk (Suranyi Y, Freier S, Dolberg L, unpublished data). Measurement of radioactivity within the lumen of the intestine was not altered.

Although in all groups there was a significant difference between control and test animals, this difference usually was more marked in the Hooded Lister strain, suggesting that this strain may have slight advantages over the Charles River strain used.

There was a significant reduction in alkaline phosphatase after the challenge in the test groups. Alkaline phosphatase is a brush border enzyme, and as such is liable to be affected even by superficial damage. Similarly, there was a significant reduction of maltase and sucrase activities. These two enzymes also are brush border enzymes. In the human, reductions of these enzymes in CMPH have been suggested by Iyngkaran *et al.* (12). In an experimental model similar to ours, Perdue *et al.* (13) found a reduction in sucrase following challenge. These authors also found impaired absorption of sodium, potassium, chloride, and water. Despite this, levels of $(Na^+\text{-}K^+)$-ATPase were unaffected. The preservation of lactase activity is difficult to explain in the face of a reduction sucrase and maltase activities. We observed a similar phenomenon in children suffering from chronic nonspecific diarrhea with minimal morphologic changes (Goldstein R, Freier S, unpublished data). It must be remembered that although the enzymes in question are brush border enzymes, we measured activity in the whole enterocyte. Sucrase is produced in the microsomes and Golgi apparatus and transported via the endoplasmic reticulum to the brush border (14). Cytosolic prosucrase can be synthesized within 15 min (14), has disaccharidase activity, and is subject to numerous stimuli such as circadian rhythm and food intake (15). Similar studies on the dynamics of lactase production have not yet been performed, and in their absence the explanation of the dissociation of the

behavior of the disaccharidases in our experimental model remains a moot point.

The absence of morpohologic changes by light microscopy in our model probably is due to the short time which elapsed after challenge. Using a similar model, Perdue *et al.* (unpublished data) found that while morphologic changes could not be identified 1½ h after challenge, after 4 h, edema and shredding of enterocytes could be demonstrated.

REFERENCES

1. Kuitunen P, Visakorpi JK, Hallman N 1965 Histopathology of duodenal mucosa in the malabsorption syndrome induced by cow's milk. Ann Paediatr 205:54–61
2. Loeb H, Vanisel M, Cadranel S, Jacky A, Diederick M, Walter R 1971 Malabsorption and villous atrophy in four infants: relationship to cow's milk intolerance. Acta Paediatr Scand 60:372–380
3. Kletter B, Gery I, Freier S, Noah Z, Davies AM 1971 Immunoglobulin E antibodies to milk proteins. Clin Allergy 1:249–255
4. Weil S, Kuperman O, Ilfeld D, Finelt M, Freier S 1982 Non-specific suppressor cell activity and lymphocyte response to beta-lactoglobulin in cow milk hypersensitivity. J Pediat Gastroenterol Nutr 1:389–393
5. Byars N, Ferraresi RW 1976 Intestinal anaphylaxis in the rat as a model of food allergy. Clin Exp Immunol 24:352–356
6. Bloom G, Kelly JW 1960 The copper phthalocyamine dye Astrablau and its staining properties, especially the staining of mast cells. Histochemie 2:48–57
7. Dahlquist A 1968 Assay of intestinal disaccharidases. Anal Biochem 22:99–107
8. Goldstein R, Klein T, Freier S, Menczel J 1970 Alkaline phosphatase and disaccharidase activities in the rat intestine from birth to weaning. I Effect of diet on enzyme development. Am J Clin Nutr 24:1224–1231
9. Pesce MA, Strande CS 1973 A new micromethod for determination of protein in cerebrospinal fluid and urine. Clin Chem 19:1265–1268
10. Jarrett EEE 1978 Stimuli for the production and control of IgE in rats. Immunol Rev 41:52–76
11. Belut D, Moneret-Vantrin DA, Nicolas JP, Grilliat JP 1980 IgE levels in intestinal juice. Dig Dis Sci 25:323–328
12. Iyngkaran N, Davis K, Robinson MJ, Boey CG, Sumithran E, Yadav M, Lam SK, Puthucheary SD 1979 Cow's milk protein sensitive enteropathy: an important contributing cause of secondary sugar intolerance in young infants with acute infective enteritis. Arch Dis Child 54:39–43
13. Perdue MH, Chung M, Gall DG 1984 Effect of intestinal anaphylaxis on gut function in the rat. Gastroenterology 86:391–397
14. Cezard JP, Conklin KA, Das BC, Gray GM 1979 Incomplete intra-cellular forms of intestinal surface membrane sucrase-isomaltase. J Biol Chem 254:8969
15. Saito M, Murakami E, Suda M 1976 Circadian rhythms in disaccharidases of rat small intestine and its relation to food intake. Biochem Biophys Acta 421:177–179

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Diagnostic and Therapeutic Implications of Medium-Chain Acylcarnitines in the Medium-Chain Acyl-CoA Dehydrogenase Deficiency

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ABSTRACT. The medium-chain acyl-coA dehydrogenase deficiency is one of several metabolic disorders presenting clinically as Reye syndrome. Evidence is presented for a characteristic organic aciduria that distinguishes this disorder from Reye syndrome and other masqueraders characterized by dicarboxylic aciduria. The key metabolites, suberylglycine and hexanoylglycine, are excreted in high concentration only when the patients are acutely ill. More significantly, using novel techniques in mass spectrometry, the medium-chain defect is shown to be characterized by excretion of specific medium-chain acylcarnitines, mostly octanoylcarnitine, without significant excretion of a normal metabolite, acetylcarnitine, in four patients with documented enzyme deficiency. Similar studies on the urine of two patients reported with Reye-like syndromes of unidentified etiology have suggested the retrospective diagnosis of medium-chain acyl-coA dehydrogenase deficiency. Administration of L-carnitine to medium-chain acyl-coA dehydrogenase deficiency patients resulted in the enhanced excretion of medium-chain acylcarnitines. Octanoylcarnitine is prominent in the urine both prior to and following L-carnitine supplementation. The detection of this metabolite as liberated octanoic acid, following ion-exchange chromatographic purification and mild alkaline hydrolysis, provides a straightforward diagnostic procedure for recognition of this disorder without subjecting patients to the significant risk of fasting. In view of the carnitine deficiency and the demonstrated ability to excrete the toxic medium-chain acyl-coA compounds as acylcarnitines, a combined therapy of reduced dietary fat and L-carnitine supplementation (25 mg/kg/6 h) has been devised and applied with

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