Normal Variation in Intraepithelial Lymphocytes of the Terminal Ileum

Smiljana Istvanic, MD,1 Rhonda K. Yantiss, MD,2 Stephen P. Baker, MScPH, PhD,3 and Barbara F. Banner, MD4

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Abstract

The number of intraepithelial lymphocytes (IELs) is often increased in the terminal ileum of patients with immune-mediated inflammatory diseases of the colon. However, data regarding their number in normal ileal mucosa of asymptomatic patients are lacking. We aimed to establish the acceptable range of IELs in biopsy specimens of normal ileal mucosa.

Ileal mucosal biopsy specimens obtained during colonoscopy of 61 asymptomatic patients without endoscopic or pathologic evidence of colitis were immunostained for CD3 to assess the number of IELs present in each specimen. CD3+ lymphocytes were counted in 3 well-oriented villi and results expressed as the average for each biopsy specimen. The study group included 25 males and 36 females, ranging in age from 10 to 84 years (mean, 44.4 years). The mean ± SD number of ileal CD3+ cells per 100 enterocytes for the group was 3.8 ± 2 (median, 3; range, 0-9). In addition, there was a significant inverse relationship between the number of villus IELs and increasing age.

Occasional IELs (approximately 4/100 villus enterocytes) are normally present in ileal biopsy specimens from asymptomatic patients. One should avoid overinterpreting the importance of occasional IELs in ileal biopsy specimens from asymptomatic patients.

The terminal ileum is commonly sampled during coloscopic procedures performed for routine cancer screening and for symptoms of diarrhea and the evaluation of inflammatory bowel disease. Occasionally, the number of intraepithelial lymphocytes (IELs) seems to be increased in villi in biopsy specimens from the terminal ileum that are otherwise pathologically and endoscopically normal, raising the question of unsuspected ileitis. However, the clinical importance of mild lymphocytic infiltrates in the ileal mucosa is unclear because baseline data defining the number of IELs normally present in the ileum have not been established.

The normal number of villus IELs may vary throughout the gastrointestinal (GI) tract. IELs normally range from 2 to 25 per 100 enterocytes in the duodenal villi, whereas the upper limit of normal is 20 per 100 enterocytes in the jejunum.1-7 IELs in the ileum may be fewer than elsewhere in the small intestine, as reported in studies of IELs in immune disorders that included normal ileal mucosal biopsy specimens as control samples.8,9 However, the distribution of IELs in the ileal mucosa among asymptomatic patients is not established. Therefore, the aim of this study was to count IELs detected by immunostaining for CD3 antigen to determine the acceptable range of IELs present in mucosal biopsy specimens from the normal ileum.

Materials and Methods

Case Selection

Cases with ileal mucosal biopsy specimens obtained at colonoscopy were retrospectively identified from the surgical pathology files of UMass Memorial Health Care, Worcester,
MA. Ileal biopsy specimens diagnosed as normal from asymptomatic patients, which were obtained during routine screening colonoscopy or the evaluation of abdominal complaints, were identified and reviewed, and 61 specimens that contained 3 well-oriented villi, each with at least 100 enterocytes per villus, were selected for study. Clinical, demographic, and endoscopic data were obtained from the patients’ medical records to identify patients who had no disorders that might affect the number of IELs in the ileum. The study was performed within the guidelines and with the approval of the UMass Memorial Healthcare Institutional Review Board.

**Immunohistochemical Studies**

We cut 5-µm-thick tissue sections from the formalin-fixed, paraffin-embedded tissue blocks. Sections were then deparaffinized and rehydrated with graded ethanol solutions. Immunohistochemical stains were performed with antibodies directed against CD3 (pan–T cell marker, rabbit polyclonal, DAKO, Carpinteria, CA) at a 1:100 dilution using standard techniques on an automated immunostainer (DAKO Autostainer, Universal Staining System, DAKO). Normal tonsillar tissue was used as a positive control sample, and negative controls were accomplished by omitting the primary antibody with each batch run.

**Evaluation of Immunostaining**

The CD3+ IELs were counted using the same standard microscope. Villi were considered well-oriented if they were cut longitudinally and were continuous with an adjacent crypt. All of the villi that were evaluated were at least 1 high-power field (40× objective) from any lymphoid aggregates. Either membranous or cytoplasmic staining for CD3 was interpreted as a positive staining reaction. In each case, the total number of CD3+ IELs per 100 enterocytes was counted at ×400 magnification in each of 3 well-oriented villi, and the mean number was recorded for each case.

**Statistical Analysis**

The association between patient age and IEL counts was evaluated by using the Spearman correlation for nonparametric data and the Pearson correlation coefficient, using the patient age and natural log of the counts. The IEL counts were compared by sex by using a Student t test. The IEL counts were analyzed by sex and age together in a multivariate analysis using a multiple linear regression test.10,11

**Results**

The 61 patients included 25 males and 36 females ranging in age from 10 to 84 years (mean, 44.4 years). The indications for endoscopy included routine screening (n = 5); patient complaints of diarrhea, bloating, or constipation (n = 21), bleeding (n = 14), and abdominal pain (n = 15); or clinical indications of anemia (n = 4) or suspicion of a mass (n = 2). The endoscopic findings included normal (n = 27), hemorrhoids or diverticula (n = 25), and the presence of colonic polyps (n = 9). All ileal biopsy specimens showed normal villous architecture without evidence of ileitis, and none of the concomitant colonic biopsy specimens showed evidence of colitis.

The mean ± SD number of ileal CD3+ IELs per 100 enterocytes was 3.8 ± 2 (median, 3; range, 0-9). A villus with CD3+ IELs is shown in **Image 1**. Analysis of the correlation between the mean number of IELs per 100 enterocytes and patient age showed a decrease in CD3+ IELs with increasing age (P = .002). There was no identifiable association between the number of IELs per 100 enterocytes and patient sex, initial complaint, or any other clinical parameter.

**Discussion**

The mucosal immune system of the GI tract includes the dispersed lymphoid follicles and Peyer patches and the diffuse distribution of T and B lymphocytes in the lamina propria and mucosal epithelium. IELs are almost exclusively T lymphocytes, predominantly of the CD8+ suppressor phenotype (80%-90%), and are present in the surface epithelium of the entire GI tract. In contrast, most (60%-70%) T-helper lymphocytes with a CD4+/CD8– phenotype are confined to the lamina propria.3,6,12,13

**Image 1** Ileal mucosal biopsy specimen immunostained for CD3+ intraepithelial lymphocytes showing rare positively staining cells within the villus epithelium (original magnification ×400).
The number of IELs normally present in the small bowel mucosa may vary from one anatomic area to another. For example, recent studies have established that the normal range of IELs in duodenal mucosal biopsy specimens is 2 to 25 IELs per 100 enterocytes. The finding of greater than 30 IELs per 100 enterocytes in duodenal villi has been shown to be a sensitive pathologic marker of gluten-sensitive enteropathy. Previous studies have shown that the number of IELs normally present in the jejunum rarely exceeds 20 IELs per 100 enterocytes. On the other hand, preliminary evidence suggests that the normal range of IELs may be much less in the ileal mucosa. Studies of ileal IELs in various immune-mediated disorders have reported, for the control groups, ranges of 0 to 4 and 4.7 to 14.7 IELs per 100 enterocytes.

Anecdotally, we have noticed that the numbers of IELs occasionally seem to be increased in terminal ileal biopsy specimens from patients without clinical or endoscopic evidence of ileitis. The significance of inflammatory activity in these biopsy specimens is difficult to assess because the baseline number and range of ileal IELs normally present has not been established. It is well known that the GI tract mucosa may show increased numbers of inflammatory cells in areas away from the primary site of involvement in a number of diseases. For example, gluten-sensitive enteropathy may be associated with lymphocytic gastritis and/or lymphocytic colitis. In addition, recent studies have demonstrated increased numbers of duodenal IELs in concomitant chronic gastritis or esophagitis. However, the number of IELs present in these situations is usually less than that seen in association with gluten sensitivity. Whether such increases in IELs are clinically significant or simply represent a subclinical manifestation of the dispersed mobile nature of the GI immune system is not known.

Therefore, for the present study we selected a group of ileal biopsy specimens from patients who, as far as we could determine, had no GI or other disorders that could influence the number of IELs in the ileum. Our study group was further defined by having normal histologic findings on examination of the ileal and colonic biopsy specimens submitted from the procedures. We did not have data about the medications the patients received or their dietary histories and, thus, could not evaluate the group based on these factors.

Our findings are consistent with those in previous studies. We found that the mean ± SD number of IELs per 100 enterocytes in the ileal mucosa was 3.8 ± 2, with a range of 0 to 9. It is interesting that even though the number of IELs per villus was small, there was a trend toward a decrease in number among older patients. When the group was analyzed statistically, the trend was significant, and the rate of decrease was calculated as 1 IEL per 20 years. One explanation for this finding could be that the indications for colonoscopy among younger patients were often medical, such as diarrhea or bloating, whereas the indications among older patients were more often for routine screening. However, this did not seem to be the case. In our patients younger than 30 years, the indications for colonoscopy included constipation (n = 1), pain (n = 2), bleeding (n = 2), diarrhea (n = 2), and possible colitis (n = 1), whereas in patients older than 70 years, the indications were 1 each for screening for polyps, anemia, bleeding, pain, and diarrhea. Furthermore, in all cases, the endoscopic findings were normal in the ileum and colon, and the biopsy specimens were also normal. Therefore, it seems that other factors may account for the apparent decrease in villus IELs with advancing age.

Villus IELs in terminal ileal biopsy specimens from a carefully constructed group of people 10 to 84 years old with no identifiable GI or systemic disorders were evaluated using immunostaining for the T-lymphocyte marker CD3. Our findings indicate that ileal mucosal biopsy specimens from healthy people contain approximately 4 IELs per 100 villus enterocytes (range, 0-9) and that the number of IELs decreases slightly with advancing age.

From the Departments of Pathology, University of Pittsburgh Medical Center, St Margaret’s Hospital, Pittsburgh, PA; Pathology, Weill Medical College of Cornell University, New York, NY; Information Services and Cell Biology, University of Massachusetts Medical School; and Pathology, UMass Memorial Medical Center, Worcester.

Address reprint requests to Dr Banner: Dept of Pathology, UMass Memorial Medical Center, Three Biotech, One Innovation Dr, Worcester, MA 01650.

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