Neutrophil and Eosinophil Involvement of the Small Bowel in Patients with Celiac Disease and Crohn's Disease: Studies on the Secretion Rate and Immunohistochemical Localization of Granulocyte Granule Constituents

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Purpose: The concentrations of myeloperoxidase (MPO), a neutrophil granule constituent, and eosinophil cationic protein (ECP), a specific eosinophil granule protein, were measured in jejunal perfusion fluid in an attempt to elucidate the neutrophil and eosinophil involvement of the small bowel in health and disease.

Patients and methods: The control group consisted of 14 males and two females. Ten patients (seven males and three females) with Crohn's disease and seven patients (two males and five females) with celiac disease were also studied; in addition, one patient with relapsing giardiasis, one patient with giardiasis and complete absence of plasma cells in small intestinal lamina propria, and one patient with selective IgA deficiency and no IgA plasma cells in duodeno-jejunal lamina propria were evaluated. Segmental perfusion of the jejunum was performed according to a previously described method. MPO and ECP were measured by radioimmunoassays.

Results: In healthy control subjects, the concentrations of both granule proteins were in a narrow range and much higher than would have been anticipated from passive leakage from circulating blood. In patients with celiac disease, the perfusion fluid concentrations of MPO and ECP were on average 3.5 and eight times, respectively, higher than the values seen in the controls. The jejunal segment perfused in patients with Crohn's disease was endoscopically and histologically normal. The perfusion fluid concentrations of MPO and ECP were increased 3.5 and two times, respectively, compared with that in the control subjects. Both patient groups and the control group had similar perfusion fluid concentrations of albumin. Data on MPO and ECP expressed as jejunal secretion rates gave the same differences between patients and controls as just described for the jejunal fluid concentrations. Immunohistochemical studies of jejunal biopsy specimens from another group of patients with celiac disease demonstrated a prominent extracellular deposit of ECP in the lamina propria of the atrophic intestinal mucosa, whereas the release of neutrophil constituents (cathepsin G, MPO) was scarce. In Crohn's disease, an extracellular degranulation of ECP and, to a lesser extent, of cathepsin G was observed in relation to ulcerations only.

Conclusion: Data obtained indicate that the local release of neutrophil and eosinophil granule components is enhanced in the jejunal tissue from patients with celiac sprue and Crohn's disease. The prominent extracellular deposit of eosinophil granule constituents with cytotoxic properties at the site of inflammatory intestinal lesions in celiac sprue might reflect a pathophysiologic mechanism. The deposit of eosinophil and neutrophil granule components in ulcerated areas in Crohn's disease suggests that granulocytes may contribute to the inflammatory lesions also in this disease.

The granules of neutrophil granulocytes contain a number of enzymes, e.g., myeloperoxidase (MPO), of importance in the combat against bacteria [1]. The protein content of the granules of eosinophil granulocytes is dominated by the presence of highly cationic proteins, e.g., eosinophil cationic protein (ECP) and major basic protein [2,3]. These proteins have cytotoxic properties useful in the protection against parasites [4,5]. The appearance of neutrophil and eosinophil granulocytes in the mucosa of the small intestine has been considered a protection against bacteria and other agents penetrating the thin surface epithelium. However, the destructive mechanisms of the granulocytes might also be turned against the host, resulting in organ lesions. Previous studies have indicated enhanced granulocyte involvement in inflammatory bowel diseases. In the celiac sprue mucosa, the polymorphonuclear population is markedly expanded [6,7], and rectal biopsy specimens from patients with active ulcerative colitis and Crohn's disease are reported to contain increased amounts of neutrophil granule enzymes [8,9]. Another study [10] reported the apparent discharge of major basic protein-containing cores from the eosinophils in surgical Crohn's disease specimens examined by electron microscopy. The present study was performed in order to further elucidate the...
activity of neutrophil and eosinophil granulocytes in the healthy and inflamed intestine by measurements of MPO and ECP in jejunal perfusion fluid from healthy control subjects and patients with celiac sprue and Crohn's disease. Furthermore, intestinal biopsy specimens from patients with celiac sprue and Crohn's disease were examined by immunohistochemical methods. The antibodies used were directed against ECP and MPO and also against cathepsin G (chymotrypsin-like cationic protein), another neutrophil granule constituent [11]. The obtained results indicate an enhanced jejunal secretion of neutrophil and eosinophil components in these disorders and a prominent extracellular release of eosinophil constituents, in particular, in areas of the gut with pathologic lesions.

PATIENTS AND METHODS

Control Subjects and Patients in Whom Jejunal Perfusion Was Performed

The control group (14 males and two females; mean age 40 years, range, 25 to 52) included 16 patients who were referred to the hospital because of abdominal pain. A complete investigation, including proximal jejunal biopsy by means of endoscopically obtained biopsy specimens, revealed no evidence of organic disease or immunologic abnormality.

Ten patients with Crohn's disease (seven males and three females; mean age 31 years, range, 18 to 52) were studied. The median duration of the disease was two years (range, four months to six years). The localization of the disease was ileum (n = 2), colon (n = 4), ileum-colon (n = 3), and colon-rectum (n = 1). Previous resection of the ileum and right colon had been performed in one patient. The mean Crohn's disease activity index [12] of the patients was 211 (range, 40 to 428). All patients had normal jejunal mucosa, both by fiberoptic endoscopy and by endoscopically obtained biopsy specimens, at the time of the jejunal perfusion, and none of them received any drug treatment at the time of the investigation.

Seven patients (two males and five females, mean age 44 years, range, 16 to 77) with celiac disease were also studied. Six of the patients had active disease, i.e., symptoms and subtotal villous atrophy of the jejunal mucosa, and one patient had clinical, endoscopic, and histologic remission on a gluten-free diet. All patients had a symptomatic and histologic response to gluten restriction.

Lastly, we also investigated one patient with relapsing giardiasis, one with giardiasis and complete absence of plasma cells in small intestinal lamina propria but only moderately decreased levels of circulating immunoglobulins, and another with selective IgA deficiency and no IgA plasma cells in duodenal-jejunal lamina propria.

Jejunal Perfusion

Segmental perfusion of the jejunum was performed according to Rambaud et al [13], using a four-lumen tube with a proximal occluding balloon. The length of the test segment was 40 cm. The tube was swallowed by the subject before dinner and the perfusion started after overnight fasting, the infusion point being located near the duodeno-jejunal junction under the inflated balloon (as checked fluoroscopically). The gut was perfused with a 115 mM sodium chloride, 10 mM potassium chloride, and 35 mM mannitol solution containing 1 g/liter of polyethylene glycol 4,000, at a rate of 10 ml/minute. Consecutive 20-minute samples were collected after a 60-minute equilibration period. Perfusate samples were recovered at 0°C. One millimole of disopropylfluorophosphate, a potent protease inhibitor, was added to an aliquot of each sample, which was homogenized and stored at -20°C until assayed. Previous studies with the same technique have shown that the coefficients of variations of secretion rates of water, electrolytes, and various endogenous proteins were small [14]. Thus, in the present experiments, one or two 20-minute samples chosen in a random order were used for analytic evaluation for each subject. During perfusion, duodenal contents proximal to the balloon were continuously collected and discarded. Contamination of the jejunal samples by duodenal fluid bypassing the balloon was controlled by using two semiquantitative methods: detection of bromosulphophthalein, which was infused above the balloon into the duodenum, and detection of chymotrypsin and lipase activity in the perfusate. Any contaminated sample was discarded after detection. Absence of blood contamination was confirmed in all samples (Hemotest, Ames, France).

Control Subjects and Patients in Whom Immunohistochemistry of Endoscopic Biopsies and Resected Specimens Was Performed

Histologically normal biopsy specimens from the small intestine were obtained by endoscopy from one child and six adults without clinical signs of small intestinal disease. Their clinical diagnoses were cholecystolithiasis (n = 2), irritable bowel (n = 1), chronic alcoholism (n = 1), benign stenosis of the papilla Vater (n = 1), bile acid-induced diarrhea (n = 1), and subvalvular aortic stenosis (n = 1). Biopsy specimens were also collected by endoscopy from the terminal duodenum or initial jejunum in 10 patients (five children and five adults; mean age 27 years, range, two to 65) with clinically active celiac disease. The biopsies showed crypt hyperplasia and subtotal villous atrophy. Remission of the symptoms was achieved in all patients on a gluten-free diet; in four of the patients, biopsy specimens were obtained endoscopically in the inactive phase of the disease. Two of these patients later underwent provocation with gluten and reinvestigation by endoscopic biopsies. Ileo-colic resected specimens were collected from five patients (three males and two females; mean age 38 years, range, 14 to 52) with Crohn's disease of the terminal ileum. The investigated specimens showed ulcerative, transmural inflammation and epithelioid cell granulomas. Colonic biopsy specimens were obtained from non-ulcerated mucosa of another three patients (two males, one female, aged 21 to 57 years) with Crohn's disease of the colon. The biopsies revealed chronic inflammation and epithelioid cell granuloma.

Immunohistochemistry

The antibodies used for immunohistochemistry were polyclonal antisera against eosinophil cationic protein.
protein (ECP), cathepsin G (chymotrypsin-like cationic protein), and MPO. These antisera have previously been described [11,15]. Monoclonal antibodies against ECP [16], called EG2, were generously supplied by Dr S. Spry and Dr P.C. Tai, Department of Immunology, St. George's Medical School, London. Paraffin sections were dewaxed, rehydrated, and subjected to immunostaining with the antibodies used.

The immunoperoxidase method was used with the polyclonal primary antibodies against ECP and cathepsin G. The endogenous peroxidase activity was blocked in 50 ml 3 percent hydrogen peroxide in 200 ml absolute methanol for 20 minutes. Following soaking for 30 minutes in Triton TBS (Tris-buffered saline, pH 7.4, containing 1 percent Triton X-100 [Sigma]), sections were exposed to a 1 percent solution of swine serum for 30 minutes, rinsed briefly in Triton TBS, and exposed to optimally diluted antisera (1:2,200 for ECP-antiserum and 1:2,000 for cathepsin G-antiserum) for 20 hours at 4°C followed by two hours of re-equilibration at room temperature. The site of antigen-antibody reaction was demonstrated by the peroxidase-antiperoxidase procedure of Sternberger [17].

The mouse monoclonal antibody, EG2, was detected by means of the unlabeled bridge technique (APAAP procedure). Sections were incubated for 30 minutes at room temperature with the primary mouse monoclonal antibody (1:2). After a short rinse, antimouse immunoglobulin (Dakopatts) at a 1:25 dilution was added and the sections were incubated for 30 minutes. Then the sections were washed for two minutes and incubated with the APAAP complex (Dakopatts, at a 1:50 dilution). Incubation with an alkaline phosphatase substrate (Naphtol AS-MX phosphate and fast red TR salt [F1500, Sigma] with levamisole to inhibit endogenous alkaline phosphatase) developed a red color. A mouse monoclonal antibody of the same isotype (IgG) was used as a control.

MPO was detected by the indirect two-stage technique of immunoenzymatic staining by alkaline phosphatase-conjugated antibodies. Sections were incubated with anti-MPO at a dilution of 1:100. After a short rinse, alkaline phosphatase-conjugated swine antibody (Dakopatts), diluted 1:2, was added and incubated for 30 minutes. Incubation as previously described developed a red color.

Conventional staining controls as detailed by Sternberger [17] were used. Specificity controls were performed by blocking the reactivity of the antisera with the relevant purified antigens (ECP, MPO, and cathepsin G). The cell-bound and the extracellular immune reactivities of the granular proteins were distinguished as previously described [18,19].

The ECP immunoreactivity was detected by the use
of polyclonal antibodies against ECP and a monoclonal antibody, EG2. In case of single eosinophils in the tissue sections, extracellular deposits of ECP were characterized as ECP immunoreactivity outside the cell membrane with a reduction of ECP-positive granules within the cell. The normal eosinophil is densely packed with ECP-positive granules. In areas rich in eosinophils, cell-bound immune reactivity of ECP was defined as a distinct staining of the cells for ECP on an unstained background, and extracellular immune reactivity was defined as a distinct staining of the background. The extracellular deposits of ECP delineated by these criteria were always immunoreactive for EG2, indicating the secreted form of ECP [16].

Analytic Methods

MPO and ECP were measured by radioimmunoassays [20,21]. The jejunal fluid samples were analyzed in duplicate and in sequence. The sensitivity for the MPO assay was 4 µg/liter, and 2 µg/liter for the ECP assay. The variability was less than 10 percent for both methods. The specificity of the assays applied to jejunal fluid was assured by means of dilution and recovery experiments demonstrating parallelity to the standard curves and complete recovery of added proteins.

Albumin in jejunal perfusion fluid was measured by turbidic nephelometry (Multistat III, Instrumental, Lexington, Massachusetts) at the Department of Clinical Chemistry, University Hospital of Uppsala. Polyethylene glycol was assayed by the turbidic method of Hyden [22].

Calculation, Expression of Results, and Statistics

Water, MPO, and ECP flow rates at the sampling point, and thus the secretion of MPO and ECP in the test segment, were calculated according to the usual formulas [15]. When two samples were analyzed in a subject, the arithmetic mean was used for calculation. Statistical comparisons between control subjects and patients were performed by t-test.

RESULTS

Jejunal Perfusion Studies

Neutrophil and eosinophil granule constituents were measurable in the jejunal perfusate from healthy control subjects (n = 16); the mean MPO concentration was 15 ± 2.5 (SEM) µg/liter (actual range, 4 to 38 µg/liter) and the mean ECP concentration was 8.5 ± 1.6 (SEM) µg/liter (actual range, 2 to 24 µg/liter). The perfusion fluid from patients (n = 10) with Crohn's disease contained increased concentrations of both granule proteins: the mean MPO concentration was 52 ± 19 µg/liter (actual range, 4 to 194 µg/liter) (p <0.05) and the mean ECP concentration was 15.9 ± 3.8 µg/liter (actual range, 2 to 32 µg/liter) (p <0.05). Patients with celiac disease had similar MPO concentrations as patients with Crohn's disease; the mean MPO value was 54 ± 19 µg/liter (actual range, 10 to 161 µg/liter) (p <0.01). The group with celiac disease had the highest ECP concentrations in the perfusion fluid; the mean ECP concentration was 65 ± 27 µg/liter (actual range, 3 to 176 µg/liter) (p <0.01).

The albumin concentration in the perfusion fluid was 15.2 ± 5.4 (SEM) mg/liter in the control subjects. Similar albumin concentrations were measured in patients with Crohn's disease (12.5 ± 4.5 mg/liter) and celiac disease (15.3 ± 2.8 mg/liter). The flow rates during intestinal perfusion were 9.3 ± 0.1 (SEM) ml/minute in the control group, 9.8 ± 0.2 ml/minute in the group with Crohn's disease, and 10.6 ± 0.3 ml/minute in the group with celiac disease.

In order to compensate for differences in flow rate, the secretion rates of MPO and ECP were calculated. The individual data of the patients are illustrated in Figure 1. The mean secretion rate of MPO in the control group was 152 ± 26 (SEM) ng/minute/40 cm jejunal segment (actual range, 48 to 376 ng/minute/40 cm jejunal segment). Corresponding values in the group with Crohn's disease and the group with celiac disease were 508 ± 197 ng/minute/40 cm jejunal segment (p <0.05) and 666 ± 263 (p <0.01) ng/minute/40 cm jejunal segment, respectively. Five of seven patients with active Crohn's disease, i.e., Crohn's disease activity index (CDAI) more than 150, had MPO values outside the normal range, whereas three of three with inactive disease (CDAI less than 150) had normal values. Three of seven patients with celiac disease had significantly increased MPO values (Figure 1).

The mean secretion rate of ECP in the control group was 77 ± 18 ng/minute/40 cm jejunal segment (actual range, 13 to 238 ng/minute/40 cm jejunal segment). Corresponding values in the group with Crohn's disease and the group with celiac disease were 162 ± 40 (p <0.05) and 666 ± 263 (p <0.01) ng/minute/40 cm jejunal segment, respectively. Four of 10 patients with Crohn's disease had ECP values outside the normal range. Six of seven patients with celiac disease had significantly increased ECP values (Figure 1). No relation between ECP secretion rate and disease activity was apparent.

The values obtained in jejunal perfusion fluid from individual patients with other diseases affecting the small bowel are listed in Table I. Two of these patients had giardiasis and both were characterized by increased secretion rates of ECP.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>MPO (µg/liter)</th>
<th>MPO (ng/minute/40 cm)</th>
<th>ECP (µg/liter)</th>
<th>ECP (ng/minute/40 cm)</th>
<th>Albumin (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.6</td>
<td>146</td>
<td>35</td>
<td>319</td>
<td>59</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>1</td>
<td>592</td>
<td>5,967</td>
<td>90</td>
<td>907</td>
<td>18</td>
</tr>
<tr>
<td>Celiac</td>
<td>1</td>
<td>17</td>
<td>167</td>
<td>2</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16</td>
<td>15 ± 10*</td>
<td>152 ± 106*</td>
<td>9 ± 7*</td>
<td>77 ± 62</td>
<td>15 ± 22*</td>
</tr>
<tr>
<td>Giardiasis</td>
<td></td>
<td></td>
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</table>

*Mean ± SD.
rate of MPO was seen only in the patient who also had absence of plasma cells in the lamina propria of the small intestine. One patient with agammaglobulinemia A had normal secretion rates of MPO and ECP.

Immunohistochemical Studies

Immunohistochemical biopsy studies were performed in patient and control groups other than those investigated by jejunal perfusion.

Jejunal biopsy specimens from patients (n = 10) with active celiac disease revealed crypt hyperplasia and subtotal villous atrophy of the mucosa (Figure 2A and B). The prominent cellular infiltrate of the lamina propria was dominated by lymphocytes and eosinophils, whereas only a few neutrophils were seen. Degranulation of eosinophils was observed and confirmed with the immunoperoxidase staining for ECP (Figure 2C). A heavy deposition of immunoreactive ECP was present in the lamina propria (Figures 2C and 3A) and especially towards the surface of the flattened villi (Figure 2C). The same pattern was revealed using the monoclonal antibody EG2. Immunoreactive MPO was observed in only a small number of cells in some of the sections. Positive control slides stained well for MPO (Figure 3D). Immunoreactive cathepsin G was seen in a varied number of neutrophils; very few cathepsin G-positive cells were documented in most sections, but in two of the patients the biopsies demonstrated accumulation of neutrophils with scattered degranulation of cathepsin G. Tissue from patients on a gluten-free diet (n = 4) revealed a normal mucosa (Figure 2D). The immunoreactivity of ECP was confined to a small number of eosinophils (Figure 2E). In one of the patients, the biopsy specimen revealed small isolated areas with extracellular ECP in the deep part of the lamina propria. Two of the patients whose condition was in remission during a gluten-free diet later underwent provocation with gluten. The biopsies demonstrated relapse of morphologic changes typical for celiac disease and return of heavy
ECP deposits especially beneath the surface epithelium of the villi.

Transmural inflammation was present in resected ileo-colic specimens from patients with Crohn's disease (n = 5). Ulcers and fissures were infiltrated with inflammatory cells. In non-ulcerated mucosa, lymphocytes and plasma cells were the dominating infiltrating cells, but many neutrophils and eosinophils were observed as well. Immunoreactive ECP was located in eosinophils present throughout all layers, but in high numbers in the mucosa in particular (Figure 3B and C). Many eosinophils were seen passing through the surface epithelium into the lumen (Figure 3C). The eosinophilic ECP showed immunoreactivity to the antibody EG2. A prominent extracellular release of ECP was demonstrated in relation to ulcers and fissures but not in intact mucosa. MPO- and cathepsin G-immunoreactive cells were seen throughout all layers (Figure 3D). However, the number of MPO-positive cells was few compared with cathepsin G-positive cells. An extracellular deposit of cathepsin G but not of MPO was seen in relation to ulcers and fissures. The colonic biopsy specimens from patients with Crohn's disease of the colon (n = 3) revealed the same immunohistochemical pattern as observed in the ileo-colic specimens, but the number of cathepsin G-positive cells was few compared with the number of immunoreactive eosinophils.

The jejunal biopsies from the control subjects (n = 7) showed a normal morphology with scattered eosinophils, lymphocytes, and plasma cells. In three cases, the immunoperoxidase method for the detection of ECP revealed isolated areas with scarce extracellular ECP deposits in the deep part of the lamina propria. In these areas, the same distribution of ECP immunoreactivity could be demonstrated with the antibody EG2. Immunoreactive MPO was not demonstrated in the control biopsy specimens. A few cells were reactive for cathepsin G but no extracellular deposits of this granule protein were seen.
The eosinophil component ECP appeared in the jejunal perfusion fluid from control subjects in concentrations corresponding to about 15 to 20 percent of the normal serum values [2,20]. In contrast, the albumin concentrations in the perfusion fluid were about 0.05 percent of the normal serum albumin levels. Thus, it is not likely that granulocyte granule constituents in jejunal fluid originate from the plasma compartment due to passive mechanisms. Rather, the presence of significant amounts of MPO and ECP in the jejunal perfusate should reflect the turnover of the neutrophil and eosinophil masses located in the jejunal wall itself.

It is reasonable to attribute a preparedness of neutrophils and eosinophils in the small bowel to a physiologic role in the defense against invading microorganisms. MPO occurs in the azurophil granules. During degranulation, and in the presence of either iodine or chlorides, MPO can use hydrogen peroxide to kill bacteria [1]. The present observation of very high MPO concentrations in the perfusion fluid from the patient with no plasma cells in the cells in the small intestine lamina propria may in fact reflect compensatory mobilization of neutrophils to the intestine when one defense line of the intestine is missing. An anti-inflammatory role has previously been attributed to the eosinophils because of its ability to inhibit mast cell and basophil-mediated reactions [23-25]. The eosinophil release of ECP and other cytotoxic components such as major basic protein and eosinophil peroxidase is considered an important defense mechanism against parasites [4,5,26,27]. The finding that our patients with giardiasis had increased ECP but normal MPO secretion rates may illustrate such a role of eosinophils. Intestinal eosinophils are also reported to be actively phagocytic, and are major phagocytic cells at least in the lamina propria of the colon [28].

Granulocytes may also participate in inflammatory-induced tissue injury. The potential hazard of persistent local neutrophil activation relates to the ability of the neutrophil to release enzymes and toxic oxygen radicals [29-31]. The azurophilic granules of the neutrophil contain a number of preformed proteolytic enzymes, including collagenase, elastase, and cathepsin G, which have the capacity to derange the connective tissue matrix. The very toxic hypohalide ion catalyzed by the enzyme MPO may also cause tissue damage [32]. The cytotoxic potential of eosinophilic leukocytes may likewise be turned against the host. Eosinophils have been demonstrated to be effector cells in antibody-dependent cytotoxicity models [33,34]. The eosinophils probably mediate cytotoxic damage by a number of mechanisms, but the demonstration that isolated granule proteins from eosinophils, including ECP, major basic protein, and eosinophil peroxidase, may be toxic to a variety of mammalian cells is exciting [2,3,35-39]. The hypothesis that eosinophils and in particular their cytotoxic products might be involved in the development of organ lesions has gained clinical and experimental support by recent studies [18,35-46]. The demonstration of toxic concentrations of ECP and major basic protein in cerebrospinal fluid [42], bronchoalveolar lavage fluid [43], sputum [44], and synovial fluid [45] defines a potential role for these proteins in inflammatory human disease in, for example, the brain, lung, and joints. Thus, neutrophils as well as eosinophils have a potent armamentarium of products that may be involved in the development of damage to various organs, including the intestine.

There is good reason for supposing that celiac sprue results from local inflammatory reactions to gluten in genetically predisposed persons. However, the mechanisms of the inflammatory damage remain a controversial issue. Until now, the dominating work in celiac disease has been focused on the content and role of lymphocytes and plasma cells infiltrating the upper intestinal mucosa [47,48]. However, the infiltration of polymorphonuclear leukocytes and in particular eosinophilic leukocytes in the lamina propria is a prominent finding [6,7]. Our jejunal perfusion studies in patients with celiac disease showed that the jejunal secretion rate of ECP was increased on average eight times compared with that in the control subjects, whereas the secretion rate of MPO was increased about three times. These findings are compatible with enhanced local granulocyte involvement mainly consisting of eosinophils and, to a lesser degree, neutrophils. The immunohistochemical studies on jejunal biopsy specimens demonstrated that ECP from eosinophils was not only released into the jejunal lumen but also into the intestinal tissue. Thus, large amounts of extracellular ECP were seen in the lamina propria infiltrated with eosinophils. The extracellular deposit of ECP was in particular localized in areas just beneath the surface epithelium of the flattened villi. This pattern of extracellular release was absent in the morphologically normalized mucosa seen after a period on a gluten-free diet but returned again after gluten provocation. The observed localization of ECP in the affected mucosa might reflect its cytotoxic potential and offer a possible explanation for the cell damage typical of celiac disease. In contrast to the histologic findings of ECP, no extracellular deposit of MPO was seen in the intestinal biopsies, but a scattered and rather discrete deposit of cathepsin G was observed in areas with neutrophil accumulation, indicating that a minor extracellular deposit of neutrophil granule components may in fact occur in the affected mucosa in celiac disease.

The inflammation in Crohn's disease may affect any part of the gastrointestinal tract, although involvement of the terminal small intestine and the colon is most common. However, a more systemic nature of the disease has been suggested. Previous investigators have reported transport defects in normal ileal mucosa from patients with Crohn's disease [49] as well as subclinical inflammatory affection of the lung [50]. Furthermore, increased intestinal permeability to polyethylene glycol seen in patients with Crohn's disease is also present in clinically unaffected relatives [51]. The present jejunal perfusion studies in patients with Crohn's disease may be another illustration of a latent disease manifestation. Thus, although the biopsy specimens taken from the perfused jejunal area were histologically normal, we observed an increased secretion rate of MPO linked to the disease activity. A minor increase of the secretion rate of ECP was observed as well. Examination of specimens from affect-
ed areas of the ileum and colon demonstrated a historically typical picture for Crohn's disease. In non-ulcerated mucosa, lymphocytes and plasma cells were the dominating infiltrating cells. Increased numbers of neutrophils immunoreactive for MPO and cathepsin G were seen throughout all layers but in particular in the mucosa; however, no extracellular deposits of these components were found. Eosinophils immunoreactive for ECP were also observed in increased numbers in the non-ulcerated mucosa. Many eosinophils were seen passing through the surface epithelium into the lumen. In contrast to the findings in celiac sprue mucosa, the accumulated eosinophils had not released their granule contents since no extracellular deposits of ECP could be demonstrated. However, in relation to ulcers and fissures, extracellular deposits of both neutrophil and eosinophil constituents were found. The findings of a local eosinophil granule protein release are in accordance with the observations in this disease by Dvorak [10] of absent granule cores in the granules of undamaged eosinophils present in gut specimens with overt pathologic lesions. Taken together, the jejunal secretion data and the immunohistochemical studies in Crohn's disease suggest an enhanced granulocyte involvement in non-affected intestine and an extracellular release and tissue deposition of neutrophil and eosinophil granule constituents at sites of inflammatory lesions.

In summary, this study has demonstrated that the neutrophil and eosinophil activity of the small bowel can be elucidated in health and disease by analysis of the concentrations of granulocyte granule components in jejunal perfusion fluid. The significance of enhanced jejunal secretion of eosinophil and neutrophil constituents in celiac sprue and Crohn's disease is uncertain. It might reflect an unspacific enhanced turnover of granulocytes in the intestine without a defined pathophysiologic role. However, the possibility that eosinophil and neutrophil tissue damaging products may contribute to certain inflammatory intestinal lesions is supported by the immunohistochemical localization of large amounts of eosinophil cytotoxic products in atrophic intestinal lesions in celiac disease and the localization of eosinophil and neutrophil granule components in ulcerations in Crohn's disease.

REFERENCES


