

Immunoreactivity for CD25 in Gastrointestinal Mucosal Mast Cells is Specific for Systemic Mastocytosis

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Abstract: Systemic mastocytosis (SM) is characterized by the accumulation of neoplastic mast cells in bone marrow and other organs. Gastrointestinal (GI) symptoms are common in both SM and cutaneous mastocytosis [urticaria pigmentosa (UP)], and are usually caused by the release of histamine and other inflammatory mediators. Occasionally, neoplastic mast cells may also directly infiltrate the GI tract. Previous studies have suggested that enumeration of the mast cells in GI biopsies may help establish the diagnosis of SM. However, mast cells have been reported to be increased in various inflammatory diseases, and mast cell density has not been systematically evaluated in other GI disorders. Recently, expression of CD25 by mast cells in bone marrow has been shown to be specific for SM. The purpose of this study was (1) to quantitate and compare mast cells in mucosal biopsies from patients with SM involving the GI tract, UP with GI symptoms, and a control group of diverse inflammatory disorders, and (2) to determine whether immunostaining for CD25 can be used to distinguish neoplastic from reactive mast cells in GI biopsies. Seventeen GI biopsies from 6 patients with SM; 17 GI biopsies from 5 patients with UP; and 157 control cases including 10 each normal stomach, duodenum, terminal ileum, and colon, *Helicobacter pylori* gastritis, bile reflux gastropathy, peptic duodenitis, celiac disease, Crohn disease, ulcerative colitis, lymphocytic colitis, and collagenous colitis, 20 biopsies from 16 patients with irritable bowel syndrome, 8 biopsies from 5 patients with parasitic infections, and 9 biopsies from 7 patients with eosinophilic gastroenteritis were immunostained for mast cell tryptase, c-kit (CD117), and CD25. Mucosal mast cells were quantitated, and the presence or absence of CD25 expression on mast cells was determined. In SM patients, mast cells in the small intestine and colon numbered > 100/high-power field (HPF) in nearly all cases (mean 196/HPF; range 74 to 339). This was significantly higher than in GI biopsies from UP patients (mean 17/HPF; range 8 to 32, $P < 0.0001$) and all inflammatory diseases ($P < 0.01$). Mast cell density in other disorders ranged from a mean of 12/HPF in *H. pylori* gastritis to 47/HPF in parasitic infections. Interestingly, all SM biopsies (and none of the other cases) contained

aggregates or confluent sheets of mast cells. In addition, mast cells in all SM cases were positive for CD25, whereas GI mucosal mast cells in UP and all other control cases were negative. In conclusion, quantitation of mast cells can be helpful to diagnose SM in GI mucosal biopsies, although mast cells are also markedly increased in parasitic infections. Aggregates or sheets of mast cells are only seen in SM. Immunoreactivity for CD25 in GI mucosal mast cells is specific for SM and can be used to confirm the diagnosis.

Key Words: mast cell disease, mastocytosis, urticaria pigmentosa, immunohistochemistry, gastrointestinal, CD25, c-kit, CD117

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Mastocytosis encompasses a group of disorders characterized by the proliferation and accumulation of mast cells in one or more organs. Systemic mastocytosis (SM) is characterized by mast cell infiltration of multiple extracutaneous organs (most commonly bone marrow) with or without skin involvement.³⁹ In contrast, cutaneous mastocytosis is limited to the skin, and can manifest as urticaria pigmentosa (UP), telangiectasia macularis eruptiva perstans, diffuse cutaneous mastocytosis, or a solitary mastocytoma.³⁹ Gastrointestinal (GI) symptoms are common in both patients with systemic and cutaneous mastocytosis. Up to 70% of patients with SM have GI symptoms including nausea, vomiting, abdominal pain, and diarrhea, whereas GI complaints have been reported in approximately 25% of UP patients.³⁸ The pathophysiology of GI symptoms in patients with mastocytosis has not been clearly elucidated, but has been hypothesized to be due to either peptic ulcer disease from histamine-induced gastric acid hypersecretion, the effects of increased circulating mast cell mediators, and/or direct infiltration of the GI tract by mast cells.²² In addition, despite the high frequency of GI symptoms in patients with mastocytosis, the histologic findings in GI biopsies in these patients have not been well characterized. The previously published studies have been primarily case reports,^{1,2,6,8,9,11,12,16,21,30,33,37} and the results of mast cell quantitation in these studies have been conflicting. Compounding this confusion is increasing evidence that mucosal mast cells play a role in either the pathogenesis or evolution of various inflammatory diseases of the GI tract. However, mast cell density has not been systematically evaluated in GI disorders, and the

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acceptable limits of “normal” numbers in reactive mast cell infiltrates are, therefore, not well established.

According to the World Health Organization classification system, the major diagnostic criterion for bone marrow involvement by SM is the presence of dense aggregates (> 15 cells) of mast cells.³⁹ Expression of CD25, a low-affinity receptor for interleukin-2 (IL-2), is a reliable diagnostic tool for distinguishing neoplastic mast cell aggregates from reactive proliferations,^{26,34,39} and has therefore recently become a minor criterion for the diagnosis of SM.³⁹ However, measurement of CD25 expression has been performed primarily on mast cells collected from peripheral blood or bone marrow biopsies. The purpose of this study was 2-fold: (1) to quantitate and compare mast cells in mucosal biopsies from patients with SM involving the GI tract, UP with GI symptoms, and a control group of diverse inflammatory disorders, and (2) to determine whether immunostaining for CD25 can be used to distinguish neoplastic from reactive mast cells in GI biopsies.

MATERIALS AND METHODS

Study Groups

GI mucosal biopsies from 6 patients with SM and 5 patients with UP were compared with normal biopsies from 80 patients and biopsies from 92 patients with inflammatory diseases of the GI tract, and 16 patients with a clinical diagnosis of irritable bowel syndrome (IBS). Representative hematoxylin and eosin (H&E)-stained sections were reviewed by both authors in all cases to confirm the diagnoses.

Study cases were selected by identifying patients diagnosed with SM who also underwent GI endoscopy and biopsy. Most patients had previously undergone bone marrow biopsy ($n = 4$), and the diagnosis of SM was confirmed in all cases by the presence of either 1 major and 1 minor World Health Organization diagnostic criterion or 3 minor criteria.³⁹ Briefly, the major diagnostic criterion is the presence of multifocal mast cell aggregates (> 15 mast cells per cluster) in bone marrow or extracutaneous organs. Minor diagnostic criteria include spindled or atypical morphology of mast cells, *KIT* point mutation at codon 816, aberrant expression of CD2 and/or CD25 by neoplastic mast cells, and serum total tryptase > 20 ng/mL.³⁹ In total, 17 GI mucosal biopsies from 6 patients with SM (2 gastric, 6 small intestinal, and 9 colonic) were evaluated in this study.

All patients with UP had confirmatory skin biopsies showing aggregates of dermal mast cells highlighted by chloroacetate esterase stains and mast cell tryptase immunostains. In some patients ($n = 2$), bone marrow biopsy had also been performed, often prompted by GI symptoms suggesting possible systemic involvement. All bone marrow biopsies in the UP patients were negative for involvement by SM. Overall, 17 GI biopsies from 5 patients with UP (4 gastric, 7 small intestinal, and 6 colonic) were selected for this study.

Control groups included histologically normal biopsies of the stomach, duodenum, terminal ileum, and colon (10 patients each; 40 biopsies total) in patients without diarrhea, nausea, or vomiting.

To evaluate mucosal mast cell density in inflammatory diseases of the GI tract, biopsies from patients with a variety of common and uncommon diseases were retrieved for this study. Biopsies showing *Helicobacter pylori* gastritis, bile reflux gastropathy, peptic duodenitis, celiac disease, ulcerative colitis, Crohn colitis, collagenous colitis, and lymphocytic colitis (10 patients each; 80 biopsies total) were evaluated. Colonic and appendiceal tissue from 5 patients (8 biopsies total) with parasitic infections (4 strongyloidiasis; 1 enterobiasis) were evaluated. In addition, biopsies from patients with eosinophilic gastroenteritis (7 patients; 9 biopsies) were also studied. Finally, colonic and terminal ileal biopsies (10 biopsies each) from 16 patients with a clinical diagnosis of IBS were examined.

Immunohistochemical Studies

Immunohistochemical studies were performed on 4- μ m thick formalin-fixed paraffin-embedded tissue sections. The antibodies, clones, dilutions, pretreatment conditions, and sources are listed in Table 1. Briefly, immunoperoxidase studies were performed on paraffin sections after deparaffinization and pretreatment with aqueous hydrogen peroxide to eliminate endogenous peroxidase activity and retrieval with either ethylene diaminetetraacetic acid solution in a steamer (CD25) or trypsin at 37 degrees (mast cell tryptase). No antigen retrieval was used for c-kit (CD117). Slides were incubated with primary antibody for 1 hour then washed and incubated with peroxidase-labeled polymer conjugated to goat antimouse (for monoclonal antibodies) or goat antirabbit (for c-kit) immunoglobulin antibody (Envision+, Dako, Carpinteria, CA) for 30 minutes. Slides were washed and antibody localization was effected using a peroxidase reaction with 3,3'-diaminobenzidine tetrahydrochloride (DAB+, Dako) as chromogen. Slides were washed with water, counterstained, dehydrated, and mounted. Appropriate positive and negative controls were used throughout.

TABLE 1. Panel of Antibodies Used in This Study

Antibody	Clone	Dilution	Antigen Retrieval	Source
Mast cell tryptase	AA1	1:500	Trypsin	Dako, Carpinteria, CA
c-kit (CD117)	Polyclonal	1:200	None	Dako
CD25	4C9	1:200	EDTA + steamer	Novocastra, Newcastle upon Tyne, UK

EDTA indicates ethylene diaminetetraacetic acid.

Mast Cell Quantitation and Statistical Analysis

Mast cells were quantitated after immunostaining by counting the number of stained mast cells per high-power field (HPF) using a 40× objective and a 10× ocular lens (Olympus BX41 microscope; field size: 0.25 mm²). Ten contiguous HPF were counted per biopsy, and the mean was calculated. Only intact mast cells with visible nuclei were counted for analysis.

P values were determined using the student *t* test. A *P* value < 0.05 was considered statistically significant. The study was approved by the Institutional Review Board of Brigham and Women's Hospital.

RESULTS

Detailed clinical history was available for 5 of the 6 patients with SM. All 5 patients had GI symptoms including diarrhea (5/5) and epigastric pain with vomiting (2/5). The UP patients had similar symptoms with either diarrhea (4/5) or abdominal pain and vomiting (1/5). The single asymptomatic UP patient underwent screening upper endoscopy because of a family history of gastric cancer.

Histologic abnormalities were present in many of the biopsies from SM patients (Fig. 1). For example, villous blunting was seen in 4 of 6 (67%) small intestinal biopsies, and 4 of 6 (67%) also showed increased eosinophils in the lamina propria and occasionally within the epithelium (Fig. 1A). Expansion of the lamina propria by a mixed inflammatory infiltrate of mast cells, eosinophils, lymphocytes, plasma cells, and histiocytes was seen in 5 of 6 (83%) small intestinal biopsies, ranging from mild (*n* = 3), to moderate (*n* = 1), to marked (*n* = 2). In cases where the lamina propria expansion was mild, mast cells were often difficult to identify on H&E stain (Fig. 1B). Interestingly, 2 of 6 (33%) small intestinal biopsies also showed marked lymphangiectasia. In the colon and rectum, increased lamina propria eosinophils were seen in 4 of 9 (44%) biopsies. All colorectal biopsies showed expansion of the lamina propria (Fig. 1D), ranging from mild (*n* = 5), to moderate (*n* = 2), to marked (*n* = 2). Architectural distortion was also seen in some of the colonic biopsies, including irregular crypt spacing (3 of 9; 33%) and crypt foreshortening (5 of 9; 56%). However, histologic features of other inflammatory diseases of the GI tract, such as neutrophilic cryptitis or crypt abscess formation, intraepithelial lymphocytosis, granulomas, or subepithelial collagen table abnormalities, were not seen.

Mast cells were highlighted by immunostains for c-kit (strong membranous staining) (Fig. 1C) and mast cell tryptase (strong cytoplasmic staining) (Fig. 1E). Mast cell counts obtained with both stains were similar (within 5%). Therefore, our final analysis was performed using only the mast cell counts obtained from slides immunostained for c-kit.

The results are summarized in Table 2. In normal control biopsies, mast cells were present as scattered single cells in the lamina propria and muscularis mucosae.

The average mast cell density from all sites (stomach, duodenum, terminal ileum, and colon) was 23/HPF (range: 4 to 51). As expected,³¹ the greatest concentration of mast cells was seen in the terminal ileum (mean: 32/HPF; range: 21 to 40), although this was not significantly higher than mast cell densities in other sites. When compared with other inflammatory diseases of the GI tract, only cases of parasitic infection had significantly increased numbers of mast cells (mean: 47/HPF; range: 31 to 70) compared with normal colonic control biopsies (mean: 21/HPF; range: 10 to 31; *P* < 0.001). Mast cell density in other inflammatory diseases was not significantly higher than normal controls.

Biopsies from UP patients were similar to control cases, with only scattered single mast cells identified on immunostains for c-kit and mast cell tryptase (Fig. 2). In contrast, all small intestinal and colonic biopsies from SM patients (Fig. 1) contained aggregates and/or sheets of mast cells within the lamina propria. As summarized in Table 2, mast cell density in the small intestinal mucosa (mean: 175/HPF; range: 74 to 339) and colonic mucosa (mean: 209/HPF; range: 110 to 301) of SM patients was significantly higher than normal controls (*P* < 0.02 and *P* < 0.0001, respectively) and all other GI inflammatory diseases evaluated (ranging from *P* < 0.0001 to *P* < 0.02). In addition, GI biopsies from patients with UP (small intestine, mean: 22/HPF; range: 12 to 32; *P* < 0.02; colon, mean: 13/HPF; range 8 to 19; *P* < 0.0001) also showed significantly lower mast cell densities than biopsies from SM patients.

In one case, a diagnosis of SM was initially suggested because the biopsy contained increased mast cells, and the patient had a history of rash, hives, diarrhea, and abdominal pain. This patient's colonic biopsy contained markedly increased eosinophils, including eosinophilic microabscesses and degranulated forms. Mast cell tryptase stains highlighted the presence of scattered lamina propria mast cells, numbering up to 70/HPF. The patient was initially lost to follow-up. Eight years later, the patient again presented with similar clinical symptoms, and a repeat colonoscopy was performed. The subsequent colonic biopsy showed similar histologic findings with marked eosinophilic infiltration and increased mast cells (42/HPF). However, deeper sectioning through the block revealed inflammation surrounding a mucosal helminth, and a definitive diagnosis of colonic strongyloidiasis was rendered.

Mucosal mast cells in all 15 small intestinal and colonic biopsies from SM patients aberrantly coexpressed CD25 in a membranous pattern with moderate staining intensity (Fig. 1F). The CD25 expression on mast cells could be easily differentiated from CD25 expression on T cells, because the T cells contained scant cytoplasm and showed strong staining. In contrast, mucosal mast cells in all of the normal controls, biopsies from UP patients (Fig. 2C), and biopsies from other inflammatory diseases of the GI tract were negative for CD25. Interestingly, in the 2 gastric biopsies from SM patients, only 1 showed markedly increased mast cells compared with normal controls

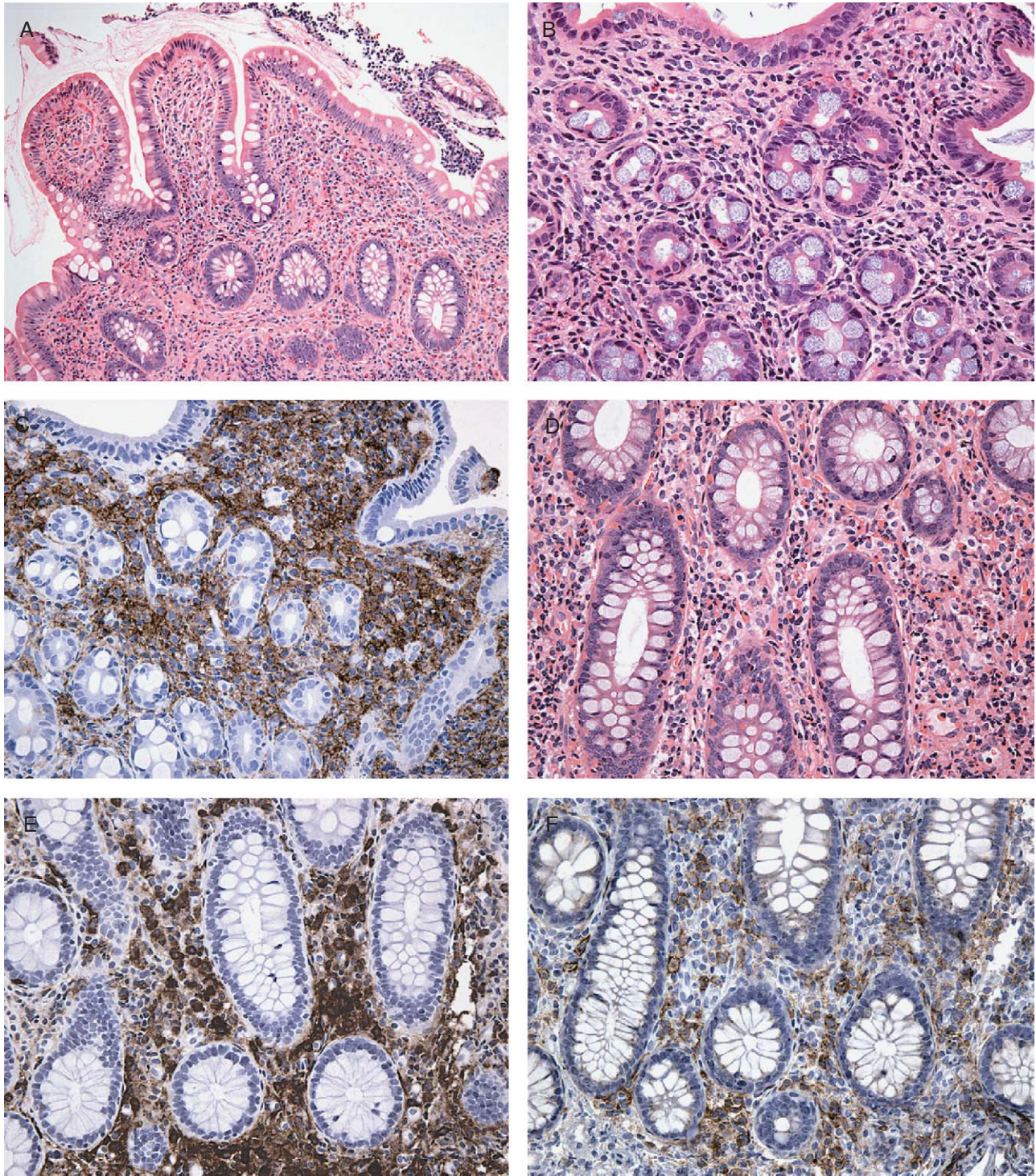


FIGURE 1. A, SM involving the small intestine with mild villous blunting and lamina propria expansion with increased eosinophils. B, Mast cell infiltrates are inconspicuous on H&E stain in this small intestinal biopsy, but are highlighted by an immunostain for c-kit (C). D, Mucosal mast cells are more prominent in this colonic biopsy and can be identified by their abundant pale cytoplasm, as well as on a mast cell tryptase immunostain (E). F, The neoplastic mast cells are positive for CD25.

(90/HPF), whereas the other showed a mild increase in the numbers of mast cells (24/HPF). Mast cells in the former biopsy aberrantly coexpressed CD25, confirming involve-

ment by SM. However, the scattered mast cells in the latter biopsy were negative for CD25, consistent with a reactive, rather than neoplastic, proliferation.

TABLE 2. Mast Cell Quantitation and CD25 Immunostaining Results

	Mast Cell Density (Mean/HPF; Range)	P (vs. SM)	CD25 Expression (Number Positive; %)
Stomach			
Normal (N = 10)	12 (5-21)	NS	0 (0%)
<i>H. pylori</i> gastritis (N = 10)	12 (2-30)	NS	0 (0%)
Bile reflux gastropathy (N = 10)	12 (7-23)	NS	0 (0%)
Eosinophilic gastritis (N = 4)	14 (9-17)	NS	0 (0%)
UP (N = 4)	14 (10-17)	NS	0 (0%)
SM (N = 2)	57 (24-90)		1 (50%)
Small intestine			
Normal duodenum (N = 10)	27 (4-51)	< 0.02	0 (0%)
Normal terminal ileum (N = 10)	32 (21-40)	< 0.02	0 (0%)
Peptic duodenitis (N = 10)	17 (6-28)	< 0.01	0 (0%)
Celiac disease (N = 10)	24 (7-43)	< 0.02	0 (0%)
IBS terminal ileum (N = 10)	25 (13-39)	< 0.02	0 (0%)
Eosinophilic enteritis (N = 3)	15 (6-22)	< 0.01	0 (0%)
UP (N = 7)	22 (12-32)	< 0.02	0 (0%)
SM (N = 6)	175 (74-339)		6 (100%)
Colon			
Normal colon (N = 10)	21 (10-31)	< 0.0001	0 (0%)
Ulcerative colitis (N = 10)	16 (10-26)	< 0.0001	0 (0%)
Crohn colitis (N = 10)	13 (3-41)	< 0.0001	0 (0%)
Lymphocytic colitis (N = 10)	18 (11-25)	< 0.0001	0 (0%)
Collagenous colitis (N = 10)	17 (6-29)	< 0.0001	0 (0%)
IBS (N = 10)	20 (12-31)	< 0.0001	0 (0%)
Parasitic infection (N = 8)*	47 (31-70)	< 0.0001	0 (0%)
Eosinophilic colitis (N = 2)	12 (12)	< 0.0001	0 (0%)
UP (N = 6)	13 (8-19)	< 0.0001	0 (0%)
SM (N = 9)	209 (110-301)		9 (100%)

*Parasitic infection vs. normal colon, $P < 0.001$.

NS indicates not significant.

DISCUSSION

GI symptoms are common in patients with SM. A review of the literature reveals an overall mean percentage of SM patients with GI complaints of 51% (ranging from 14% to 85%), making the frequency of GI symptoms almost as high as the frequency of pruritus (reviewed in Ref. 22). For example, in a prospective study of 15 patients with SM, Cherner et al¹⁰ found that 80% had GI symptoms, most commonly abdominal pain, nausea, vomiting, and diarrhea. The pathogenesis of these symptoms is unclear. It has been hypothesized that GI symptoms in SM patients may be secondary to direct infiltration of the GI tract by neoplastic mast cells, increased circulating mast cell mediators, or some combination of the two.²² Low fasting serum gastrin

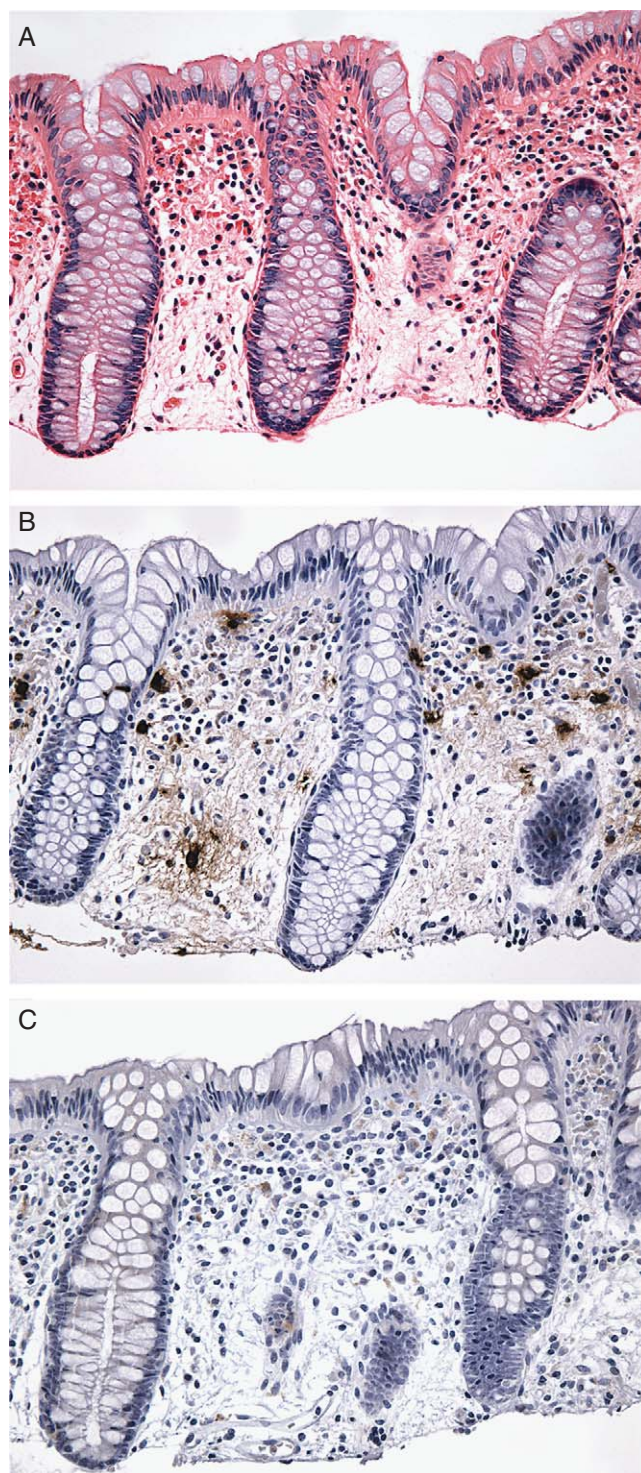


FIGURE 2. A, Colon biopsy from an UP patient with diarrhea. The scattered reactive mast cells identified on mast cell tryptase immunostain (B) are negative for CD25 (C).

levels in SM patients has provided some indirect evidence that increased histamine levels and consequent increased acid production may produce dyspeptic pain in some SM

patients.¹⁰ However, the histologic appearances of the GI mucosa in SM patients are incompletely described. Most of the existing literature consists of case reports^{1,6,8,9,11,14,16,21,30,37} with very few case series,^{2,12,24,33} and the results of these studies have been quite varied and in some cases, conflicting. Results of mucosal mast cell quantitation have similarly been mixed with some studies reporting increased,^{6,8,11,12,14,21,37} decreased,³³ and normal numbers of mucosal mast cells^{1,2,9,16,30} in patients with SM compared with controls.

In the current study, we examined endoscopic GI mucosal biopsies from 6 patients with SM. Clinical history was available for 5 of the 6 patients, all of whom had ongoing GI symptoms at the time of endoscopy. Histologically, approximately half of the biopsies from SM patients showed an eosinophilic infiltrate in the lamina propria. Other histologic findings included villous blunting, crypt architectural distortion, and lamina propria expansion. However, in some cases, only minimal histologic changes could be identified on H&E stains alone. In such cases, definitive identification of mast cells required immunostaining with either c-kit or mast cell tryptase. All of the small intestinal and colonic biopsies from SM patients showed aggregates and/or sheets of mast cells with mean mast cell densities of 175 ± 94 and $209 \pm 68/\text{HPF}$, respectively, which were significantly higher than normal controls ($P < 0.02$ and $P < 0.0001$).

Similar to our findings, previous reports have found the histologic appearances of the GI mucosa in SM patients to be quite variable. For instance, villous blunting^{6,8} and a mixed inflammatory infiltrate including eosinophils, neutrophils, and plasma cells were seen in some patients.^{2,6,8,24,27} However, normal villous architecture,³⁰ even in the presence of a dense mast cell infiltrate²¹ has also been observed.

Mast cell quantitation has also yielded variable results. Similar to our study, some groups have found sheets and aggregates of mast cells that number up to 100/HPF.^{6,8,21} Other studies have reported scattered but increased numbers of mast cells,^{11,12,14,27,37} normal numbers of mast cells,^{1,2,9,16,30} and decreased numbers of mast cells³³ compared with normal controls. Although our study cannot address the overall prevalence of increased mast cells in the GI tract in patients with SM, our results support the hypothesis that direct infiltration of the mucosa by mast cells may cause GI symptoms in at least some SM patients. It is possible that some of the differences in previously published mast cell quantitation could be due to different methods used to highlight mast cells. Giemsa and toluidine blue stains, which require the presence of mast cell granules, may underestimate the number of mast cells as compared with immunostaining for c-kit or mast cell tryptase.^{17,18} It is also likely that neoplastic mast cell infiltration may show an uneven distribution, thus, sampling may also be an issue. For example, Ferguson et al¹¹ found increased mast cell density in gastric and duodenal biopsies from SM patients, but a normal number of mast cells in the

rectum. Without a systematic endoscopic biopsy study of symptomatic patients with SM, this issue cannot be resolved.

Patients with limited skin involvement have cutaneous mastocytosis, most commonly UP. However, although infiltration of mast cells is limited to the skin, it is common for these patients to have GI symptoms. For instance, in a previous study of 30 patients with UP, 11 had GI complaints.³⁸ Studies of the GI tract in patients with UP have also yielded conflicting results, as histologic examination of mucosal biopsies in some studies has shown increased mast cell infiltrates (possibly resulting in reclassification of some of these patients as SM).^{21,27,37} In a recent study, Siegert et al³³ examined gastric and duodenal biopsies from 12 patients with UP and found that they had greater numbers of mucosal mast cells compared to 15 patients with SM. However, the concentration of mucosal mast cells in UP patients was not significantly different from normal controls. In our study, we quantitated mast cell density in GI mucosal biopsies from 5 UP patients, the majority of whom (4/5) had documented GI symptoms. We also found that GI mucosal mast cell concentrations in patients with UP were similar to normal controls.

Two of the SM patients in our study underwent upper endoscopy with gastric biopsy. Only 1 patient had gastric mucosal infiltration by neoplastic mast cells, with a dense mast cell infiltrate (90/HPF) that aberrantly coexpressed CD25. The other SM patient had a mild reactive (CD25-negative) mast cell proliferation. Interestingly, both of these patients showed small intestinal and colonic involvement by SM. Given our results and the results of the study by Siegert et al,³³ it is possible that infiltration of the stomach by neoplastic mast cells in SM may be less common than involvement of the small and large intestines. However, additional studies of larger series of SM patients will be required to further investigate this possibility.

There is increasing evidence that mast cells play an important role in inflammatory diseases of the GI tract (reviewed in reference Ref. 32). Increased numbers of mast cells have been reported in biopsies from patients with *H. pylori* gastritis,^{13,15} celiac disease,³⁵ ulcerative colitis, and Crohn disease.^{3,7,23} In addition, in a recent study, mastocytic enterocolitis has been proposed as a distinct disorder defined by chronic intractable diarrhea and increased GI mucosal mast cells.²⁰ Despite the possible importance of mast cells in the pathogenesis of many of these diseases,^{3,7,13,15,20,23,35} in our current study, we did not find a significant increase in mucosal mast cells in patients with most inflammatory disorders compared with normal controls. Because GI mucosal eosinophils are frequently increased in patients with SM, the absence of significantly increased mucosal mast cells in biopsies from patients with eosinophilic gastroenteritis is particularly notable, given this potentially problematic differential diagnosis.

Mast cells may be involved in the pathophysiology of IBS. However, whether the number of mucosal mast

cells in patients with IBS is increased compared with normal controls is controversial. Some studies have reported increased numbers of mast cells in the terminal ileum^{29,40} and/or colon,^{28,29} whereas others have reported normal numbers of mast cells in IBS patients.^{19,36} In fact, in most studies that reported increased mast cells in such patients, the increase did not clearly correlate with patient symptoms.^{29,40} In our current study, we evaluated mast cell density in 20 terminal ileal and colonic biopsies from 16 patients with IBS. We did not find a significant increase in mast cells compared with normal controls. Recent studies have suggested that the location of mast cells, rather than the absolute numbers, may be more important in the excitation of visceral sensory nerves by mast cell mediators in IBS.^{4,5}

In our study, we found that colonic biopsies from patients with parasitic infections showed significantly increased mucosal mast cells compared with normal controls, and this therefore may be a potential diagnostic pitfall in the diagnosis of SM, particularly given the similarities in clinical presentation. Of note, other than SM, these were the only cases with significantly increased mucosal mast cells. As an example of this diagnostic problem, we identified a case previously diagnosed as suspicious for involvement by SM that was years later found instead to be colonic strongyloidiasis. Similar to some of the SM cases, this biopsy showed a marked increase in both lamina propria eosinophils and mast cells (up to 70/HPF). However, upon extensive sectioning of the subsequent colonic biopsy, strongyloides was identified. In retrospect, parasitic infection could have been favored over SM, because the numbers of mast cells were lower than those seen in the biopsies from SM patients, and the mast cells were present singly and never in aggregates or sheets.

Finally, recent studies have shown that immunohistochemical detection of CD25 on mast cells is a reliable marker of neoplastic mast cell infiltrates in bone marrow biopsies.^{26,34} However, although CD25 expression by neoplastic mast cells in extracutaneous organs is a minor diagnostic criterion for SM,³⁹ there is very little literature on the use of CD25 as an immunohistochemical marker for neoplastic mast cells outside of the bone marrow. A recent study using flow cytometry on freshly isolated GI mast cells suggested that normal GI mast cells do not express CD25.²⁵ To our knowledge, only 2 previous studies have reported the results of CD25 immunohistochemistry in GI biopsies from patients with SM. In their study of 15 patients with SM, Siegert et al³³ did not find aberrant expression of CD25 by GI mucosal mast cells and concluded that they were reactive in nature. Unfortunately, only 3 biopsies from their patient cohort were stained with CD25; thus, it is difficult to reach definitive conclusions on the basis of these results. In contrast, a recent case report of a SM patient with GI tract involvement showed aberrant CD25 expression by the dense mast cell infiltrates in the colonic and small intestinal mucosa.⁶ However, our current study is the first to systematically evaluate

CD25 expression on GI mucosal mast cells. Mast cells in normal control biopsies, biopsies from UP patients, and biopsies of other inflammatory diseases were all negative for CD25. In contrast, we found that the mucosal mast cell infiltrates in all patients with SM aberrantly expressed CD25. Of note, in one of our study patients, GI biopsies were performed 1 year before the diagnosis of SM, and were initially thought to represent eosinophilic gastroenteritis. Retrospective analysis revealed the presence of dense mast cell infiltrates that were positive for CD25, confirming involvement by SM.

In summary, quantitation of mast cells can be helpful to diagnose SM in GI mucosal biopsies, although mast cells are also markedly increased in parasitic infections. Aggregates or sheets of mast cells are only seen in SM. Immunoreactivity for CD25 in GI mucosal mast cells is specific for SM and can be used to confirm the diagnosis in the appropriate clinical context.

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