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Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in *H. pylori* gastritis

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We have observed expansions of intraepithelial lymphocytes in duodenal biopsies from patients with *Helicobacter pylori* gastritis. This study was undertaken to prospectively evaluate, unselected, paired gastric and duodenal biopsies from 50 patients with *H. pylori* gastritis and a comparison group of 30 patients with other types of gastritis (10 autoimmune and 20 reactive) to: (1) quantify duodenal intraepithelial lymphocytes, determine their distribution patterns, epithelial location, and phenotype, and (2) correlate the intraepithelial lymphocyte elevations with various features of gastric and duodenal pathology. Intraepithelial lymphocytes were analyzed with antibodies including CD3, CD8, and TIA-1. A stain for *H. pylori* was performed on all gastric and duodenal biopsies. Duodenal intraepithelial lymphocytes from patients with *H. pylori* gastritis (using CD3) ranged from 3 to 42 lymphocytes/100 epithelial cells (mean 18.5) compared to 3 to 18 lymphocytes/100 epithelial cells (mean 6.6) in the comparison group. Intraepithelial lymphocyte elevations were seen in 44% of the duodenal biopsies from patients with *H. pylori* gastritis (using CD3). Significant differences in the intraepithelial lymphocyte counts between patients with *H. pylori* gastritis and the comparison group were seen for all three T-cell antigens ($P<0.001$ for CD3 and CD8 and $P<0.002$ for TIA-1). Duodenal intraepithelial lymphocytes in the *H. pylori*+ cases had a latent cytotoxic phenotype, *H. pylori* was not visualized in any of the duodenal biopsies from patients with *H. pylori* gastritis, and no patient had clinical evidence of celiac disease. Our study highlights frequent duodenal intraepithelial lymphocytosis in individuals with *H. pylori* gastritis and the lymphocyte distribution patterns (and numbers) overlapped with those described for celiac disease patients. *H. pylori* gastritis must be considered as a possible explanation for duodenal intraepithelial lymphocytosis with normal villous architecture, especially when lymphocytosis is patchy, intraepithelial lymphocytes display a 'latent' cytotoxic phenotype, and the clinical findings and serologic profile does not fit celiac disease.
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Helicobacter pylori, a Gram negative bacterium, is one of the most prevalent human pathogens, infecting up to 30% of individuals in developed countries[1] and >50% of the population in developing countries,[2] making *H. pylori* gastritis the most common infectious disease worldwide.[3] Infected individuals most commonly develop nonatrophic pan-gastritis. Duodenal ulcer disease is uncommon in individuals with this pattern of gastritis but occurs more often in people with antral predominant gastritis.[4] Other patterns of *H. pylori* gastritis, including lymphocytic gastritis, are also well recognized.[5] *H. pylori* is responsible for 90–95% of all cases of duodenitis,[6] histologically characterized as either ulcer-associated or nonspecific. Duodenal biopsies from patients with *H. pylori* gastritis, taken from areas of erythema or endoscopically normal-appearing mucosa, demonstrate a variable expansion of the lamina propria due to an increase in the number of plasma cells, lymphocytes, and macrophages. Neutrophillic infiltrates are seen occasionally, but foci of gastric metaplasia, with or without *H. pylori* colonization, are only rarely identified due to their patchy distribution.[7]

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While evaluating duodenal biopsies from patients with *H. pylori* gastritis, we have observed increased numbers of intraepithelial lymphocytes in a significant number of cases that had no alterations of villous architecture. Moreover, differentiating the various patterns of lymphocytic infiltrates, in many of these cases, from those observed in celiac disease and other small bowel diseases, was not possible on H&E examination. Proximal small bowel intraepithelial lymphocytosis is most commonly observed in biopsies from patients with celiac disease, often accompanied by alterations of the villous architecture.[8] Increasing awareness of a high prevalence of celiac disease in the West, including the United States,[9] has led to both an increase in the number of small bowel biopsies performed by gastroenterologists, as well as a heightened sensitivity of pathologists to detect subtle architectural alterations of the duodenal mucosa. Numerous studies, over the past decade, have highlighted the frequent finding of isolated duodenal intraepithelial lymphocytosis with minimal or no villous architectural alterations, described as the 'infiltrative lesion' (Marsh stage 1) by Marsh and Crowe,[10] as the sole abnormality, in biopsies from celiac disease patients.[11,12] Even though duodenal (or jejunal) intraepithelial lymphocytosis, as an isolated finding, has a high sensitivity for celiac disease, it lacks sufficient specificity for this entity, as elevations of intraepithelial lymphocytes are observed in a variety of gastrointestinal (and extra-intestinal) diseases (Table 1).

Surgical pathologists commonly encounter duodenal biopsies from individuals with *H. pylori* gastritis but there is a paucity of information regarding the incidence, extent, phenotype, or the pattern of intraepithelial lymphocytes that can be observed in these biopsies. We carried out a prospective study that entailed: quantifying duodenal intraepithelial lymphocytes, determining their patterns of distribution (villous-tip, villous-base, or uniform) and epithelial location (supranuclear, subnuclear, or both), phenotypic characteristics, and correlating intraepithelial lymphocyte elevations with various gastric and duodenal histologic parameters in patients with *H. pylori* gastritis. Duodenal intraepithelial lymphocyte counts were also performed in other types of gastritis (reactive and autoimmune) for comparison. We discuss the patterns of intraepithelial lymphocyte distribution and their phenotype, in relation to those reported for celiac disease[13,14] and other small intestinal diseases,[15,16] to highlight similarities as well as differences.

Table 1 Etiology of increased duodenal intraepithelial lymphocytes in the absence of gluten sensitivity

Etiology	*Reference*
Food allergy	Mavromichalis *et al*[53]
Primary immunodeficiency diseases	Klemola,[54] Nilsen *et al*[55]
Viral enteritis	Goldstein,[28] Cutz *et al*[61]
Giardiasis	Mavromichalis *et al*[53]
Blind loop syndrome	Burrows *et al*[56]
Tropical (postinfectious) sprue[a]	Ansari[57]
Crohn's disease	Wright and Riddell[58]
Autoimmune diseases	Kakar *et al*,[15] Holden *et al*[60]
NSAIDS	Kakar *et al*[15]
Irritable bowel disease	Tornblon *et al*[59]

[a]Usually in distal small bowel.

Materials and methods

Tissue Samples and Patient Characteristics

We evaluated 50 consecutive, unselected, paraffin-embedded, formalin-fixed (10% neutral-buffered formalin) paired gastric antral and duodenal biopsies from patients with *H. pylori* gastritis (19 M, 31 F, age range 27–81 years, mean 58 years) and 30 unselected, and paired gastric antral and duodenal biopsies from patients (16 M, 24 F, age range 15–83 years, mean 54 years) with other forms of gastritis (10 cases of autoimmune gastritis and 20 cases of reactive gastritis, including five with an acute inflammatory component). In 32 cases (64%) of *H. pylori* gastritis, fundus/body biopsies were also available for review. The duodenal biopsies were taken from areas of erythema, mucosal nodularity, or endoscopically normal mucosa. Clinical information regarding associated gastrointestinal (and non-GI) symptoms and conditions, and history of NSAID use were obtained from the hospital information system. There was no clinical evidence of giardiasis or any other parasitic infection, celiac disease, or food allergy in any of the patients with *H. pylori* gastritis. Serologic testing, for endomysial or tissue transglutaminase antibodies, prompted by the histologic findings, was performed in four patients.

Immunohistochemistry

An initial subset of duodenal biopsies ($n = 20$) from patients with ($n = 10$) and without ($n = 10$) *H. pylori* infection were stained with the antibodies listed (Table 2) to: (1) characterize the intraepithelial and lamina propria lymphocytes and (2) detect any phenotypic alterations of the epithelium. The remaining cases were only stained with antibodies against CD3, CD8, TIA-1, and *H. pylori*. Briefly, 4 μm formalin-fixed, paraffin-embedded sections were deparaffinized and subjected to antigen retrieval (10 mM citrate buffer (pH 6) and microwaved for 25 min). Immunohistochemical staining was performed on a Dako autostainer (Dako, Carpinteria, CA, USA). Slides were incubated with the primary antibodies (30 min, room temperature) and detection was carried out using the Envision plus system (Dako, Carpinteria, CA, USA) with DAB as

Table 2 Primary antibodies used for immunohistochemical analysis

Antibody	*Clone*	*Dilution*	*Supplier*
CD3	F7.2.38	1:800	Dako, Carpinteria, CA, USA
CD20	L26	1:500	Dako, Carpinteria, CA, USA
CD8	C8/144B	1:40	Dako, Carpinteria, CA, USA
CD45	PD7/26 and 2B11	1:200	Dako, Carpinteria, CA, USA
CD4	4BI2	1:50	Novocastra, Burlingame, CA, USA
TIA-1	2G9A10F5	1:1000	Immunotech, Westbrook, ME, USA
E-cadherin	36B5	1:40	Chemicon, Temecula, CA, USA
MIB-1	KI-S5	1:50	Dako, Carpinteria, CA, USA
Perforin	5B10	1:50	Novocastra, Burlingame, CA, USA
Granzyme B	GrB7	1:20	Chemicon, Temecula, CA, USA
Helicobacter pylori		1:10	Abcam, Cambridge, MA, USA

chromogen. Positive controls consisted of sections of tonsil and normal small bowel.

Biopsy Assessment and Scoring

Semiquantitative scoring (1 to 3+) of gastric biopsies was performed according to the revised Sydney criteria[17] in addition to other parameters, including: (1) presence of lymphoid follicles, (2) extent of intestinal metaplasia (0 to 3+), (3) density of *H. pylori* organisms (1+ to 3+), and (4) number of lymphocytes/100 epithelial cells, an upper limit of 25 lymphocytes/100 epithelial cells was considered normal.[16] Duodenal biopsies were assessed for: (1) grade of acute and chronic inflammation, (2) presence of *H. pylori*, (3) presence of lymphoid follicles, (4) villous atrophy, (5) crypt hyperplasia, (6) gastric metaplasia, and (7) number of lymphocytes/100 epithelial cells, an upper limit of 20 lymphocytes/100 epithelial cells was considered normal.[13] Biopsy pieces with the highest number of lymphocytes were chosen to calculate the intraepithelial lymphocyte counts, as these tend to catch the attention of pathologists and are used for a subjective estimation of intraepithelial lymphocytosis. Intraepithelial lymphocyte quantification was performed on serial sections stained with CD3, CD8, and TIA-1. The number of intraepithelial lymphocytes was assessed in five different villi by counting the number of lymphocytes/100 epithelial cells in each villus, and calculating the mean. Predominant distribution (villous-base, villous-tip, or even distribution along the villous-length) and epithelial location (supranuclear, subnuclear, or both supra and subnuclear) of the lymphocytes was noted. The percentage of villi/biopsy piece with increased intraepithelial lymphocytes was also assessed and data segregated into four groups: 0–25, 26–50, 51–75, and 75–100%. All duodenal biopsies had at least two well-oriented pieces (range 2–9, mean 3.12) where villous architecture could be evaluated.

Statistics

Fisher's exact test and χ^2 tests were performed and $P<0.05$ was considered significant.

Results

Immunohistochemistry

In the initial subset of duodenal biopsies (20 cases), the majority of lamina propria T cells were CD4+ and only rare CD4+T cells were seen in the epithelium. The intraepithelial lymphocytes were: CD3+, CD8+, and TIA-1+ (Figures 1 and 2); only a few lymphocytes stained (weakly) with perforin or granzyme B, consistent with a 'latent cytotoxic' phenotype. CD20 highlighted scattered B cells and lymphoid follicles in the lamina propria. Rare intraepithelial lymphocytes stained with MIB-1 in duodenal biopsies from both groups and no difference in the proliferation rates was noted between the two groups. No alteration in the pattern of E-cadherin staining was detected (membranous in all cases). Immunohistochemical staining for *H. pylori* identified the bacillus in all antral but none of the duodenal biopsies from patients with *H. pylori* gastritis. In addition, *H. pylori* was not identified in any of the gastric or duodenal biopsies from patients with other forms of gastritis.

Intraepithelial Lymphocyte Counts and Distribution

Duodenal intraepithelial lymphocytes (on CD3-stained sections) from patients with *H. pylori* gastritis ranged from 3 to 45 lymphocytes/100 epithelial cells (mean 18.5) compared to a range of 3 to 18 lymphocytes/100 epithelial cells (mean 6.6) in patients with other types of gastritis. Significant differences in the intraepithelial lymphocyte counts were observed between patients with *H. pylori* gastritis and other forms of gastritis for all three T-cell antigens ($P<0.001$ for CD3 and CD8 and $P<0.002$ for TIA-1). Increased numbers of intraepithelial lymphocytes were documented in 44% of the duodenal biopsies of patients with *H. pylori* gastritis on staining for CD3, 46% with CD8, and 42% with TIA-1. These differences were statistically nonsignificant; most likely reflecting a sampling variation due to scoring of different levels with the different antibodies (Table 3). Intraepithelial lymphocyte elevations limited to <25% of the villi were

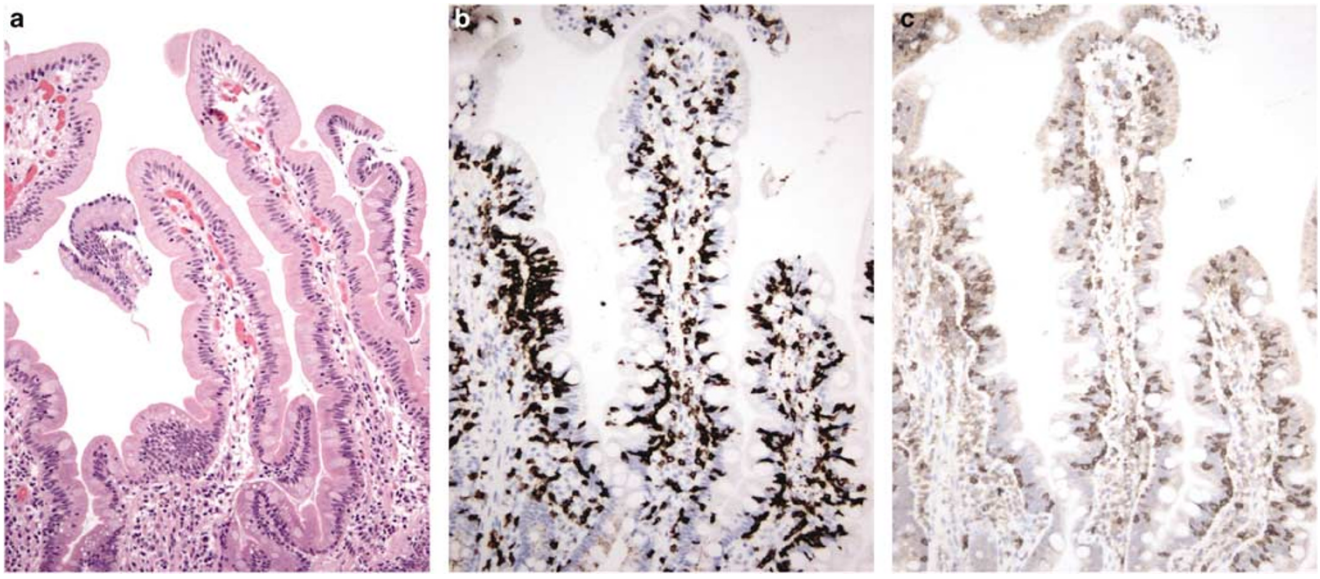

Figure 1 An example of an even distribution pattern of intraepithelial lymphocytes along the length of an architecturally normal villous: (**a**) H&E, (**b**) CD3, (**c**) CD8 (all $\times 10$).

observed in only one biopsy while increased intraepithelial lymphocytes involving >75% of villi were seen in 10 biopsies (20%). Data regarding the percentage of villi/biopsy-piece with increased intraepithelial lymphocytes are provided in Table 4. There was no singular, predominant pattern of intraepithelial lymphocyte distribution along the crypt–villus axis (Table 5). No statistical difference was found between a basal or villous-tip predominant pattern (36 *vs* 22%). An even distribution of intraepithelial lymphocytes, along the villous length, was as common as a basal predominant pattern. Figure 1 and 2b represent examples of the diffuse or even distribution pattern of intraepithelial lymphocytes while Figure 2a illustrates the villous-tip predominant pattern. Superficial/supranuclear location of the intraepithelial lymphocytes was as common as a subnuclear location, the majority of cases, however, showed an equal distribution of lymphocytes in these two locations (Table 6). An example of the latter pattern is shown in Figure 2b.

Correlation of Duodenal Intraepithelial Lymphocytes with Duodenal and Gastric Pathology

The majority of patients, 42/50 (84%), had antral predominant gastritis and only 8/50 (16%) had evidence of pan-gastritis. The number of duodenal intraepithelial lymphocytes did not correlate with the density and location (antrum, body, or both) of *H. pylori* in the gastric mucosa, severity of acute or chronic inflammation of the gastric antrum or duodenum, presence of gastric mucosal lymphoid follicles, or the presence of intestinal metaplasia in the stomach. The gastric antrum had <25 lymphocytes/100 epithelial cells (range 2–9 lymphocytes/100 epithelial cells, mean 4 lymphocytes/100 epithelial cells) in all cases of *H. pylori* gastritis. No formal count was performed in biopsies from the body/fundus as only rare intraepithelial lymphocytes were identified in this location. The villus–crypt ratio was >3:1 in all duodenal biopsies and no villous blunting, mucin depletion, or gastric metaplasia was identified in any of the duodenal biopsies from patients with *H. pylori* gastritis or the comparison group. Scattered neutrophils were seen in the duodenal lamina propria (but not the surface epithelium) in only 4/50 (8%) biopsies from patients with *H. pylori* gastritis and in none of the biopsies from the comparison group. Lymphoid follicles or aggregates were present in 7/50 (14%) of duodenal biopsies from patients with *H. pylori* gastritis but in none of the biopsies from the control group. There was no histologic evidence of Crohn's disease, giardiasis, or food allergy in duodenal biopsies from both groups. No significant differences, in the severity of duodenal inflammation, were observed between the two groups.

Patient Characteristics

No significant differences were noted regarding other gastrointestinal (as well as non GI) symptoms and conditions between the two groups (Table 7). A history of NSAID use was only documented in a minority of patients with *H. pylori* gastritis (10%) but in none of the patients from the comparison group. Owing to the nature of associated diseases or medical conditions, it is likely that use of anti-inflammatory agents was under-reported in both patient groups. The results of serologic testing for celiac disease were negative in all four patients

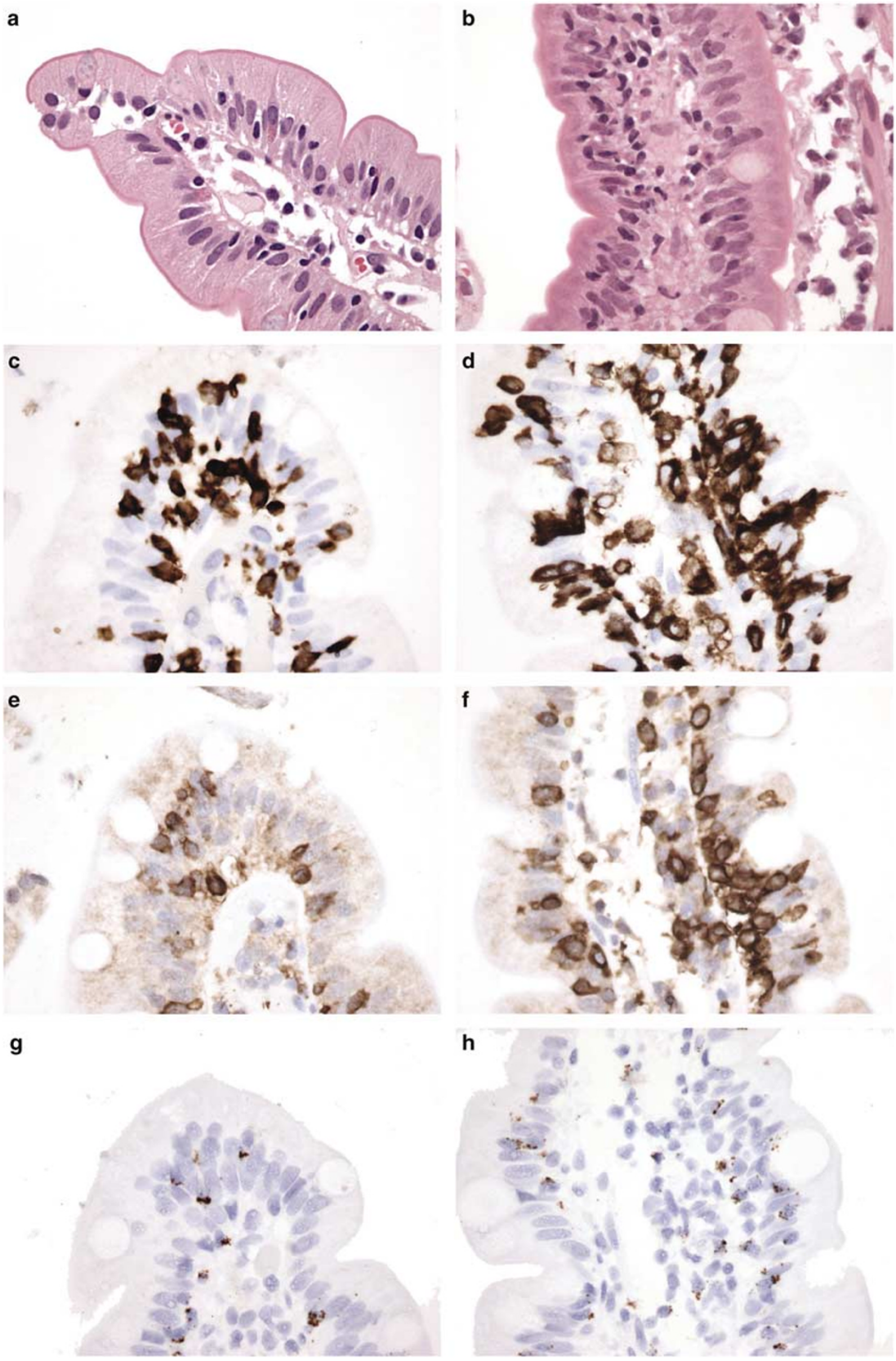

Figure 2 (**a**) Increased intraepithelial lymphocytes present in the villous tip. Intraepithelial lymphocytes stain with (**c**) CD3, (**e**) CD8, and (**g**) TIA-1. (**b**) Intraepithelial lymphocyte distribution along the villous edge; lymphocytes are present in both supranuclear and subnuclear locations, (**d**) CD3, (**f**) CD8, and (**h**) TIA-1 (all $\times 40$).

Table 3 Duodenal intraepithelial lymphocyte elevations in *H. pylori*+ cases

	20–25/100 EC	*26–30/100 EC*	*>30/100 EC*
CD3 ($n=22$)	8	8	6
CD8 ($n=23$)	12	6	5
TIA-1 ($n=21$)	9	8	4

EC, epithelial cells.

Table 4 Percentage of villi with increased intraepithelial lymphocytes in *H. pylori*+ cases

	0–25%	*26–50%*	*51–75%*	*76–100%*
CD3+ ($n=22$)	1	5	6	10
CD8+ ($n=23$)	0	4	10	9
TIA-1+ ($n=21$)	1	6	8	6

Table 5 Intraepithelial lymphocyte distribution along the crypt–villous axis in *H. pylori*+ cases

	Base	*Tip*	*Both*
CD3 ($n=22$)	8 cases	5 cases	9 cases
CD8 ($n=23$)	9 cases	6 cases	8 cases
TIA-1 ($n=21$)	8 cases	5 cases	8 cases

Table 6 Epithelial location of lymphocytes in *H. pylori*+ cases

	Basal/ subnuclear	*Superficial/ supranuclear*	*Basal and superficial*
CD3 ($n=22$)	7 cases	5 cases	10 cases
CD8 ($n=23$)	8 cases	6 cases	9 cases
TIA-1 ($n=21$)	6 cases	4 cases	11 cases

Table 7 Associated symptoms, signs, and diseases in patients with *H. pylori* and other types of gastritis

	H. pylori+ *cases with intraepithelial lymphocytosis*	H. pylori+ *cases without intraepithelial lymphocytosis*	H. pylori– *cases*
GI symptoms/conditions			
Abdominal or epigastric pain	13	7	11
Diarrhea	1	5	5
Dyspepsia	0	3	5
GE reflux	1	2	3
Anemia	2	4	2
Irritable bowel disease	1	1	0
History of Crohn disease	1	0	1
History of duodenal ulcer	1	1	0
History of colon cancer or polyps	2	4	3
History of pancreatitis	0	1	0
Other diseases/conditions			
Hematemesis	0	1	0
Arthritis	1	2	1
HIV	0	1	0
Nephrotic syndrome	0	0	1
Cardiovascular disease	2	3	0
Diabetes	0	1	0
Asthma	1	1	0
History of neurologic disease	0	0	1
History of breast cancer	0	0	1
History of prostate cancer	1	0	1
History of GYN cancer	0	1	0
History of Hodgkin's lymphoma	1	0	0

tested. None of the patients were referred to the celiac disease clinic due to resolution of symptoms post triple drug therapy for *H. pylori*. Post-therapy duodenal biopsies were not performed.

Discussion

The triad of villous atrophy, intraepithelial lymphocytosis, and chronic inflammation of the duodenum has been referred to as the 'celiac lesion.' These histologic features are considered the 'gold standard' for diagnosing celiac disease or gluten-sensitive enteropathy.[18] Fry *et al*,[19] more than three decades ago, had brought attention to the diagnostic significance of increased intraepithelial lymphocytes in patients with celiac disease. Intraepithelial lymphocytosis was found to be more reliable in detecting gluten sensitivity than the endoscopic appearance of small bowel mucosa. An increase in jejunal intraepithelial lymphocytes is one of the earliest histologic abnormalities in response to gluten challenge before any epithelial and structural alterations are noted.[20,21] Duodenal biopsies from patients with the 'silent' or latent celiac disease,[22] dermatitis herpetiformis,[23] and first-degree relatives of patients with celiac disease[24] often show isolated intraepithelial lymphocytosis. Even biopsies from symptomatic patients, at times, demonstrate increased intraepithelial lymphocytes as the sole

mucosal abnormality.[12,14] Recent studies have, however, reported a low specificity of 'isolated intraepithelial lymphocytosis' for diagnosing celiac disease.[13,15,25,26] This is not surprising as numerous etiologic agents can elicit an increase in duodenal intraepithelial lymphocytes, in association with a spectrum of mucosal changes that mimic celiac disease[15,26–28] (Table 1).

Hasan *et al*[29] reported increased intraepithelial lymphocytes in association with both ulcer-associated and nonspecific duodenitis in 1983, prior to the discovery of *H. pylori* as the major etiologic agent. They found significantly higher intraepithelial lymphocyte counts in biopsies from areas of duodenal ulceration and severe nonspecific duodenitis, compared to controls. Hayat *et al*[30] found raised intraepithelial lymphocyte counts, in the second part of the duodenum, in 4/13 (31%) patients with *H. pylori*-associated lymphocytic gastritis. Gastric intraepithelial lymphocyte counts decreased after treatment of *H. pylori* infection but the duodenal intraepithelial lymphocytosis persisted. The authors suggested the possibility of underlying celiac disease in these patients (2/4 patients had villous atrophy and 70% possessed the HLA-DQ2 allele).

We observed a high rate of duodenal intraepithelial lymphocytosis (44%, using CD3) in patients with *H. pylori* gastritis who had otherwise normal villous architecture (Figure 1, Table 3). Intraepithelial lymphocytosis was patchy but 16/22 (73%) biopsies (using CD3) had increased intraepithelial lymphocytes in $>50\%$ of villi/biopsy piece (Table 4). A villous-base predominant or diffuse pattern of lymphocyte distribution was more commonly observed but a villous-tip predominant pattern (Figure 2) was seen in 23% of cases (Table 5). In contrast to Hasan *et al*,[29] who described a subnuclear location of intraepithelial lymphocytes in the three cases (200 cell count) analyzed, we observed all three patterns: subnuclear (32%), supranuclear (23%), and mixed (45%).

Duodenal biopsies from patients with celiac disease often show a uniform distribution of intraepithelial lymphocytes. Goldstein and Underhill[14] found a high sensitivity but a low specificity of the 'even intraepithelial lymphocyte distribution pattern' for celiac disease. The extent of duodenal intraepithelial lymphocytosis is also not helpful in making the distinction between celiac disease and other small bowel diseases with increased intraepithelial lymphocytes.[13] Intraepithelial lymphocyte expansions in celiac disease can occasionally be patchy[31–33] that is, only one or a few biopsy pieces have increased intraepithelial lymphocytes. A top heavy or villous-tip predominant pattern of intraepithelial lymphocyte distribution, with the loss of the 'decrescendo' pattern,[14,26] has been used to suggest the diagnosis of celiac disease in such cases (as well as in biopsies with only mild intraepithelial lymphocytosis). This distribution pattern, however, also lacks sufficient specificity for diagnosing celiac disease.[13,14] In the study by Mino and Lauwers[13] CD3+ tip-intraepithelial lymphocyte scores were only marginally different from the nonceliac disease group with intraepithelial lymphocytosis ($P=0.054$). These authors, however, pointed out the utility of calculating villus-tip to base intraepithelial lymphocyte ratios to increase the specificity for celiac disease.

Our findings demonstrate that the spectrum of duodenal intraepithelial lymphocyte distribution patterns in patients with *H. pylori* gastritis overlaps to quite a considerable extent with the patterns described for celiac disease, in agreement with the observations of Goldstein.[26] Interestingly, in both the studies of Goldstein and Underhill[14] and Mino and Lauwers,[13] the non-gluten-sensitive groups with increased intraepithelial lymphocytes, included patients with *H. pylori* gastritis. Mahadeva *et al*[25] observed intraepithelial lymphocyte elevations in 14/626 (2.2%) of consecutive duodenal biopsies with a normal villous architecture over a 12-month period. Celiac disease was discovered in a minority of their cases but no etiology could be determined in 8/14 (57%) biopsies. Kakar *et al*[15] observed a significant association of isolated intraepithelial lymphocytosis with a variety of immunological diseases, including Hashimoto's thyroiditis, Graves' disease, rheumatoid arthritis, psoriasis and multiple sclerosis, and NSAID use (in addition to celiac disease). Both these studies, however, did not report any patients with *H. pylori* gastritis. This could relate to the more stringent criteria employed in these studies; only inclusion of biopsies with a generalized intraepithelial lymphocytosis in the former study and a high 'normal' cutoff value for intraepithelial lymphocytes (>40 lymphocytes/100 epithelial cells) in the latter.

The 'normal' range of intraepithelial lymphocytes in the duodenal mucosa has not been well established. Many studies cite the upper limit of normal as 40 lymphocytes/100 epithelial cells, based on the 'normal' range of 6–40 lymphocytes/100 epithelial cells published by Ferguson and Murray[34] in 1971. It is pertinent to point out that in this study (1) the normal range of intraepithelial lymphocytes was established for the jejunum (not the duodenum), (2) using capsule biopsies (a procedure no longer performed) and (3) numerous diseases/conditions associated with small bowel intraepithelial lymphocytosis were not known at that time. Other reports have used counts >30 lymphocytes/100 epithelial cells to 'arbitrarily' define elevations of intraepithelial lymphocytes.[35,36] Mahadeva *et al*[25] and Hayat *et al*[30] found substantially lower intraepithelial lymphocytes counts in duodenal biopsies from normal patients; range 2–20 (mean 12.4, s.d. 4.6) and 1.8–26 (mean 11, s.d. 6.8), respectively. Based on their results, 22[25] and 25 lymphocytes/100 duodenal epithelial cells[30] were suggested as the upper limits of normal (mean + 2s.d.). A count of 20

lymphocytes/100 epithelial cells was chosen as the upper limit of normal in our study, as we have observed that an increase above this level correlates well with the subjective visual impression of an elevation in intraepithelial lymphocytes (unpublished observations) and because we and others[35] have observed that not only individuals with latent or treated celiac disease but even symptomatic patients can, at times, present with only mild elevations of intraepithelial lymphocytes (see below). The histologic changes in biopsies from such patients are not sufficient to make the diagnosis of celiac disease according to the established criteria[37] and the interpretation of these biopsies is further compounded by the fact that serologic tests are often negative at this stage.[38,39] Mino and Lauwers,[13] presumably, also used a cutoff of 20 lymphocytes/100 epithelial cells for similar reasons. If we use 25 lymphocytes/100 epithelial cells as the upper limit of normal, an increased number of intraepithelial lymphocytes would still be seen in a substantial number (28%) of duodenal biopsies from patients with *H. pylori* gastritis (Table 3). Intraepithelial lymphocyte counts >40/100 epithelial cells were only seen in 4% (2/50) of our patients.

A comparison of duodenal intraepithelial lymphocyte distribution patterns and phenotypes, between patients with *H. pylori* gastritis and celiac disease, was not the intent of our study. Although, in order to assess the range of duodenal intraepithelial lymphocyte counts that can be observed in patients with untreated celiac disease, who have similar ethnic and racial characteristics as the patients with *H. pylori* gastritis, we retrospectively performed intraepithelial lymphocyte counts (using CD3 stained sections) on 38 consecutive duodenal biopsies taken from individuals with newly diagnosed and serologically confirmed celiac disease at our institute (during the same period). Demographics of the celiac disease patients were similar to those of the other study groups (data not shown) and the range of intraepithelial lymphocyte counts was 20–104/100 epithelial cells (mean 48.1). Four (10%) of the celiac disease patients had intraepithelial lymphocyte counts between 20 and 25 (two had counts between 26–30 and 32 had counts >30 lymphocytes/100 epithelial cells). However, none of these patients had normal villous architecture (partial villous atrophy was present in 36% and subtotal or total villous atrophy in 64% of the biopsies). Mino and Lauwers[13] also reported intraepithelial lymphocyte counts in the range of 20–40/100 epithelial cells in 3/8 (37%) biopsies from patients with celiac disease that had preserved villous architecture. Thus our observations, in conjunction with those of others,[13,15,25] further reinforce the fact that a substantial overlap in intraepithelial lymphocyte counts exists, between celiac disease and other diseases/conditions, both at the low as well as at the high end of the range of intraepithelial lymphocytosis. Since it has been observed that *H. pylori* gastritis related intraepithelial lymphocyte elevations decrease in the second part of the duodenum,[26] jejunal biopsies might be of utility in distinguishing these cases from celiac disease. Gluten challenge might also help identify patients with gluten sensitivity, especially in cases where the biopsies have normal mucosal architecture and only a borderline elevation in intraepithelial lymphocytes.[35]

Intraepithelial lymphocytes, in both our study and comparison groups, were: CD3+, CD8+, TIA-1+, and perforin and granzyme-B (weak, focal)+. This phenotype is consistent with alpha–beta T-cell receptor bearing lymphocytes with 'latent' cytotoxic potential. Lymphocytes with this phenotype represent the predominant intraepithelial lymphocyte population of normal human small intestinal mucosa[40,41] and expansions of this subset have been described in giardiasis.[42] In contrast, duodenal intraepithelial lymphocytes in celiac disease[42] as well as gastric intraepithelial lymphocytes in celiac disease-associated lymphocytic gastritis[43] have an 'activated' cytotoxic phenotype, that is, the intraepithelial lymphocytes express perforin and granzyme-B. The presence of activated cytotoxic T cells has been documented in the gastric lamina propria in *H. pylori* gastritis and some cases of NSAID-induced acute gastritis[43,44] but we are not aware of any studies that have demonstrated this phenotype of the intraepithelial lymphocytes in *H. pylori*-associated lymphocytic gastritis. No significant expansions of the CD3+ CD8− subset, representing gamma–delta T cells or a rare subset of CD8- alpha–beta T cells,[45] were seen in any of the biopsies in our study. Gamma–delta T cells are often expanded in biopsies from patients with active celiac disease,[46] autoimmune diseases,[47] and primary B-cell immunodeficiency diseases.[55]

The source of duodenal intraepithelial lymphocytes in *H. pylori* gastritis is not known. Lymphoid aggregates were only seen in a minority (14%) of cases and thus an origin of intraepithelial lymphocytes from duodenal mucosa-associated lymphoid tissue (MALT) cannot be proposed with certainty. Since an increase in gastric intraepithelial lymphocytes was not observed in any case of *H. pylori* gastritis, an anterograde spread of gastric intraepithelial lymphocytes cannot be invoked either. In addition, *H. pylori*-induced lymphocytic gastritis tends to be corpus predominant.[5] T-cell-mediated immune responses against *H. pylori* contribute to gastric pathology;[48] however, there are no data regarding the specificity of duodenal intraepithelial lymphocytes for *H. pylori*. In contrast to the observations in celiac disease,[49] we only detected rare MIB-1+ intraepithelial lymphocytes in duodenal biopsies from *H. pylori*+ patients. In conjunction with the absence of *H. pylori* in the duodenum and lack of an activated intraepithelial lymphocyte phenotype, our findings support a passive, perhaps trans-endothelial, migration and accumulation of

intraepithelial lymphocytes, rather than local antigen-induced proliferation. Water extracts of *H. pylori* have been shown to induce neutrophil adhesion and emigration from rat mesenteric venules;[50] however, a similar inductive effect has not been described for duodenal lymphocytes. Further studies are required to determine whether antigens shed or secreted by *H. pylori* from the gastric antrum play a role in the epithelial migration of duodenal lymphocytes. Duodenal intraepithelial lymphocytosis has recently been reported in association with NSAID use[15] and increased intestinal permeability and inflammation have also been documented for certain types of NSAID's.[51] A history of NSAID use was obtained for only 10% of our patients with *H. pylori* gastritis and no elevation in duodenal intraepithelial lymphocytes was seen in any of the cases of reactive gastritis. Future studies should investigate the possible roles of the type of NSAID, dosage, or duration of drug exposure, in inducing duodenal intraepithelial lymphocytosis. Lastly, acid-induced injury must also be considered as a possible explanation for intraepithelial lymphocytosis. Hasan *et al*[29] reported normalization of intraepithelial lymphocyte counts (<10/100 epithelial cells) after treatment with cimetidine. Endoscopic and histologic evidence of acid-induced injury were lacking in the majority of our patients. We could not assess the response of intraepithelial lymphocytes to treatment, as no follow-up biopsies were performed in any of the patients.

Neutrophils are considered the major mediators of duodenal inflammation and epithelial damage and the severity of duodenal injury is determined by interplay between host (cytokine polymorphisms) and bacterial factors (VacA toxin production and presence of the CagA pathogenecity determinant).[52] The role of T cells, specifically intraepithelial lymphocytes, in the pathogenesis of duodenal disease is not known. Hasan *et al*[29] attributed the duodenal mucosal damage to mediators of acute inflammation and considered the intraepithelial lymphocyte elevations to represent a nonspecific reaction. Since serological markers of celiac disease were only evaluated in four patients (all were negative), we cannot exclude the possibility that a few patients may have had celiac disease. We believe this supposition to be unlikely for the majority of the patients as: (1) all patients responded to the triple drug regimen for *H. pylori* and (2) the incidence of intraepithelial lymphocytosis (44%) in our study was much higher than the incidence of celiac disease in the US population (approximately 1%).[9]

The purpose of this study is to alert pathologists to the frequent occurrence of duodenal intraepithelial lymphocyte expansions in individuals with *H. pylori* gastritis and the considerable overlap of the intraepithelial lymphocyte counts as well as the distribution patterns with those described for celiac disease. *H. pylori* gastritis could be an explanation for duodenal intraepithelial lymphocyte elevations, when duodenal mucosal alterations are absent, lymphocytosis is patchy, and the intraepithelial lymphocytes have a latent cytotoxic profile. Further studies are needed to address the significance of the intraepithelial lymphocyte expansions and the extent to which other variables, such as host susceptibility factors (HLA and non-HLA-associated) and NSAID use, could contribute to this phenomenon.

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