

Clinical aspects of allergic disease

Intestinal reactivity in allergic and nonallergic patients: An approach to determine the complexity of the mucosal reaction

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Background: To determine whether inflammatory markers and mediators were released in response to different intestinal antigens, studies were performed in atopic patients allergic to birch, patients allergic to psyllium powder (ispaghula), and patients intolerant to milk.

Methods: Allergy to birch and psyllium powder was documented by the presence of circulating IgE antibodies and positive skin tests. Patients intolerant to milk had negative outcomes of radioallergosorbent tests and skin tests but positive results of double-blind, placebo-controlled tests. Challenge of the intestine with different antigens was achieved by perfusion of a jejunal segment occluded between balloons. Basal and antigen-activated release of mast cell/basophil and eosinophil products and of substances emanating from the plasma and interstitial fluid was compared in perfusate fluid from patients ($n = 8$) and matched healthy controls ($n = 8$).

Results: Perfusate levels of albumin and hyaluronan (previous name hyaluronic acid) were increased threefold to fivefold by antigen in all patients, but not in controls. Eosinophilic cationic protein increased in patients but also in ispaghula controls. Histamine was released in response to milk, but not in patients allergic to birch or ispaghula. Prostaglandin E_2 increased in milk- and birch-sensitive patients. In response to ispaghula, however, it was released in both patients and controls.

Conclusions: We conclude that subclinical intestinal challenge with antigen induces an increase in the appearance rate of albumin and hyaluronan in the intestinal lumen both in atopic patients (with target organs such as the lungs or nose and eyes) and in patients with intestinal intolerance to milk. These changes in permeation are similar to those reported from other organs such as the lung. They may reflect a common response in early phase I reactions that are either IgE-mediated or occur in response to food antigens without any obvious involvement of an IgE-mediated mechanism. Subclinical provocation with intestinal antigens should prove useful for further elucidation of mechanisms underlying intestinal mucosal reactions to exogenous stimuli. (*J ALLERGY CLIN IMMUNOL* 1993;91:553-9.)

Key words: Albumin, allergy, birch, eosinophils, food intolerance, histamine, hyaluronan, intestinal anaphylaxis, mast cells, segmental intestinal perfusion

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The nature of the intestinal reaction in patients challenged with food antigens is still not well understood.¹ Any association between ingestion of a particular food component and different symptoms has commonly been considered to reflect a causal relationship. Systemic anaphylactic reactions to food do not ordinarily present a medical diagnostic problem, because the syndrome is so dramatic. However, the local gastrointestinal reaction is considerably more difficult to recognize. The symptoms are often nonspecific, and ingestion of the food does not produce well-charac-

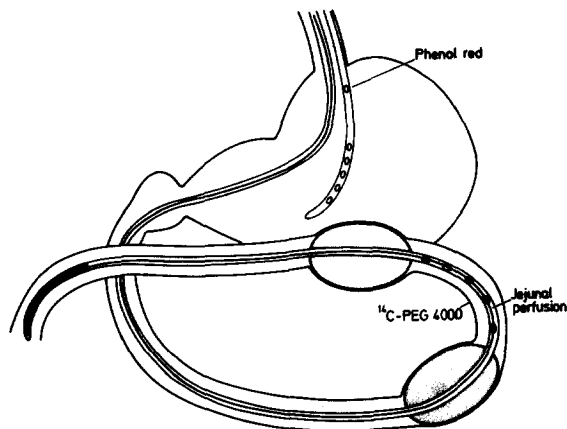


FIG. 1. The technique with a double balloon system exposes only a limited segment (10 cm) of the jejunal mucosa to the antigen. Buffered perfusion solution is infused through a hole in the middle of the segment and evacuated through holes in both ends of the segment.

terized reactions such as angioedema, urticaria, or shock. Even so, patients who believe themselves to be allergic may select diets lacking in several major food groups, which leaves them nutritionally inadequate. The proper approach to management of these patients would be to ascertain the appropriate diagnosis and then prescribe treatment based on this knowledge.² However, several difficulties are associated with objective confirmation of a diagnosis of food intolerance. Classic procedures involve elimination diets and stepwise readministration of the food-stuffs under suspicion. Neither the patient nor the clinician should be aware of what food is being consumed. The use of double-blind, placebo-controlled challenges in studies of food intolerance has eliminated some of the contradictions in this area.^{3,4} Still, this technique is based on elicitation of systemic reactions, reactions that at any moment can turn into dangerous conditions such as angioedema, pulmonary tract constriction, and shock.

In a disease where the gastrointestinal tract is the target organ, studies of local inflammatory events would seem much more appropriate. The antigens first encounter the immune system at the luminal surface of the intestinal epithelium. These primary reactions have not been well elucidated with respect to food antigens in humans, although it has been possible to use an investigative approach in animal models.⁵⁻⁷ When food antigens come in contact with the gastrointestinal mucosa, they trigger reactions in a multitude of different cell types and also induce permeability changes.

The aim of this study was to establish a system for

Abbreviations used

ECP:	Eosinophilic cationic protein
IgE:	Immunoglobulin E
PEG:	Polyethylene glycol
PGE ₂ :	Prostaglandin E ₂
RAST:	Radioallergosorbent test
RIA:	Radioimmunoassay
ST:	Skin test

segmental intestinal challenge with antigens suspected of causing alimentary hyperreactivity in humans. We describe an approach for determining intestinal mucosal and secretory changes in patients in whom intolerance and sensitivity to different food or naturally occurring antigens have been established by clinical methods.

MATERIAL AND METHODS

A tube made of polyvinylchloride with an outer diameter of 16F (5.3 mm) was used (LOC-I-GUT; Kabi-Pharmacia AB, Uppsala, Sweden). A detailed description of the technique is given elsewhere.⁸ In brief, the tube includes six channels and is provided distally with two elongated rubber balloons, 40 mm long, each separately connected to one of the smaller channels. The two wider channels in the center of the tube are for infusion and aspiration of perfusate from the segment. The two remaining peripheral smaller channels are used for administration of marker substances or for drainage. This permits isolation and perfusion of a 10 cm segment (Fig. 1). A Teflon-coated guide wire is used for insertion of the tubes. To further facilitate passage, an 8 cm long weight made from short tungsten plates is attached to the distal end of the tube. The tube was introduced orally, and after its positioning in the desired part of the small intestine, checked fluoroscopically, the guide wire was withdrawn. Continuous gastric drainage was achieved by a separate sump tube (12F). The subjects were free to choose a comfortable position, either seated or lying down. The balloons were inflated with air, and the test segment was rinsed with 154 mmol/L NaCl, at 3 ml/min for 30 minutes with a syringe pump. After the rinsing period, the segment was continuously perfused at 3 ml/min with a solution containing 10 mmol/L glucose, 5.4 mmol/L KCl, 120 mmol/L NaCl, 2 mmol/L Na₂HPO₄, 1 gm/L polyethylene glycol (PEG) MW 4000, and 35 mmol/L mannitol. The osmolality of the perfusion fluid was 290 mOsm/L. ¹⁴C-labeled PEG (¹⁴C PEG, MW 4000 d, 2.5 µCi/L, Amersham Lab., Little Chalfont, England) was added to the perfusate as a volume marker.⁹ Ten milliliters of aprotinin (10,000 KIU/ml Trasylol; Bayer AG, Leverkusen, Germany) was added to every liter of perfusion solution to inhibit any proteolytic activity of trypsin that might have contaminated the effluent. Phenol red solution was infused into the stomach through the sump line of the sump tube at a rate of 1 ml/min and was analyzed in the effluent from the intestinal

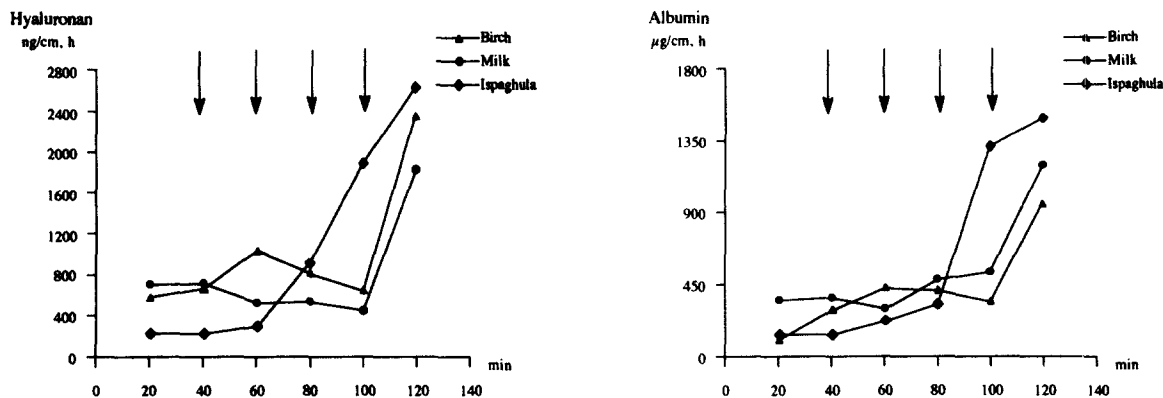


FIG. 2. Increased permeation of hyaluronan (*left*) and albumin (*right*) after titration with antigen in individual patients allergic to birch (▲) or ispaghula (◆) or intolerant to milk (●). The antigen was given with a $\times 10$ increase in concentration at each step (*arrows*).

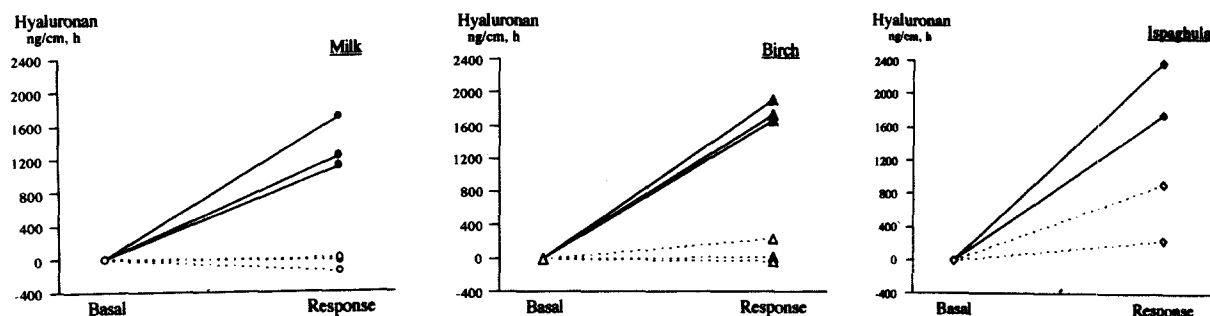


FIG. 3. Maximal net increase (basal subtracted) of hyaluronan in each patient group (*filled*) compared with controls (*unfilled*).

segment. This was done to rule out leakage, that is, contamination from intestinal fluid bypassing the proximal balloon into the segment. All solutions were infused at 37° C; they were collected from the stomach by suction (Ameda Suction Pump Type 23, Ameda Ag, Zug, Switzerland) and from the segment by passive evacuation through gravity drainage. Samples of perfusate were collected on ice at 20-minute intervals and frozen at -70° C in plastic vials. Perfusions were performed for a total period of 120 minutes.

Allergens

The different allergens used were the same in the patients as in their healthy controls. Patients allergic to ispaghula (psyllium powder), a fiber laxative from the seeds of *Plantago ovata*, were challenged with 1/100, 1/10, 10 ml, and finally 50 ml of ispaghula solution (10 mg/ml). Birch allergen (Pharmalgen Birch, 100,000 BE/ml; Pharmacia Diagnostics, Uppsala, Sweden) was serially diluted to concentrations of 1/100,000, 1/10,000, 1/1000, and 1/100 in the intestinal perfusion fluid. The milk allergen was commercial pasteurized cow's milk that was diluted to 1/1000, 1/100, and 1/10, and finally 10 ml of undiluted milk. After

a 40-minute baseline period the antigens were infused in a volume of 10 ml (3 ml/min), starting with the lowest concentration and then increasing the concentration every 20 minutes.

Analytic measurements

¹⁴C-PEG was determined by liquid scintillation spectrometry, and phenol red was measured spectrophotometrically.^{10, 11} Because some of the radioimmunoassays (RIAs) could be influenced by serine proteases in the effluent, all samples were carefully thawed on ice with the addition of 2 mmol/L (10 µl of a 0.2 mol/L stock solution) of phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.), dissolved in absolute alcohol, before any RIA analyses. PMSF is an irreversible serine protease inhibitor. The samples of effluent were analyzed in duplicate for albumin, eosinophilic cationic protein (ECP), histamine, hyaluronan, and prostaglandin E₂ (PGE₂). Albumin, histamine,⁸ and ECP¹² in the perfusion fluid (50 µl sample) were measured in duplicate by RIAs (Pharmacia Diagnostics AB), as was PGE₂ (NEN, Du Pont, Dreieich, Germany). The coefficient of variation for the histamine assay was below 13% and for ECP below 10%. Hyaluronan was mea-

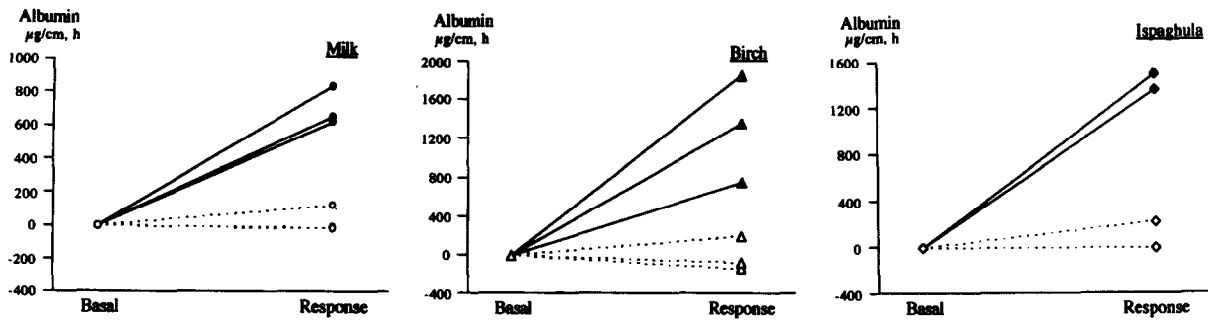


FIG. 4. Maximal net increase (basal subtracted) of albumin in each patient group (filled) compared with controls (unfilled). As in the case of hyaluronan, Fig. 3, there was a pronounced increase in all patients but not in the control subjects.

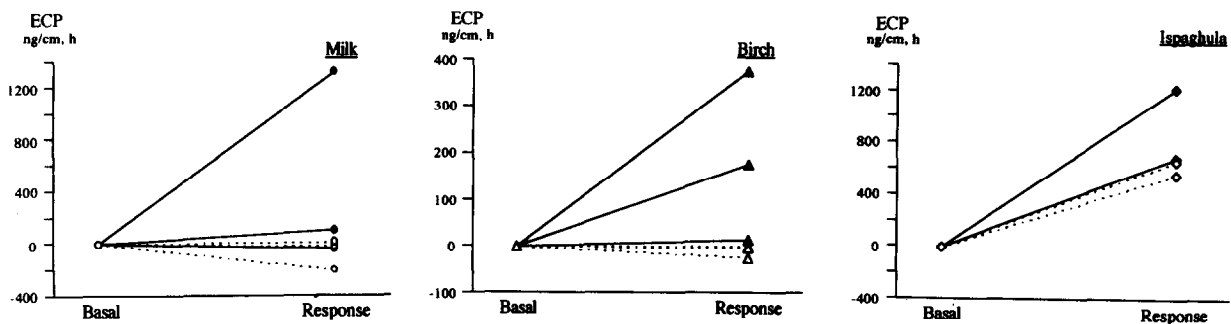


FIG. 5. Maximal net increase (basal subtracted) of ECP in each patient group (filled) compared with controls (unfilled). Not all patients showed an increase, but in the ispaghula group there was a concomitant increase in the controls and patients.

sured by a RIA according to the manufacturers' specifications (Pharmacia Diagnostics AB^{13, 14}). The coefficient of variation for this modified method was below 8%.

Calculations

Appearance rates of the analyzed substances were calculated after correlation for the minimal ¹⁴C losses with the formula: concentration in perfusion fluid $\times$ 3 ml/minute $\times$ 60 minutes/10 cm. Data are presented as secretion per centimeter of bowel per hour. The results are expressed as absolute values in the initial dose-response curves (Fig. 2) and as the maximal net increase (basal subtracted) after challenge in Figs. 3 to 5. The results of the histamine and PGE₂ determinations are expressed as maximal net increase (basal subtracted) in the table.

Patients and controls

Studies were performed in three groups of patients with proven allergy to different antigens. Three patients with proven allergy to birch pollen all had a positive skin test (ST) performed the same day as the intestinal study. These patients had all reacted with asthmatic symptoms to inhalation of birch pollen during the pollen season and they had positive RASTs. The studies on these patients were all carried out during the winter, when there is no exposure to birch.

Three patients with food intolerance to milk were also studied. These patients had shown positive double-blind, placebo-controlled test results when challenged with cow's milk, although they all had negative outcomes on ST and RAST. The symptoms after exposure to milk included abdominal distention, discomfort, nausea, and diarrhea.

Finally, two patients allergic to ispaghula, a dietary fiber, were studied. They were members of the hospital staff, and sensitivity to the allergen developed when they were giving the fiber powder to patients. Their symptoms were rhinoconjunctivitis and angioedema, and they had positive results on RAST and ST. Each patient was compared with a matched, healthy control. The controls all had a negative history of allergy and also had negative results on ST and RAST. The patients and controls completed a standardized questionnaire regarding previous allergic reactions, and the study was approved by the Ethical Committee of the Medical Faculty, Uppsala University.

RESULTS

Recovery of volume and markers

The perfusate instilled into the segment was effectively recovered by gravity drainage and did not escape from the test segment to any appreciable extent. The effluent volume in 20 minutes (instilled volume-

period was 60 ml) was on average 56.2 ± 2.5 ml in the controls and 57.0 ± 4.0 ml in the patients. The percentage recovery of the ^{14}C -PEG marker infused into the segment was $96.6\% \pm 1.3\%$ in the controls, compared with $91.2\% \pm 5.0\%$ in the patients. The amounts of proximal contents entering the segment per 20-minute period, as tested by the appearance of phenol red in the segment, were $1.6\% \pm 0.2\%$ and $2.1\% \pm 0.4\%$ of the amounts infused into the stomach in the controls and patients, respectively.

Intestinal antigen titration

Dose-response titrations were initially performed with increasing concentrations of luminal antigens in both patients and controls and are presented for hyaluronan and albumin in Fig. 2. This was done to confirm that the concentration of antigen used did not provoke systemic reactions. None of the patients had symptoms that could be attributed solely to the antigen rather than to the discomfort of the investigation. The two patients provoked with ispaghula, however, had some abdominal discomfort, as did one of the controls in this group. For milk and birch only the highest concentrations of antigen increased the permeation of the two macromolecules albumin and hyaluronan into the lumen. Ispaghula initiated an increase in permeation even at a lower concentration. This antigen, however, could not be standardized and was also given as a larger volume.

Basal appearance rate and maximal net increase in response to antigen

The mean basal values of the different inflammatory mediators for the first two 20-minute periods were as follows: (1) Albumin ($\mu\text{g}/\text{cm}$, hour): birch-allergic patients 472 ± 141 , control subjects 789 ± 343 ; milk-intolerant patients 323 ± 44 , controls 361 ± 128 ; ispaghula-allergic patients 246 ± 104 , controls 180 ± 40 . (2) Hyaluronan (ng/cm , hour): birch-allergic patients 1324 ± 351 , control subjects 1265 ± 358 ; milk-intolerant patients 566 ± 79 , controls 400 ± 102 ; ispaghula-allergic patients 959 ± 729 , controls 413 ± 181 . (3) ECP (ng/cm , hour): birch-allergic patients 111 ± 16 , control subjects 97 ± 28 ; milk-intolerant patients 112 ± 24 , controls 73 ± 33 ; ispaghula-allergic patients 123 ± 27 , controls 86 ± 26 . (4) Histamine (ng/cm , hour): birch-allergic patients 79 ± 18 , control subjects 46 ± 4 ; milk-intolerant patients 113 ± 8 , controls 45 ± 50 ; ispaghula-allergic patients 151 ± 105 , controls 73 ± 56 . (5) PGE_2 (pg/cm , hour): birch-allergic patients 22 ± 2 , control subjects 26 ± 3 ; milk-intolerant patients 26 ± 5 , controls 18 ± 7 ; ispaghula-allergic patients 25 ± 11 , controls 12 ± 2 .

The maximal net increase (basal subtracted) after challenge with the highest concentration of antigen is

TABLE I. Net (basal subtracted) increase in histamine and PGE_2 expressed as secretion per centimeter of bowel per hour in individual patients and control subjects

	Subject	Histamine ng/cm. hour	PGE_2 pg/cm. hour
Milk			
Patients	1	327	78
	2	104	35
	3	114	-6
Controls	1	-18	-7
	2	11	1
	3	25	-4
Birch			
Patients	4	-27	21
	5	-7	31
	6	-9	-3
Controls	4	-3	-2
	5	-19	-4
	6	-18	-7
Ispaghula			
Patients	7	-31	413
	8	6	199
Controls	7	140	85
	8	228	155

illustrated for patients and controls in Figs. 3 to 5. All patients reacted with a distinct increase in the intestinal appearance of albumin and hyaluronan compared with the controls. The appearance rate increased to a value three to five times the basal secretion with all three antigens. The maximal response occurred in the first 20-minute period after the administration of the highest concentration of the antigen.

The determination of mediators likely to arise from eosinophils and mast cell and basophils illustrates the response from mucosal effector cells. Two of three patients allergic to birch and two of three patients intolerant to milk, but not the controls, reacted to allergen challenge with a release of ECP. Challenge with ispaghula induced such a response in both patients and controls (Fig. 5). This nonspecific effect was also observed when mast cell and basophil degranulation was assessed by histamine determination. Of the different antigens used for intestinal challenge, only ispaghula induced an increased release of histamine in controls. Histamine release occurred in all patients intolerant to milk, but not in patients allergic to birch (Table I). The same two of three patients who showed an increase in ECP when challenged with milk or birch also displayed an increase in PGE_2 , with no reaction in the controls. The pronounced release of PGE_2 in patients after challenge with ispaghula was noted in both patients and controls.

DISCUSSION

In an attempt to clarify some of the mechanisms underlying intestinal immune reactions to food, we have used a segmental perfusion system in humans to characterize biochemical events and products of antigen-antibody reactions in tissues. The local release of high and low molecular weight substances derived from interstitial tissue and lymph and plasma, and of granule components from eosinophils and mast cells and basophils, was examined. An occluded segment of the proximal jejunum was studied in patients intolerant to different antigens and in control subjects. A group of patients with immunoglobulin E (IgE)-mediated, positive asthmatic disease on RAST and positive to birch on ST was compared with a group with intolerance to milk. These latter patients displayed positive responses to placebo-controlled, double-blind provocation tests, but were negative to milk on ST and RAST, indicating that they probably had local but not systemic reactions to milk antigens. A third group consisted of patients reacting to the powder of the dietary fiber ispaghula with rhinoconjunctivitis; they were positive on RAST and ST. The target organs in the three groups of patients were (1) upper airways and lungs, (2) intestinal mucosa, and (3) a combination of 1 and 2 with preference for the nose and eyes, respectively.

The technique used here was developed to produce local but not systemic reactions. When patients, but not controls, were challenged with the highest concentration of antigen, the mucosa responded with a release of several inflammatory mediators. The parallel increase in albumin and hyaluronan in all patients studied illustrates the local anaphylactic inflammatory reaction. Dietary fiber such as ispaghula, however, not only provokes an allergic intestinal reaction in predisposed patients, but can also achieve part of its effect through nonspecific irritation. This was evident by an increase in hyaluronan and albumin in one of the control subjects.

Signs of increased leakage of albumin and hyaluronan over the jejunal mucosa during challenge with gliadin in patients with celiac disease has recently been described.¹⁵ In the present study the increased appearance rate of albumin probably reflects an increased leakage from plasma to lumen, as is also seen in other IgE-dependent allergic reactions. Hyaluronan is known to be abundant in mesenteric lymph and also in interstitial fluid.¹⁶ Presumably the rapid increase in the rate of appearance of hyaluronan after challenge mainly reflects an increased passage of this large glucosaminoglycan from mesenteric lymph and interstitial fluid.

Mast cells and basophils are known to mediate allergic reactions.^{17, 18} In fact, IgE-mediated allergic reactions are usually considered to be mediated by histamine release and/or the formation of arachidonic acid metabolites.^{19, 20} Although not IgE-mediated, mast cell and basophil degranulation with ensuing histamine release is part of the intestinal response to challenge with gluten in celiac disease,²¹ but in milk intolerance there are morphologic reports of both degranulated and nondegranulated mast cells.^{22, 23} In the present study we failed to demonstrate an increase in intestinal histamine secretion as a sign of mast cell and basophil activation after antigen challenge of patients with IgE-mediated allergy to birch, although there was a release in patients intolerant to milk (Table I). Milk intolerant patients might have an intestinal IgE-dependent reaction that is not reflected systemically. Whether this difference in the activation of mucosal cells really reflects disparate modes of action in IgE- and nonIgE-mediated reactions remains to be studied.

Intestinal anaphylaxis can, however, induce release of other inflammatory mediators stored in secretory granules. Eosinophils are known to mediate allergic and inflammatory reactions through the release of several cytotoxic conditions mediating cell damage.²⁴ This is important in the defense against intestinal parasites,^{25, 26} and an increase in eosinophils and in microvascular permeability has also been reported after intrabronchial challenge of the lung.²⁷ In patients with celiac disease intraluminal challenge with gliadin evoked eosinophil degranulation, with a maximum increase in ECP at 60 minutes.²¹ Furthermore, morphometric studies have revealed increased numbers of intraepithelial eosinophils in cow's milk allergy with circulating IgE antibodies.²⁸ In the present study, however, both patients with IgE-mediated allergy (birch) and those with nonatopic intolerance (milk) reacted with an increase in ECP. This release of granule components from eosinophils corresponded in time with enhanced transport of albumin and hyaluronan into the studied intestinal segment. In the same patients reacting with an increase in prostaglandins (two of three patients in the milk and birch group, respectively), part of the local anaphylactic reaction was also mediated through a release of the cytotoxic compound ECP. In fact, activation of eosinophils results in the release of several potentially cytotoxic substances as well as prostaglandins.²⁹ The activation of mucosal effector cells was further demonstrated in control subjects given ispaghula, in whom the challenge induced a release of ECP that was nearly as high as in the patient group.

In summary, an approach to determine the local response in intestinal anaphylaxis after challenge with antigen in allergic patients and in patients with no identified circulating IgE antibodies, was evaluated. The determination of mediators derived from mast cells/basophils and eosinophils, such as histamine and ECP and also PGE₂, did not always differentiate between patients and controls. However, the appearance of albumin and of the glucosaminoglycan hyaluronan in the intestine increased rapidly in patients, but not in controls, when the highest concentration of the antigen was used. We propose that determination of local intestinal release and permeation of these substances can be used for elucidation of the early stages of intestinal anaphylaxis in humans.

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