Eosinophil cationic protein and histamine after intestinal challenge in patients with cow's milk intolerance

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Background: Mast cells and eosinophils are key cells in the development of active symptoms in allergic diseases and other inflammatory conditions, and they mediate their action through the release of very potent granule constituents.
Methods: Five patients with milk-related gastrointestinal symptoms diagnosed by double-blind placebo-controlled milk challenges, but with negative responses to skin prick tests and RASTs with milk, and eight healthy control subjects were investigated. Repeated perfusion studies were performed with a two-balloon, six-channel tube by using milk, casein, and whey as antigens. Luminal eosinophil cationic protein, histamine, and albumin were measured by radioimmunoassay.
Results: Luminal cow's milk induced a pronounced increase in intestinal secretion of histamine and eosinophil cationic protein in patients, but not control subjects, during the first 20 minutes after challenge (histamine from 123 ± 12 to 543 ± 175 ng/cm, hr; eosinophil cationic protein from 80 ± 23 to 686 ± 262 ng/cm, hr). Albumin, as a marker of plasma leakage, was also significantly increased.
Conclusion: These data indicate that mast cells and eosinophils are effector cells not only in patients with allergic disease but also in patients intolerant to foods and lacking circulating antibodies. The underlying mechanisms may be a reaction mediated by locally appearing antibodies or an immunologic activation resembling that found in intestinal disorders such as celiac disease. (J Allergy Clin Immunol 1997; 100:216-21.)

Key words: *Albumin, allergy, eosinophils, eosinophil cationic protein, food intolerance, gastrointestinal, histamine, mast cells*

Abbreviations used
ECP: Eosinophil cationic protein
RIA: Radioimmunoassay
SPT: Skin prick test

The eosinophil granulocyte is an important effector cell in allergic disease and asthma. In fact, it has been suggested that products from eosinophil cells are major causative factors in allergic airway disease.[1] The actions of the eosinophil and its many inflammatory mediators therefore present a highly visible target for therapeutic modalities.[2] Eosinophils are essentially tissue cells that are seen, for example, in large numbers in the lamina propria of the normal gut.[3] In normal tissue they are intact, but in diseased tissue they often undergo massive degranulation and release their inflammatory mediators.[4] Eosinophil cationic protein (ECP), a cytotoxic mediator acting on mammalian cells, is a marker of eosinophil activation, although the exact mechanism of ECP in allergic disease is not known. The traditional view is that eosinophils are recruited as a consequence of the allergen-induced IgE-dependent release of chemotactic factors,[5, 6] but they can be increased, apparently, in both allergic and nonallergic asthma.[7] These and other findings indicate that local allergy-like reactions may occur.[8]

Mast cells also have important immunoregulatory functions and play a major role in immediate allergic and inflammatory reactions. Through their production of inflammatory mediators and cytokines, they contribute to the recruitment of eosinophils at the site of inflammation.[9] Mast cells may also play a role in maintaining local IgE responses in nonlymphoid organs, such as the gut, by taking over the role of T-helper lymphocytes and functioning as effector cells in acute allergic and inflammatory reactions.[10] These associations between mast cells and eosinophils have been recognized for many years, and histamine has been demonstrated to be chemotactic for eosinophils in vitro[11] and to enhance the cytotoxic capacities of these cells.[12]

Information on the participation of inflammatory mediators on eosinophils and mast cells/basophils in intestinal events in patients with a history of adverse reactions to foods is sparse or lacking. Adverse reactions to foods are divided into toxic and nontoxic reactions, and nontoxic reactions are further divided into immune-mediated reactions (food allergy) and eventually nonimmune-mediated reactions (food intolerance). New methods for clinical investigation of gastrointestinal tract functions in relation to the intestinal immune response are required for assessment of these patients. The aim of

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Supported by grants from the Swedish Association Against Asthma and Allergy and Pharmacia Diagnostics AB.
Received for publication Mar. 14, 1996; revised Mar. 5, 1997; accepted Mar. 11, 1997.
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0091-6749/97 $5.00 + 0 **1/1/81780**

TABLE I. Jejunal fluid concentrations of albumin, histamine, and ECP

	Albumin (mg/L)			Histamine (μg/L)			ECP (μg/L)		
	Milk	Casein	Whey	Milk	Casein	Whey	Milk	Casein	Whey
Patients (n = 5)									
Basal	13.7 ± 4	47.5 ± 15	26.6 ± 8	6.8 ± 0.7	5.7 ± 0.9	3.1 ± 0.4	4.4 ± 1	6.1 ± 2.6	2.8 ± 1
Peak	69.8 ± 22*†	45.9 ± 12	27.3 ± 10	33.1 ± 8*†	5.6 ± 1	4.8 ± 0.9	38.1 ± 15*†	6.1 ± 1.8	4.2 ± 1
Control (n = 8)									
Basal	24.0 ± 4	43.1 ± 7	43.0 ± 6	5.4 ± 2	2.8 ± 0.4	5.2 ± 0.9	9.6 ± 6	11.0 ± 8	3.9 ± 1
Peak	27.8 ± 6	40.6 ± 5	35.0 ± 7	9.5 ± 3	4.3 ± 0.8	6.1 ± 0.8	9.7 ± 4	12.1 ± 8	3.4 ± 0.7

Data are presented as mean values ± SEM of one basal 20-minute perfusion period compared with the highest value after challenge with the different antigens. The highest value was obtained during the first 20 minutes after challenge.
*p <0.05 versus basal values in the same patient.
†p <0.05 versus control values.

this study was to determine the local intestinal release of ECP and histamine in patients with a history of milk-induced gastrointestinal symptoms who have negative responses to skin prick tests (SPTs) and RASTs with milk, and compare the result with that in healthy control subjects.

METHODS

Patients and control subjects

Five patients with a history of milk-related diarrhea were studied. All five had a history of bronchial asthma, and three of them had a history of rhinitis and/or conjunctivitis. Clinically, they had experienced migrating arthralgia, joint swelling, and mucus in the stool; they also had abdominal distention after milk challenge. We only considered patients in whom milk-induced gastrointestinal symptoms were diagnosed by double-blind placebo- controlled milk challenge. The double-blind provocation study consisted of two active challenges with the suspected antigen and two placebo-controlled challenges in the same patient. In open challenges foods were used in their natural form. In blinded challenges the same foods were used, but they were freeze-dried and pulverized. Dextrose was used as placebo. Foods and placebo were placed in opaque gelatin capsules that were tinted with titanium oxide. For the results of an active, blinded challenge to be considered positive, symptoms the same as those that were produced in the open challenge had to be observed. Furthermore, the symptoms had to be of the same intensity and duration and followed by a placebo control challenge with negative results in the same patient. The patients had been on an elimination diet before the investigation, and antihistamines and acetylsalicylic acid were withdrawn for 48 hours before challenge.

The patients and control subjects had negative responses to SPTs and RASTs with milk, and they all tolerated lactose. Lactose tolerance was tested by an oral dose of 100 gm of lactose, which had to produce a blood glucose level of 1.2 mmol or more to be considered normal. A blood glucose level of less than 1.2 mmol indicated lactose intolerance. The oral dose of lactose induced blood glucose levels of 6.0, 6.6, 6.1, 12.2, and 7.6 mmol in the different patients, respectively. Because there are reports showing that patients with negative skin test results in response to commercial milk extracts have positive results in response to fresh skim milk, all patients were also tested with this antigen. None of the patients, however, had a positive skin reaction to skim milk.

The SPTs were performed and the reactions graded according to standard procedures. The allergen extracts were prepared and standardized in the laboratory of the Asthma and Allergy Center, Sahlgrens Hospital. For SPTs, one panel of alimentary allergens (meat, fish, shellfish, cow's milk, soy protein, egg yolk, egg white, wheat flour, rye flour, oatmeal, barley meal, hazel nut, chocolate, vegetables) and another panel of aeroallergens (mite, mold, dander, grass, herb, tree) were used, as in an earlier study.[13] All patients and control subjects were tested by Phadebas RAST (Pharmacia Diagnostics AB) for allergic reaction to milk, as described in the test kit instructions from the manufacturer.

The five patients underwent jejunal perfusion and were challenged intraluminally with the three different antigens, and the data obtained were compared with data from eight healthy volunteers studied under identical conditions. The mean age of the patients was 54 years, and the mean age of the control subjects was 28 years (ranges, 50 to 59 years and 22 to 36 years, respectively). Although we made every effort to achieve a better age match between the patients and the control subjects, it turned out that when our healthy volunteers (mostly students) became older and their financial status improved, they stopped participating as subjects in our studies because of the discomfort. This disparity in age could influence the results concerning intestinal reactivity to allergen challenge. Indicators of allergy-like skin test reactivity and peripheral blood eosinophil counts, however, show an inverse relationship to age, which means that if there were any difference between the groups in this respect, the older study group would react less strongly to challenge than would the younger group.[14]

Isolation of a jejunal test segment

A tube made of polyvinyl chloride with an outer diameter of 16F (5.3 mm) was used (LOC-I-GUT, Pharmacia AB). The precise use of the technique is explained elsewhere.[15] In short, the tube contained six channels and was provided distally with two 40 mm long, elongated latex balloons, each separately connected to one of the smaller channels. This permitted isolation and perfusion of a 10 cm long segment in the proximal part of the jejunum. The tubes were positioned under fluoroscopic guidance, and continuous gastric drainage was achieved by a separate Salem sump tube (12F; Sherwood Medical, Petit Rechain, Belgium).

Experimental design

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 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

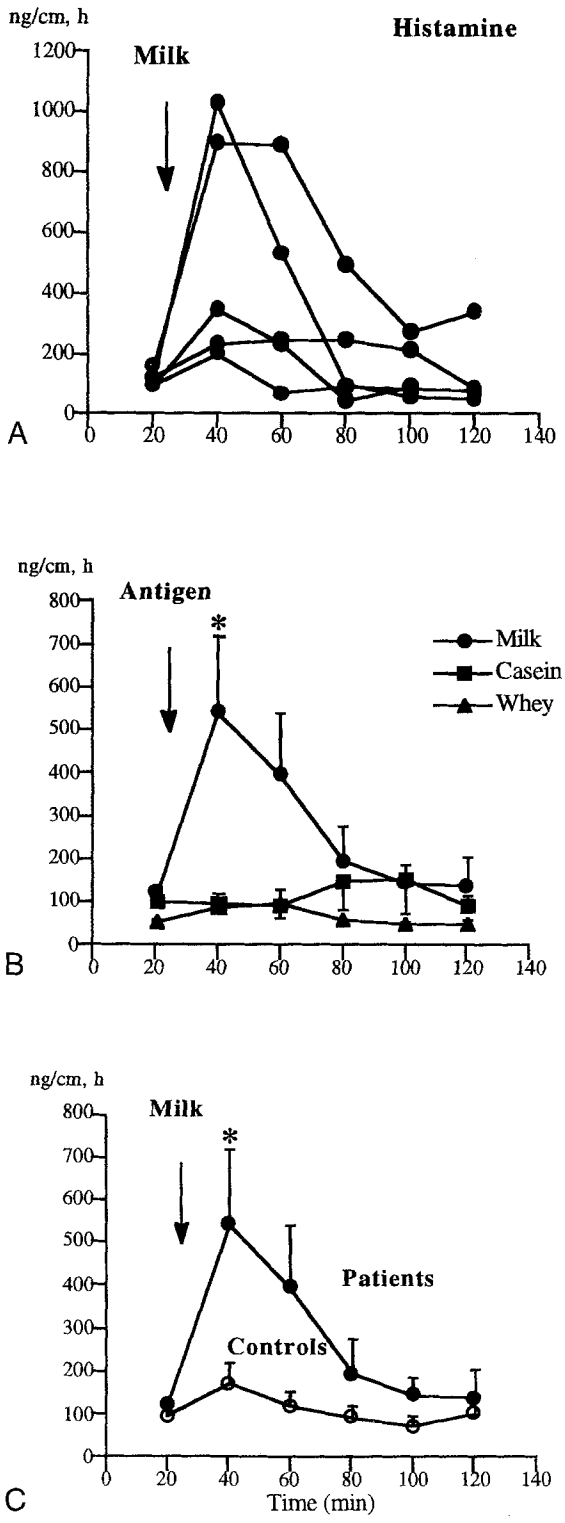

FIG. 1. Rates of histamine secretion into isolated jejunal segment in five patients. **A,** Individual secretion rates after challenge with milk as antigen *(arrow).* All five patients responded with increased secretion after challenge. **B,** Comparison of secretion after challenge *(arrow)* with three antigens: milk, casein, and whey (mean ± SEM). Only full cow's milk induced a significant increase in secretion ($*p < 0.05$). **C,** Comparison of secretion in patients and control subjects after challenge with milk (mean ± SEM; $*p < 0.05$).

Na_2HPO_4, 1 gm/L polyethylene glycol with a molecular weight of 4000 d, and 35 mmol/L mannitol. The osmolality of the perfusion fluid was 290 mOsm/L. Carbon 14-labeled polyethylene glycol (^{14}C polyethylene glycol; molecular weight, 4000 d, 2.5 μCi/L; Amersham Lab, Buckinghamshire, England) was added to the perfusion fluid as a volume marker. Ten milliliters of aprotinin (10,000 kIU/ml Trasylol; Bayer AG, Leverkusen, Germany) was added to every liter of perfusion solution in order to inhibit any proteolytic activity from proteases, which might have contaminated the effluent. Phenol red solution was infused into the stomach through the sump line of the Salem-sump tube at a rate of 1 ml/min and was measured in the effluent from the intestinal segment. This was done to rule out leakage (i.e., contamination from intestinal fluid bypassing the proximal balloon into the segment). All solutions were infused at a temperature of 37° C and were collected from the stomach by suction and from the intestinal segment by gravity drainage. Samples of perfusate were collected on ice at 20-minute intervals and frozen at −70° C in plastic vials. Perfusions were performed during a period of 120 minutes. The patients and control subjects were investigated and challenged with three different antigens (milk, casein, and whey) on three different occasions, resulting in a total of 38 perfusion studies. One patient was only given milk and casein and refused participation in the third perfusion study. Only one experiment was performed each day and experiments performed in the same subject were separated by at least 4 weeks.

Allergens

The milk allergen was commercially pasteurized cow's milk and was given as 10 ml of undiluted milk, at a rate of 3 ml/min, after a 20-minute baseline period. Whey and casein (obtained from Sigma Chemical Co., St. Louis, Mo.) were given in the same way as the milk allergen.

Analytic measurements

ECP was measured in duplicate by a radioimmunoassay (RIA) technique (Pharmacia ECP RIA).[16, 17] Histamine and albumin (50 μl samples) were assayed in duplicate by a double-antibody RIA (Pharmacia Diagnostics AB). Parallel standard curves were made for all substances by means of the respective standards mixed with either buffer or a constant volume of the perfusion fluid. The variability of the histamine assay was 13%, and the variabilities of the ECP and albumin assays were each below 10%. ^{14}C–labeled polyethylene glycol was determined by liquid scintillation and phenol red was measured spectrophotometrically.[15] Because some of the RIAs could have been influenced by the presence of even small amounts of protease 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.), dissolved in absolute alcohol, before all RIA analyses. Phenylmethylsulfonyl fluoride is an irreversible serine protease inhibitor.[18]

Calculations and statistical analyses

The results are expressed as jejunal fluid concentrations in Table I and as secretion rates of the analytes in Figs. 1 and 2 (mean ± SEM). Data are also expressed as medians and ranges in the Results section. The luminal secretion rate was calculated after correction for the minimal ^{14}C losses, by using the formula: Concentration in perfusion fluid × 3 ml/min × 60 minutes ÷ 10 cm, and expressed as secretion per centimeters of bowel per hour. All results were analyzed individually, and the

statistical significance of differences was tested by the Mann-Whitney rank sum statistic with comparisons between basal and peak values, with each patient serving as his or her own control, and between patients and control subjects. Differences were considered significant at a p value of less than 0.05. The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University.

RESULTS

The patients were openly challenged with cow's milk, casein, and whey administered in the isolated jejunal segment. The most noteworthy finding when milk was given, besides colic and diarrhea, was that in four of the five patients the abdominal circumference increased within 60 minutes after challenge. No such increase was observed when casein and whey were given. Under basal conditions there was no significant difference between the patients and the control subjects in the concentration of histamine or ECP in the isolated segment ($p > 0.05$; Table I and Figs. 1 and 2). The basal histamine secretion in the repeated studies in the five patients was 100 ng/cm, h (range, 41 to 164 ng/cm, h) (14 different studies), and in the control subjects the basal histamine secretion was 66 ng/cm, h (range, 8 to 280 ng/cm, h) (24 different studies). The basal ECP secretion was 82 ng/cm, h (range, 17 to 158 ng/cm, h) and 50 ng/cm, h (range, 18 to 1172 ng/cm, h) in patients and control subjects, respectively. These findings could be taken as confirmation that the patients had taken the recommendations of an exclusion diet seriously.

During challenge with milk, the secretion rates of ECP and histamine started to increase as early as 20 minutes after administration of the dose (Figs. 1 and 2). The maximum secretion rates of ECP and histamine were also observed during this period, and the values declined to the prestimulation levels during the rest of the observation period. The secretion rates of histamine and ECP increased in all five patients after luminal challenge with milk. Three of the five patients showed a more pronounced increase in the secretion rate of ECP, and the same patients also reacted with higher luminal histamine values. On average, the rates of albumin and histamine secretion increased fivefold, but the secretion of ECP increased almost ninefold ($p < 0.02$).

In the control subjects, administration of milk into the isolated segment did not induce any changes in the appearance rates of albumin, histamine, or ECP; nor did luminal casein or whey have any effects on these substances in the patients or control subjects.

DISCUSSION

During luminal perfusion of an isolated segment of the proximal part of the jejunum, we were able to determine the secretion rate of ECP and histamine and the effects of different milk antigens in patients intolerant to cow's milk and in healthy control subjects. We observed a fivefold increase in luminal histamine and a nearly ninefold increase in luminal ECP during the first 20 minutes after challenge with cow's milk, but not after

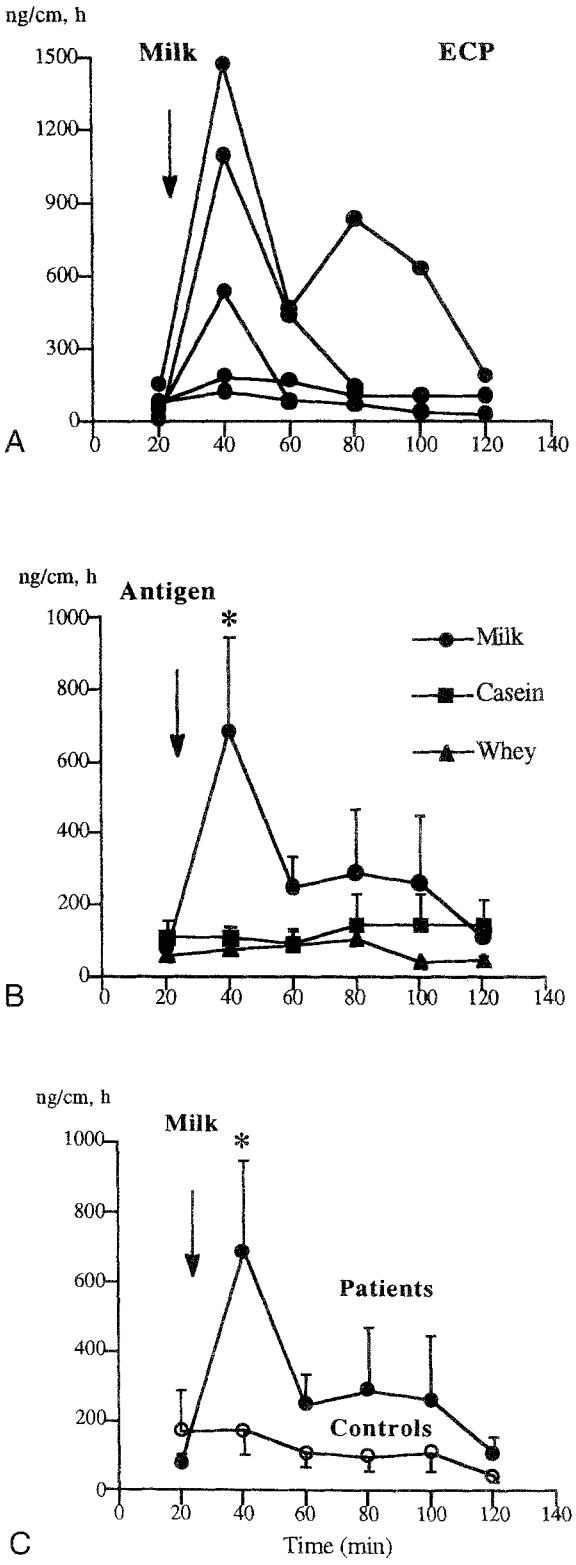

FIG. 2. Rates of ECP secretion into isolated jejunal segment in five patients. **A,** Individual increase in ECP secretion in all five patients after challenge *(arrow)*. **B,** Comparison of ECP secretion after challenge *(arrow)* with the three antigens: milk, casein, and whey (mean ± SEM). **C,** Comparison of secretion in patients and control subjects after challenge with milk (mean ± SEM; $*p < 0.05$).

challenge with casein or whey. It is reasonable to assume that the granule constituents are released mainly into the small bowel lumen from eosinophils and mast cells infiltrating the jejunal mucosa. Furthermore, it is unlikely that the appearance of ECP (molecular weight, 21,000 d) and histamine (molecular weight, 116 d) is attributable to a passive leakage from plasma. The concentration of ECP in the jejunal fluid was close to or even higher than the concentration in the bloodstream.[19] The concentrations of histamine in the perfusion fluid were considerably higher than concentrations in the plasma, and histamine does not pass through cell membranes. In contrast, the appearance of albumin in the jejunal fluid should reflect passive leakage from the plasma/interstitial fluid compartments.

Selective infiltration of eosinophilic granulocytes into local tissue is recognized as a marker of type I allergic reactions but has also been observed not only in IgE-mediated reactions but also in tissue affected by other immunologic alterations. Luminal antigens contribute to the pathogenesis of gastrointestinal disorders, such as eosinophilic gastroenteritis, celiac disease, and perhaps inflammatory bowel disease.[20, 21] In celiac disease, the pathophysiologic event that leads to the characteristic morphologic changes is primarily immunologic, including both reactions elicited by antigen-antibody complexes and cell-mediated damage involving activation of eosinophils and mast cells.[22]

Recent studies have shown that there are complicated interactions and pathways involving other cell types, chemical mediators, and cytokines in the mechanism of eosinophil attraction.[23-27] In studies of allergic mucosal responses assessed by the presence of mast cells and eosinophils in the nasal cavity, the kinetics of the cellular events have been elucidated. The numbers of eosinophils and metachromatic cells (mast cells rather than basophils) increase fairly rapidly after allergen challenge (2 to 4 hours) but much more slowly than the development of clinical symptoms, which are generally expressed 15 minutes after challenge.[28] These findings imply that cellular migration is too slow to account for the clinical attack. The symptoms have to be provoked by inflammatory mediators from cells already waiting to respond to the antigen at the site of penetration. In fact, morphometric studies of the gut have revealed increased numbers of intraepithelial eosinophils in patients with cow's milk allergy with circulating IgE antibodies.[29]

One of the four preformed mediators of human eosinophils is ECP. Challenge tests with simultaneous measurement of ECP in the serum in patients with food intolerance have been performed but with conflicting results.[30] Besides being a cytotoxic protein, ECP has the ability to alter the production of glycosaminoglycans, specifically hyaluronan, by human fibroblasts and to stimulate airway secretion.[31, 32] Hyaluronan is increased in the joints in rheumatoid disease,[33] and patients in this study complained of joint swelling. In the airways, ECP directly stimulates the release of submucosal gland products and plays a role in mucus hypersecretion, as has been described in asthma.[2] The patients in this study complained of increased amounts of mucus in the stool, which can be explained by a similar mechanism. Of potential interest also is the possible involvement of ECP in T-cell–mediated reactions in vivo such as connective tissue diseases, inflammatory bowel disease, and rheumatoid arthritis.[34]

Inflammatory lesions, irrespective of whether eosinophils are recruited into them, share a loss of impermeability of venular endothelium to plasma proteins.[7] This leads to the accumulation of plasma colloids, which was demonstrated in this study by an increased appearance rate of albumin during the first 20 minutes after challenge. Furthermore, among the effects of local histamine, increased vascular permeability and development of mucosal edema are major contributors to the inflammatory response.[35]

The association between mast cells and eosinophils has long been recognized.[36] It has been proposed that eosinophils are not initiating cells for the early phase of an allergic reaction and that factors activating eosinophils are mostly produced in and released from cells responsible for this primary reaction, such as mast cells.[37, 38] The data in this study strongly indicate that mast cells not only function as effector cells in acute allergic reactions but probably also have important immunoregulatory functions in allergic inflammation or inflammation in general.[28] Histamine, however, is not a specific marker, because the basophil is also a rich source of histamine. The pure kinetics of the cellular events in this study, with the most pronounced changes in the appearance of the inflammatory mediators occurring during the first 20 minutes after challenge, indicate a release from cells already lining the gastrointestinal tract and not transported from the blood. Cellular provocation studies of the nasal mucosa have similarly revealed that metachromatic cells lining the nasal cavity are mast cells rather than blood basophils.[39] The mast cells also contain many other inflammatory mediators, which could contribute to the pathogenesis of the mucosal reaction and participate in eosinophil activation.

In conclusion, our results indicate that the intestinal mucosa in patients intolerant to cow's milk, without circulating IgE antibodies, reacted to locally administered antigen with an increased release of ECP and histamine into the intestinal lumen. This supports the hypothesis that evaluation of the activity of inflammatory cells, and not just their numbers, provides information about the inflammatory cascade in quantitative terms and further elucidates the quality of the process and the kinetics involved.[40]

REFERENCES

1. Svensson C, Andersson M, Persson CGA, Venge P, Alkner U, Pipkorn U. Albumin, bradykinins and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. J Allergy Clin Immunol 1990;85:829-33.
2. Mehta H, Busse WW. Eosinophil as a therapeutic target in allergic disease. Compr Ther 1994;20:651-7.

3. Wardlaw AJ, Symon FS, Walsh GM. Eosinophil adhesion in allergic inflammation. J Allergy Clin Immunol 1994;94:1163-71.
4. Gleich GJ. The eosinophil and bronchial asthma: current understanding. J Allergy Clin Immunol 1990;85:422-36.
5. Metzger WJ, Richerson HB, Wasserman SI. Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late-phase asthmatic responses. J Allergy Clin Immunol 1986;72:282-90.
6. Durham SR. Allergic inflammation. Pediatr Allergy Immunol 1993; 4(suppl 4):7-12.
7. Bignold LP. The eosinophil leucocyte: controversies of recruitment and function. Experientia 1995;51:317-27.
8. Kolmanskog S, Haneberg B. Immunoglobulin E in feces from children with allergy. Evidence of local production of IgE in the gut. Int Arch Allergy Appl Immunol 1985;76:133-7.
9. Raible DG, Schulman ES, DiMuzio J, Cardillo R, Post TJ. Mast cell mediators prostaglandin-D2 and histamine activate human eosinophils. J Immunol 1992;148:3536-42.
10. Vries JE. Atopic allergy and other hypersensitivities. Editorial overview. Curr Opin Immunol 1994;6:835-7.
11. Clark RAF, Gallin JI, Kapan AP. The selective eosinophil chemotactic activity of histamine. J Exp Med 1975;142:1462-7.
12. Moqbel R, MacDonald AJ, Kay AB. Enhanced granulocyte toxicity by mediators derived from anti-IgE-stimulated human leucocytes. Immunology 1986;59:87-92.
13. Brandtzaeg P, Bengtsson U, Halstensen TS, Hvatum M, Kett K, Rognum TO, et al. Mucosal immunology and food antigens. Nutritional aspects and developments. Bibl Nutr Dieta 1991;48:30-54.
14. Mensinga TT, Schouten JP, Rijcken B, Weiss ST, Van der Lende R. Host factors and environmental determinants associated with skin test reactivity and eosinophilia in a community-based population study. Ann Epidemiol 1994;4:382-92.
15. Knutson L, Odlind B, Hällgren R. A new technique for segmental jejunal perfusion in man. Am J Gastroenterol 1989;84:1278-84.
16. Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. Br J Haematol 1977;37:331-6.
17. Peterson CGB, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vivo. Clin Exp Allergy 1991;121:561-7.
18. Hohman A, Labrooy J, Davidson GP, Shearman DJC. Measurement of specific antibodies in human intestinal aspirate: effect of the protease inhibitor phenylmethylsulfonyl fluoride. J Immunol Methods 1983;64:199-204.
19. Venge P, Dahl R, Fredens K, Hällgren R. Eosinophil cationic protein ECP and EPX in health and disease. In: Yoshida T, Torisu M, editors. Immunobiology of the eosinophil. New York: Elsevier; 1983. p. 163-77.
20. Saavedra-Delgado AM, Metcalfe DD. Interactions between food antigens and the immune system in the pathogenesis of gastrointestinal diseases. Ann Allergy 1985;55:694-702.
21. Smart HL, Mayberry JF. Atopy, food and ulcerative colitis. Hepatogastroenterology 1986;33:47-8.
22. Lavö B, Knutson L, Lööf L, Odlind B, Venge P, Hällgren R. Challenge with gliadin induces eosinophil and mast cell activation in the jejunum of patients with celiac disease. Am J Med 1989;87:655-60.
23. Bruijinzeel P, Storz E, van der Donk E, Bruijinzeel-Koomen C. Skin eosinophilia in patients with allergic asthma, patients with nonallergic asthma, and healthy controls. J Allergy Clin Immunol 1993:91: 634-42.
24. Reed CE. The importance of eosinophils in the immunology of asthma and allergic disease. Ann Allergy 1994;72:376-80.
25. Coyle AJ, Ackerman SJ, Burch R, Proud D, Irvin CG. Human eosinophil-granule major basic protein and synthetic polycations induce airway hyperresponsiveness in vivo dependent on bradykinin generation. J Clin Invest 1995;95:1735-40.
26. Weller PF. Roles of eosinophils in allergy. Curr Opin Immunol 1992;4:782-7.
27. Makino S, Fukuda T. Eosinophils and allergy in asthma. Allergy Proc 1995;16:13-21.
28. Juliusson S, Pipkorn U, Karlsson G, Enerbäck L. Mast cells and eosinophils in the allergic mucosal response to allergen challenge: changes in distribution and signs of activation in relation to symptoms. J Allergy Clin Immunol 1992;90:898-909.
29. Maluenda C, Phillips AD, Briddon A, Walker-Smith JA. Quantitative analysis of small intestinal mucosa in cow's milk-sensitive enteropathy. J Pediatr Gastroenterol Nutr 1984;3:349-55.
30. Winqvist I, Olson I, Werner S, Stenstam M. Variations of cationic proteins from eosinophil leukocytes in food intolerance and allergic rhinitis. Allergy 1981;36:419-23.
31. Hernäs J, Särnstrand B, Lindroth P, Peterson CGP, Venge P, Malmström A. Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures. Eur J Cell Biol 1992;59: 352-63.
32. Lundgren JD, Davey RT Jr, Lundgren B, Mullol J, Marom Z, Logun C, et al. Eosinophil cationic protein stimulates and major basic protein inhibits airway mucus secretions. J Allergy Clin Immunol 1991;87:689-98.
33. Engström-Laurent A, Hällgren R. Circulating hyaluronate in rheumatoid arthritis: relationship to inflammatory activity and the effect of corticosteroid therapy. Ann Rheum Dis 1985;44:83-8.
34. Hällgren R, Venge P. Clinical impact of the monitoring of allergic inflammation. In: Mattson P, Ahlstedt S, Venge P, Thorell J, editors. The eosinophil in inflammation. London: Academic Press; 1991. p. 119-40.
35. Wasserman S. The lung mast cell: its physiology and potential relevance to defense of the lung. Environ Health Perspect 1980;35: 153-64.
36. Goetzl EJ, Austen KF. Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: identification as eosinophil chemotactic factor of anaphylaxis. Proc Natl Acad Sci U S A 1975;72:4123-8.
37. Raible DG, Schulman ES, DiMuzio J, Cardillo R, Post TJ. Mast cell mediators prostaglandin D_2 and histamine activate human eosinophils. J Immunol 1992;148:3336-42.
38. Bonini S, Tomassini M, Adriani E, Magrini L, Rumi C, Bussa S, et al. Markers of eosinophilic inflammation in allergic diseases. Allergy 1993;48:133-7.
39. Bachert C, Prohaska P, Pipkorn U. Ig-E positive mast cells on the human nasal mucosal surface in response to allergen exposure. Rhinology 1990;28:149-58.
40. Venge P. Soluble markers of allergic inflammation. Allergy 1994;49: 1-8.