

Allergic Eosinophilic Gastroenteritis With Protein-losing Enteropathy: Intestinal Pathology, Clinical Course, and Long-term Follow-up

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ABSTRACT

Objectives: A subset of patients with allergic eosinophilic gastroenteritis (AEG) has anemia and hypoalbuminemia caused by protein-losing enteropathy (PLE). Our goals were to describe the response to therapy and the long-term outcome of patients in this subgroup and to evaluate their gastric and intestinal biopsies for distinguishing features that might explain their protein and blood loss.

Methods: Patients with AEG + PLE were identified retrospectively and compared with controls and with patients with AEG only. Immunohistochemical staining for tryptase, a mast cell mediator, was performed on gastric and duodenal tissues. Eosinophils identified by hematoxylin/eosin stain and mast cells identified as tryptase-positive cells were counted in one high-power field area with maximal cell infiltration.

Results: Although all patients had excellent response to therapy with amino acid–based formula and tolerated gradual introduction of some foods with time, food-responsive disease

persisted in all patients over 2.5 to 5.5 years of follow-up. Routine histological evaluation did not show any features differentiating AEG + PLE from AEG. When eosinophils and mast cells were counted in intestinal biopsies, however, significantly more mast cells were found in biopsies of the AEG + PLE group despite comparable numbers of eosinophils. In contrast, in gastric biopsies, eosinophils were more prominent in AEG + PLE, but mast cell numbers were similar in all groups.

Conclusions: Patients with AEG + PLE responded well to therapy with amino acid–based formula. Food hypersensitivities did not completely resolve over up to 5.5 years. Intestinal mast cells were significantly increased in maximally infiltrated areas of the intestine, possibly causing increased intestinal permeability and protein loss. *JPGN* 42:516–521, 2006. **Key Words:** Eosinophilic gastroenteritis—Protein-losing enteropathy—Mast cell—Eosinophil—Amino acid. © 2006 by Lippincott Williams & Wilkins

INTRODUCTION

Allergic eosinophilic gastroenteritis (AEG) is characterized by eosinophilic infiltration of the gastrointestinal tract. The clinical manifestations of this disease are variable and include abdominal pain, emesis, diarrhea, and failure to thrive (1). In some patients, protein-losing enteropathy (PLE) causes edema. The etiology of AEG is thought to be an allergic reaction to foods with a mixed immunoglobulin

(Ig) E–mediated and cell-mediated mechanism (1). The diagnosis of AEG is made if biopsy of the gastrointestinal tract demonstrates marked eosinophilic infiltration of the gastric and/or duodenal mucosa in the absence of other causes of eosinophilia such as *Helicobacter pylori* infection, parasitic infestation, or inflammatory bowel disease (2). Laboratory investigations reveal peripheral eosinophilia in approximately 50% of patients with AEG. Skin prick tests or serum tests for food-specific IgE antibodies reveal a triggering food in less than 50% of cases (1).

We have identified a subgroup of patients with AEG who had anemia and hypoalbuminemia secondary to fecal losses of blood and protein consistent with a diagnosis of PLE. Our goals were to determine this subgroup's short- and long-term clinical response to different therapies and to evaluate their gastric and intestinal biopsies for possible distinguishing features that could explain their intestinal protein and blood losses.

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MATERIALS AND METHODS

Patients and Controls

Eight patients with hypoalbuminemia and anemia were identified retrospectively from among 93 patients with AEG seen at the Mount Sinai Medical Center between January 1, 1997, and March 3, 2005 (8.6% of children with AEG). Those who had an upper endoscopy with biopsies at our center at the time of diagnosis were included in this study (AEG + PLE group, $n = 6$). The diagnosis of AEG was made in children failing to respond to H_2 blockers and proton pump inhibitors whose gastric and/or duodenal mucosal biopsies showed prominent eosinophilic infiltration [maximum eosinophils/high-power field (HPF) >20 in the antrum or >50 in the duodenum] (3,4). The 6 patients with AEG + PLE had gastrointestinal loss of protein and blood demonstrated by elevated fecal α_1 -antitrypsin and fecal occult blood, respectively. Celiac disease, immunodeficiency, and Crohn disease were ruled out clinically and/or by laboratory and radiological studies in all patients. Urinary protein loss was not present in any patient. Two comparison groups were selected from patients who underwent evaluation and biopsies at our center. The first group included randomly selected patients with AEG for whom sufficient clinical data existed and who did not have hypoalbuminemia or anemia (AEG group, $n = 6$). The second comparison group included patients with abdominal pain, emesis, or reflux symptoms who had normal findings grossly and histologically upon upper endoscopy (control group, $n = 5$).

Medical records of patients with AEG + PLE were reviewed for clinical history, physical findings, and laboratory values. Parents were contacted for clinical updates after obtaining informed consent. The Mount Sinai School of Medicine Institutional Review Board approved the study.

Immunohistochemical Staining

Paraffin-embedded antral and duodenal mucosal biopsies were retrieved, and unstained slides with 3- μ m sections were prepared for mast cells staining using an antibody to tryptase, a mast cell mediator. Sections were dried, deparaffinized in xylene, and hydrated through descending ethanol series into distilled water. Antigen retrieval was achieved by enzymatic digestion with trypsin (DAKO Corporation, Carpinteria, Calif). Endogenous peroxidase was blocked by treatment with hydrogen peroxide, and endogenous biotin was blocked by incubating slides with avidin followed by biotin (DAKO). Sections were then incubated with the primary antibody, mouse antihuman tryptase, or mouse IgG1 as an isotype control (DAKO), followed by a biotinylated secondary antibody. Streptavidin-peroxidase complex and substrate-chromogen solutions were used for staining. Hematoxylin was used as a counterstain. Specimens were evaluated in a blinded fashion. Each specimen was entirely examined under high power (400 $\times$) for mast cells (tryptase-positive cells). The HPF with the maximum infiltration of tryptase-positive cells was identified visually and the number of cells counted. Cell counting was performed independently by 2 investigators (M.C. and M.S.M.), and no major interobserver discrepancies were found. The corresponding sections stained by hematoxylin and eosin at the time of endoscopy were also retrieved. In a similar fashion, eosinophils were counted in the HPF with maximum eosinophil infiltration. We chose to focus

on maximally involved areas because AEG is a patchy disease (5). Many investigators are now using this technique for diagnostic purposes and monitoring response to therapy (6).

The median maximum number of mast cells and eosinophils per HPF in each of the 3 groups—AEG + PLE, AEG, and controls—was calculated. Comparison among the groups with respect to the medians was performed using the Mann-Whitney U test with a two-tailed P value on Prism software version 2.0 (GraphPad Inc., San Diego, Calif). A P value < 0.05 was considered statistically significant.

RESULTS

Clinical Findings

The 6 patients with AEG + PLE had a variety of gastrointestinal symptoms (median onset, 10 months of age), and all had edema (median onset, 20.5 months) (Table 1) at presentation. Their histological findings and laboratory parameters are summarized in Table 2. Median age of patients with AEG at histological diagnosis was higher than that in AEG + PLE patients (median, 54 months; range, 13–180). Inasmuch as intestinal permeability and immunologic reactivity are probably not affected by age beyond the neonatal period (7,8), we felt it was unlikely that age disparity had a significant impact on our results.

Patients with AEG + PLE received a variety of therapies after diagnosis, which produced partial, temporary, or no clinical response. These therapies included food elimination, systemic steroids, montelukast, and oral cromolyn sodium. In contrast, treatment with a diet composed exclusively of an amino acid–based formula was more effective and resulted in rapid resolution of clinical symptoms, hypoalbuminemia, and anemia in less than 4 weeks in all patients. A limited number of solid foods that were negative in allergy tests and considered of low allergenic potential were continued in the diet of 3 children (Table 1). The amino acid–based formula was taken orally without a need for tube feeding. In discussions 2.5 to 5.5 years later, parents indicated that some food-responsive symptoms persisted; however, all patients had tolerated the gradual addition of various foods to their diet after the initial improvement on amino acid–based formula therapy (Table 1).

Histological Findings

In hematoxylin and eosin–stained sections, patchy duodenal villous blunting was seen only in patient 6 from the AEG + PLE group and in one patient from the AEG group. When duodenal mucosal areas with maximal infiltration were analyzed (Fig. 1A, B), intestinal mast cells were significantly higher in patients with AEG + PLE than in those with AEG despite comparable number of eosinophils. Maximum median number of eosinophils per HPF (with interquartile

TABLE 1. Clinical characteristics, histological findings, response to therapy, and long-term follow-up of patients with AEG + PLE

Patient no.	Gender	Gastrointestinal symptoms	Atopic diseases	Age, gastrointestinal symptoms (months)	Age, edema onset (Months)	Age, biopsy diagnosis (Months)	Therapies	Response	Length of follow-up (years)	Diet at follow-up
1	F	Emesis, periorbital edema, diarrhea, growth failure	—	24	36	42	Food elimination AA formula + 1 food	— +	2	AA formula + <20 foods
2	M	Periorbital edema, occult rectal bleeding	AR	2	17	17	Food elimination AA formula	— +	4	AA formula + <20 foods
3	F	Periorbital edema, diarrhea, abdominal pain	AD, AR	31	41	42	AA formula + few foods	+	3	Avoids 6 foods
4	M	Rectal bleeding, edema	AD	10	10	16	Food elimination Oral steroids AA formula + 1 food	— + +	5.5	Avoids 4 foods
5	F	Periorbital edema, growth failure, abdominal pain, emesis	AD	8	24	35	Cromolyn sodium Food elimination Montelukast	— +/- —	5	Avoids several foods
6	M	Edema, diarrhea, occult rectal bleeding	AR	10	10	17	Food elimination AA formula Oral steroids 6-Mercaptopurine Montelukast	+/- + +/- +/-	5	Avoids 3 foods

AA indicates amino acid; AR, allergic rhinitis; AD, atopic dermatitis.

TABLE 2. Laboratory and skin test findings, growth parameters, and atopic history of patients with AEG + PLE

Patient no.	Percentile height at diagnosis	Percentile weight at diagnosis	Highest peripheral eosinophils (cells/mm ²)	Lowest serum albumin (g/dL)	Lowest hemoglobin (g/dL)	Max antral eosinophils/HPF	Max duodenal eosinophils/HPF	Serum IgG (mg/dL)	Serum IgM (mg/dL)	Serum IgA (mg/dL)	Positive foods by IgE/PST*/ foods tested
1	20	3	2516	1.8	7	300	70	118	51	31	5/9
2	50	30	1276	1.4	10.6	43	58	312	31	16	10/24
3	75	75	2100	1.6	9.3	152	71	314	62	59	22/34
4	40	30	1800	1.4	7.2	258	88	140	68	40	0/14
5	20	5	3740	1.9	10	260	30	285	33	43	40/42
6	10	20	1320	2.2	8.2	—	48 (on diet)	755	58	60	0/12
Median	30	25	1950	1.7	8.8	258	64	298	54	42	8/19
Normal range	—	—	0–800	3.5–5.5	10–14	—	—	432–1142	43–214	20–124	—

*Positive prick skin test defined as wheal diameter ≥ 3 mm; positive food-specific IgE defined as ≥ 0.35 kIU/L.

PST indicates prick skin test.

range) was 64 (39–79.5) in AEG + PLE, 61 (28–156.5) in AEG, and 31 (25.5–36.5) in controls. The medians were significantly different in AEG + PLE and controls only ($P < 0.05$). Median maximum number of mast cells per HPF was 73.5 (68–104.5) in AEG + PLE, 35.5 (30.5–50) in AEG, and 50 (43–66) in controls. The median in AEG + PLE was significantly higher than that in AEG ($P < 0.01$) and controls ($P < 0.05$).

In the antrum (Fig. 1C, D), eosinophilia was more prominent in the AEG + PLE group than in the AEG and control groups. Median maximum number of eosinophils per HPF was 258 (97.5–280) in AEG + PLE, 30 (12–55) in AEG ($P < 0.05$ compared with AEG + PLE), and 0 (0–2) in controls ($P < 0.01$ compared with AEG + PLE). Median maximum number of mast cells per HPF was not significantly different among the 3 groups. Median maximum number of mast cells per HPF in AEG + PLE was 67 (60–118), 54 (25.5–82) in AEG, and 49 (30–67.5) in controls (P not significant for all comparisons with AEG + PLE).

DISCUSSION

To our knowledge, this is the first report of a series of children with AEG + PLE successfully treated with amino acid–based formula. Amino acid–based formula therapy was previously found to be successful in patients with eosinophilic esophagitis (9,10). Similar to the experience of Katz et al. (11), our initial attempts at dietary food restriction guided by history and testing for food-specific IgE antibodies and/or prick skin tests were not successful. This is likely because of the immunologic nature of AEG, which involves both cellular and humoral immune responses (12). Our patients did have a wide range of positive tests for food-specific IgE (Table 2), and patch skin testing has been suggested as a possible testing modality for cell-mediated reactions (13,14). However, the usefulness of patch testing still requires validation by evaluating the results of food challenges. Elimination of foods based on their historical potential for allergenicity may not be adequate either, as experienced by Waldmann et al. (15). In addition, even with correct selection of permitted foods, lack of improvement may result from cross-contamination with the offending food allergens, a risk that is completely eliminated with the use of an amino acid–based formula.

Oral steroids were used successfully in our patients who opted for this therapy. This finding is in agreement with 2 referenced studies (11,15). However, symptom relapse occurred with discontinuation of steroids, and growth failure is a potential complication of prolonged therapy.

Our patients with AEG + PLE generally had mild hypogammaglobulinemia, with IgG concentrations tending to be lower than age-related norms in most patients (Table 2) and lower than those of the

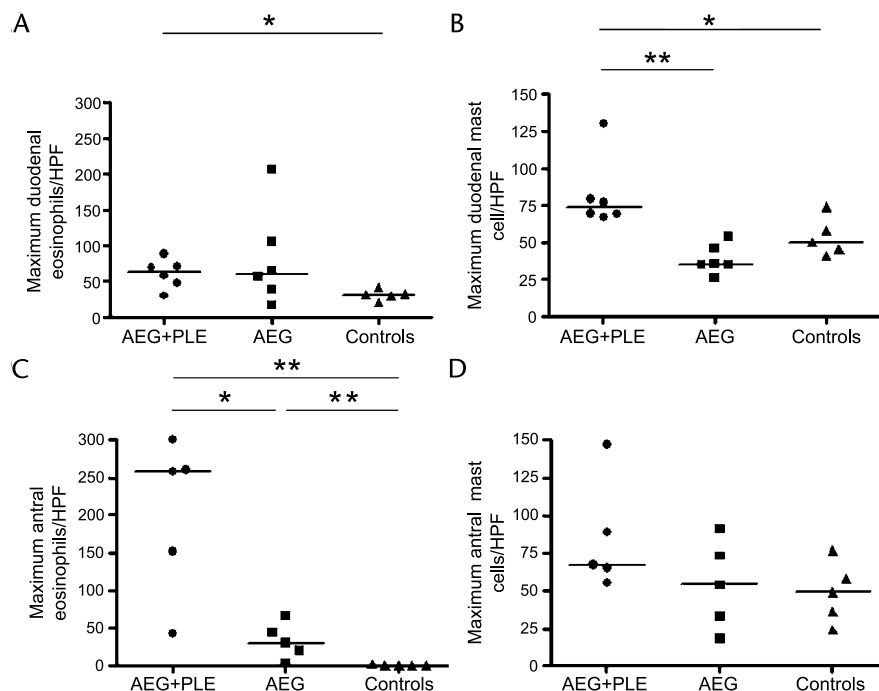


FIG. 1. When the duodenum was examined (A and B), mast cells were significantly increased in patients with AEG + PLE compared with those with AEG. In the antrum however (C and D), mast cells were not significantly increased despite an increase in gastric eosinophilia (* $P < 0.05$, ** $P < 0.01$).

AEG group. When we followed immunoglobulin levels over time in patient 2 (data not shown), we found a direct correlation between serum IgG and albumin concentrations, with IgG concentration increasing in response to therapy. This finding suggested to us that there was likely a generalized intestinal protein loss including IgG. IgM and IgA levels however did not show direct correlation with albumin in our patients with AEG + PLE (Table 2), likely because of their larger molecular size.

Historical assessment 2.5 to 5.5 years after diagnosis indicated that patients did not outgrow their food sensitivities. Many patients, however, were reported to be able to tolerate the gradual addition of various foods to their diet after the initial improvement on an exclusive amino acid-based formula. The number of foods tolerated over time did not correlate with food-specific IgE levels, again highlighting the importance of the cell-mediated allergic component of this condition.

The key distinguishing clinical feature of patients with AEG + PLE is the loss of protein due to gastrointestinal inflammation. Typically, intestinal villous blunting would be the expected histological finding if malabsorption of nutrients were the source of hypoalbuminemia. However, histological examination of duodenal specimens in our patients revealed only one with AEG + PLE who had villous blunting. Therefore, villous blunting and malabsorption are not reasonable explanations for the hypoalbuminemia and anemia in

patients with AEG + PLE. Intestinal villous blunting has been described in patients with AEG without PLE (16) and was indeed present in one patient in our AEG group who had neither anemia nor hypoalbuminemia.

Blood and protein losses are probably secondary to an increase in intestinal permeability in patients with AEG + PLE. Waldmann et al. demonstrated an increase in intestinal permeability in his patients with allergic gastroenteropathy and hypoalbuminemia with the use of a radiolabeled albumin excretion test. Injection of labeled albumin intravenously was followed by excessive fecal excretion of isotope in all patients, consistent with excessive gastrointestinal protein loss (15). In our series, patient 3 agreed to participate in an intestinal permeability study using a mixture of sucrose, lactulose, and mannitol. Urine excretion of these sugars was consistent with an increase in intestinal rather than gastric permeability (lactulose to mannitol urinary excretion ratio, 0.065; normal, <0.025 ; total sucrose, 84 mg; normal, <180 mg), even though her biopsies revealed gastric eosinophilia. Although there are limited data showing a relationship of eosinophilic inflammation with increases in bronchovascular permeability after segmental antigen challenge in allergic asthma (17), the effect of eosinophils and their products on the integrity of the gastrointestinal epithelium has not been studied. Thus, it is not clear that eosinophilic inflammation per se is the cause of, or is associated with, protein loss in these patients.

Based upon our results, it is apparent that eosinophilic inflammation and mast cell infiltration may exist differentially among patients with AEG and may be associated with different clinical outcomes. For example, eosinophilia was more prominent in the antrum in our AEG + PLE group than in the AEG group, but there was no increase in mast cells in the antrum, and this is not the site of protein loss. In contrast, in the duodenum, the numbers of eosinophils were comparable in the 2 groups; however, we found a significant increase in mast cells in patients with AEG + PLE compared with patients with AEG but without PLE. We therefore hypothesize that intestinal mast cells may be causing the increase in intestinal permeability. A potential role for eosinophils in increased permeability cannot be excluded because eosinophils may contribute to disease processes not only through increase in numbers, but also as a result of variations in the degree of their activation and degranulation (18). Mast cells, on the other hand, have been shown to play an important role in increased intestinal permeability in various animal models. Mast cells have been shown to disrupt intestinal barrier function during an enteric nematode infection (19), a disease with similarities to AEG in its T-cell mediated mechanism of inflammation and intestinal eosinophilia. In a rat model of late-phase intestinal allergy characterized by both mast cell and eosinophil intestinal infiltration, Yang et al. demonstrated a role for mast cells in antigen-induced increase in intestinal epithelial permeability by comparing mast cell-deficient mice to their littermate controls (20). Although additional studies with larger patient groups will be needed to confirm our findings, our observations, combined with those from animal studies, suggest that mast cell infiltration is related to intestinal protein loss in patients with AEG + PLE.

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