Allergy-Related Proctocolitis in Infants: Diagnostic Usefulness of Rectal Biopsy

Harland S. Winter, Donald A. Antonioli, Naomi Fukagawa, Manuel Marcial, and Harvey Goldman

Departments of Medicine (Combined Program of Pediatric Gastroenterology and Nutrition) and Pathology, The Children's Hospital, Beth Israel Hospital, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts

To evaluate the diagnostic utility of rectal mucosal biopsies in infants with proctocolitis, we compared the clinical and histologic features of an allergy-related group (N = 36) with those of normal (N = 12) and inflammatory (N = 8) controls. Clinical features were nondiscriminatory among the three groups of patients, except for an increased absolute peripheral eosinophil count in the allergic group. Similarly, morphologic evidence of proctitis (cryptitis and crypt abscesses) and small or moderate numbers of eosinophils (≤60 per ten high power fields) in the lamina propria of the biopsies did not discriminate among the three groups. However, large numbers of eosinophils (>60 per ten high power fields) and eosinophils located in the muscularis mucosae or as the predominant cell in crypt abscesses were significantly associated with allergy-related disease. No histologic features of chronic colitis were noted in the allergy-related group. Thus, in tandem with the remainder of the clinical data, rectal mucosal biopsy is a useful adjunct in the diagnosis of allergic proctocolitis.

Key words: Allergy, Proctocolitis, Eosinophils, Rectal biopsy.

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Known causes of rectal bleeding in infancy include allergic reactions, infections, fissure, ischemia (necrotizing enterocolitis), and obstruction (Hirschsprung’s disease). Although cases with no recognized etiology are often labeled as examples of idiopathic ulcerative colitis (2), this classification is usually inaccurate. Rather, most infants with proctitis and colitis of uncertain cause have a self-limited illness that typically responds to a dietary change and is presumably due to an unrecognized intolerance to dietary protein (31). In the routine review of rectal mucosal biopsies from infants with colonic bleeding, we had the impression that eosinophils were an especially prominent feature in cases that were associated with dietary protein allergy. To refine this impression, we systematically reviewed rectal biopsies of children less than 5 mo of age, many of whom presented with rectal bleeding, to determine the usefulness of histologic features in defining the etiology of infantile proctocolitis. Clinical and pathological parameters in patients with proctitis and colitis were correlated and compared with those in two age-appropriate control groups: infants suspected of having Hirschsprung’s disease (negative controls) and infants with documented intestinal infections or other known causes of enteritis (inflammatory or positive controls).

MATERIALS AND METHODS

Patient Population

Fifty-six infants were selected for the study from a group of 139 patients less than 5 mo of age who had suction rectal biopsies at The Children’s Hospital, Boston, from 1976 to 84. Children with full thickness rectal biopsies were excluded from this study to avoid bias in the interpretation of the coded specimens. These patients were selected because of adequate clinical information and sufficient biopsy material. From the 56 infants, three groups of patients were identified: 12 patients were biopsied to rule out Hirschsprung’s disease (negative control; Group I); eight patients had an infectious or other known nonallergic cause for the diarrhea (positive control; Group II); and 36 patients had no identifiable obstructive, infectious, or ischemic etiology (study patients; Group III).

Clinical Features

The following clinical parameters were assessed retrospectively: age at onset of symptoms, sex, diet, indication for biopsy, symptoms and signs (rectal bleeding, mucus in the stool, colic, fever, diarrhea, vomiting, known allergy), and laboratory results (hematocrit, white blood cell count, percentage of eosinophils and absolute eosinophil count in the peripheral blood, serum albumin, serum immunoglobulins, stool examination for ova, parasites, and rotavirus, and cultures for bacterial pathogens). The relationship between the timing of the rectal biopsy and the onset of symptoms was also noted. Seven patients in the study group had a challenge with the putative dietary allergen. However, dietary challenges were not performed using the strict criteria of Goldman et al. (6) which require three
Histologic Features

All biopsy specimens were fixed in 10% buffered formalin, and the slides were stained with hematoxylin and eosin (H&E); Giemsa or other histochemical stains were not employed to highlight eosinophils. All the slides were coded and independently evaluated by two of the authors (D. A. A. and M. M.). If more than one rectal biopsy specimen had been obtained in a particular case, the biopsy described first in the pathology report was arbitrarily chosen for review. At the completion of the slide evaluation, the two observers compared their findings. Interobserver discrepancies were minor and infrequent (15% of cases), and they were resolved by review of the biopsy specimens in question.

A specimen was considered adequate if it contained sufficient well oriented mucosa to permit evaluation of ten consecutive high power fields. The following histologic parameters were analyzed in all specimens:

1. Glandular architecture and cytology: rated as normal; active inujury (cryptitis, crypt abscesses, erosions, or ulcers; cellular degeneration or regeneration); or atrophy (shortening or abnormal branching of crypts; villiform surface; Paneth cell metaplasia).

2. Lamina propria: presence of fresh hemorrhage and edema, each graded semi-quantitatively on a scale of 0 (absent) to 3+ (severe); lymphoid nodules; increased mononuclear inflammatory cells; granulomas or giant cells; neutrophils (in lamina propria alone or also in surface or crypt epithelium or associated with crypt abscesses); and surface pseudomembranes (consisting of cellular debris, fibrin and mucus).

3. Eosinophils: total number in lamina propria in ten consecutive high power fields; location in mucosa (upper, mid, or lower third); and presence in surface or crypt epithelium, in muscularis mucose, or as part of the inflammatory component of crypt abscesses. Only intact eosinophils were counted; any cells with apparent degranulation were excluded.

Statistical Methods

Results of the clinical and pathologic evaluations were compared among infants with Hirschsprung's disease, those with intestinal inflammation due to infections or other specific causes, and the study group. All statistical data are reported as mean ± standard deviation, except for the eosinophil counts, which are reported as SEM. One-way analysis of variance was used for numerical data and $\chi^2$-square analysis for categorical data.

RESULTS

Clinical Features (Table 1)

Of the eight infants in the positive control group, three had documented intestinal infection with Salmonella ($N = 1$) or rotavirus ($N = 2$). One infant had Escherichia coli sepsis with bloody diarrhea, and the remaining four cases had objective evidence for enteritis as demonstrated by an abnormal lactose or sucrose breath hydrogen test, elevated 72-h fecal fat, clinical positive stools, and/or abnormal biopsy of the small intestine. The 36 children in Group III had negative physical examinations for rectal fissures or hemorrhoids and negative stool cultures for known infectious causes of colitis (Salmonella/Shigella ($N = 36$), Campylobacter ($N = 26$)); of 16 infants tested, all had negative stool examinations for ova and parasites. Cultures for Yersinia were not obtained.

The clinical and laboratory features of the three groups of patients are summarized in Table 1. The mean age of the patients in the study group was $1.4 \pm 0.9$ mo, which did not differ significantly from the mean age of the two control groups. Although there was a predominance of boys in the study group (28 of 36 infants), this finding did not reach statistical significance when compared with the sex ratio in either control group. The hematocrit and white blood cell count were available in 50 of the 56 patients and the differential in 49; there were no significant differences in these measurements among the three groups. However, after using the natural logarithmic transformation of the absolute peripheral eosinophil count to minimize the effect of the variance in the data (variances were equal at $P = 0.0049$), the number of eosinophils in the peripheral blood was highest in Group III patients ($P = 0.02$).

All infants in Group III had rectal bleeding, compared with only two of 12 patients in Group I ($P < 0.001$) and five of the eight patients (four with infections, one with other cause of enteritis) in Group II ($P = 0.008$). Although all the data were not available for every patient, there were no significant differences between the study and the inflammatory control group with respect to the clinical parameters of diarrhea, vomiting, colic, fever, mucus in the stool, or low serum albumin, IgG, or IgA. An insufficient number of serum IgE levels were obtained to permit comparisons.
for allergy was noted in less than half of the Group III patients in whom such a history was specifically sought.

Rectal Biopsy Features (Table 2)

The mean time at which the rectal biopsy was obtained following the onset of symptoms was 6.4 ± 10.0 d in Group III and was not significantly different from that of Group II (15.3 ± 20.6). In review of the rectal biopsies, none of the specimens contained granulomas, giant cells, or pseudomembranes. Overall architecture was normal in all specimens; none contained features of chronic colitis (28).

The pathologic features of the three groups of patients are summarized in Table 2. The presence and extent of hemorrhage and edema in the lamina propria as well as the number of lymphoid follicles did not differ among the three groups of cases. Active proctitis was identified in 12 specimens, two from the inflammatory control group and ten from the study group (differences not significant). The active proctitis was generally mild and more often focal than diffuse; it consisted of neutrophils in the lamina propria and/or infiltrating the surface-epithelium; frank crypt abscesses were identified in five of these cases (one from Group II, four from the study group), but there were no erosions or ulcerations.

The distribution of eosinophils within the lamina propria (upper, mid, or lower third) did not differ among the three groups. However, large numbers of eosinophils (greater than 60 eosinophils per ten high power fields) were noted only in specimens from Group III patients. As a result, the mean number of eosinophils per high power field differed significantly when Group I or II was compared with Group III (P = 0.0005). Similarly, eosinophils infiltrating the surface or crypt epithelium were most frequent in Group III biopsy specimens, while the feature of eosinophils as the predominant cell in crypt abscesses was identified exclusively in Group III patients. Eosinophils were not noted in the muscularis mucosae or in clusters in any biopsy specimens from patients in Group I or II, but were identified in the muscularis mucosae of 15 of 34 (44%) specimens with muscle present in Group III patients and formed clusters in ten (29%) Group III infants (Fig. 1).

Within Group III there were five infants who were breast fed and 31 who were nourished with either cow or soy protein-based formula. Several of these infants had been switched to a hydrolyzed protein formula prior to the biopsy. Nevertheless, there was no statistical difference between the mean number of eosinophils per high power field in the rectal biopsy in the breast-fed versus the formula-fed groups (8.4 ± 7.5 and 10.8 ± 9.8, respectively).

Of 16 patients evaluated in Group III, there were 12 who had decreased levels of serum immunoglobulin G or A. The mean number of eosinophils per high power field in the lamina propria of this subgroup did not differ significantly from the remainder of the patients in Group III. In the biopsies of the 12 infants with low immunoglobulins, only one biopsy contained eosinophils in the muscularis mucosae.

Based on their dietary histories, the 36 infants in Group III were presumed to have a dietary protein intolerance. Seven of these 36 patients had a dietary challenge. All seven had a positive challenge result with the putative formula and developed recurrent rectal bleeding or diarrhea.

DISCUSSION

The etiology of proctocolitis in infants is varied and includes Hirschsprung's disease and infections (with organisms such as Salmonella, Shigella, and Campylobacter or Yersinia species). Many cases are labeled as idiopathic but are presumed to be dietary-induced protein intolerance (allergic colitis or proctitis). Unlike idiopathic ulcerative colitis, allergic proctocolitis is typically a self-limited disease which resolves without sequelae by the age of 2 yr; rarely, colonic stricture may develop (26, 31). Allergic proctocolitis usually occurs in infants fed milk or soy-derived formulas, but it has been described in infants who were totally

Table 2. Rectal Mucosal Biopsies: Pathologic Features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Normal controls group I (N = 12)</th>
<th>Inflammatory controls group II (N = 8)</th>
<th>Study Group III (N = 36) III vs. I III vs. II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamina propria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>9</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Edema</td>
<td>9</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Lymphoid nodules (average no./specimen)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Active proctitis</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Eosinophils ≤60 per 10 HPF</td>
<td>12/12</td>
<td>8/8</td>
<td>15/36</td>
</tr>
<tr>
<td>Eosinophils &gt;60 per 10 HPF</td>
<td>0/12</td>
<td>0/8</td>
<td>21/36</td>
</tr>
<tr>
<td>Mean number of eosinophils per HPF</td>
<td>1.3</td>
<td>1.5</td>
<td>10.4</td>
</tr>
<tr>
<td>Location of eosinophils:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper 1/3 (%)</td>
<td>0.7 (54)</td>
<td>0.9 (60)</td>
<td>4.2 (40)</td>
</tr>
<tr>
<td>Mid 1/3 (%)</td>
<td>0.3 (23)</td>
<td>0.3 (20)</td>
<td>3.1 (30)</td>
</tr>
<tr>
<td>Lower 1/3 (%)</td>
<td>0.3 (23)</td>
<td>0.3 (20)</td>
<td>3.1 (30)</td>
</tr>
<tr>
<td>Eosinophils in epithelium</td>
<td>1/12</td>
<td>2/8</td>
<td>12/36</td>
</tr>
<tr>
<td>Eosinophils in crypt abscess</td>
<td>0</td>
<td>0/2</td>
<td>2/10</td>
</tr>
<tr>
<td>Eosinophils in muscularis mucosae</td>
<td>0/12</td>
<td>0/8</td>
<td>15/34</td>
</tr>
</tbody>
</table>

* For comparison of Group III with each of Groups I and II.

* HPF, high power field.
breast-fed (9, 16, 22, 27). It may affect as many as 7% of otherwise normal infants (4, 8).

Most colonic reactions to dietary protein in infancy have a pattern of delayed onset, in contrast to the immediate hypersensitivity reactions which are IgE-mediated (8). Since proteins in milk are the first ingested antigenic material that an infant encounters, it is not unreasonable to expect allergic reactions in this age group, even in infants who are exclusively breast-fed (5, 17, 19). The signs and symptoms associated with dietary protein intolerance usually resolve when the offending formula or food is eliminated from the child’s diet (3, 15, 23, 24, 32). Although no simple and definitive clinical laboratory test exists, increased serum IgE, positive radioallergosorbent test (RAST), elevated specific serum IgA, eosinophilia, or lymphocyte proliferation to the antigen may suggest intolerance to a specific protein (1, 11, 14, 18, 30, 33).

Unfortunately, in young infants it may be difficult to distinguish allergy from other causes of proctocolitis by ordinary clinical means. Rectal bleeding was noted in all of the patients in our study group, but was also present in over half of those with other causes of enteritis or colitis. Furthermore, the age distribution, sex ratios, other clinical symptoms, and most of the routine laboratory test results did not differentiate among the infants with Hirschsprung’s disease, nonallergic enterocolitis, and idiopathic, presumably allergic, proctitis. Only an elevated absolute peripheral eosinophil count delineated the latter group from the negative and inflammatory control groups. Of interest, a positive family history of allergy was obtained in less than 50% of the allergy-related group.

Given the clinical problems in separating allergic from infectious and other causes of proctocolitis in this age group, we decided to evaluate the diagnostic utility of histopathologic features (especially eosinophils) in rectal biopsies in resolving this dilemma. We reviewed coded specimens from cases of possible allergy as well as from inflammatory and noninflammatory control patients. We were aware of the potential limitations of our study: (a) it was retrospective in nature; (b) complete clinical information was lacking for some infants; and (c) analysis of the population of infants with possible allergy was limited to those having rectal biopsies. Nevertheless, given the rarity of the average hospital seeing many cases of allergic proctocolitis, we felt the study was worthwhile as a pilot project for the subset of infants having a tissue examination. The validity of our results can be further assessed prospectively.

Prominent edema and fresh hemorrhage in the lamina propria were equally prevalent in each of the three groups of patients, suggesting strongly that these features are the results of trauma related to the en-
ophils (up to 60 per ten high power fields) was highly specific for small or moderate number of eosinophils in the lamina propria. However, a modest numbers form a normal distribution of eosinophils in the lamina propria did not correlate with the presence of eosinophils in the rectal biopsy. The histologic findings in mucosal biopsy specimens from patients with allergic proctocolitis have been reported to vary, in general, from a normal appearance to a mild, usually focal colitis (7, 8, 9, 12, 16, 25, 27). Our findings suggest that, when present, large numbers of eosinophils in the lamina propria and/or their presence in crypt abscesses or in the muscularis mucosae help to identify cases of allergic proctitis. Accordingly, evaluation of rectal mucosal biopsies in tandem with the remainder of the clinical data can be a useful adjunct in the diagnosis of allergic proctocolitis.

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Address reprint requests to: Donald A. Antonioli, M.D., Dept. of Pathology, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

REFERENCES
Book Review

Wolf BC, Neiman RS: Disorders of the Spleen, 211 pp, Philadelphia, W. B. Saunders, 1989 ($60.00)

There have been remarkably few textbooks devoted to the pathology of the spleen. For most pathologists the spleen remains as Galen described it, “organum magnum mistrium.” Drs. Wolf and Neiman have, in “Disorders of the Spleen,” provided pathologists with a particularly useful review of splenic anatomy, physiology, and pathology and, thereby, a means of resolving much of the difficulty in the study of spleen structure. This monograph is clearly written and includes much current information, including discussions of the immunologic basis of many immunologic disorders, AIDS and its many manifestations, and the evaluation of leukemias and lymphomas with a review of the immunopathology of hematopoietic and lymphoreticular cells. Virtually every condition of importance to the pathologist in understanding the pathology and physiology of the spleen is reviewed.

One could quibble about a few topics. The macroscopic features of the various splenic disorders are presented but might receive a little more emphasis. As our tools for evaluation of tissue become more and more sophisticated, it will be increasingly important to obtain samples in different ways in order to optimize our ability to perform special studies, such as immunohistochemical analyses and gene rearrangement determinations in the study of lymphomas. Decisions for sample preparations will have to be made prior to fixation. The techniques of molecular pathology are, however, not adequately discussed. This is understandable, since DNA technology has only become readily available for diagnostic studies in the last year, but a discussion of this topic should be a goal of the second edition.

The quality of the illustrations, particularly the color plates, is not optimal. Why is it that so many of the medical books published in Europe have superb reproductions of the gross and microscopic pathology, whereas so few of the books published in the United States achieve that goal? Where are the discrete bacilli in Plate 1? The parenchyma of the Gaucher’s disease spleen has a characteristic “salt and pepper” appearance; this can’t be appreciated in Fig. 11-1. There is little value to publishing Fig. 14-7; even the experienced spleen pathologist will have trouble visualizing detail. Amyloid has a typical “smudgy” appearance after hematoxylin and eosin staining; this cannot be seen in Figs. 9-7 and 9-8.

Despite these concerns this book is highly recommended. It is an excellent starting place for the young pathologist who wants to learn about the pathology of the spleen. It is also a worthy reference book and belongs on the shelf of the seasoned pathologist.

Stephen A. Geller
Cedars-Sinai Medical Center
Los Angeles, California