

# Immunohistochemical study of chymase-positive mast cells in inflammatory bowel disease

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**Abstract.** Mast cell-derived chymase promotes inflammatory responses and tissue fibrosis. Although previous studies have reported changes in the number of mucosal mast cells in inflammatory bowel disease (IBD), the behaviour of chymase immunopositive mast cells has not been studied. In this study, we immunohistochemically investigated chymase immunopositive mast cells in the inflamed mucosa of IBD patients. Surgically-obtained or biopsy specimens from 10 patients with ulcerative colitis (UC), 10 patients with Crohn's disease (CD) and 10 normal colorectal tissue specimens were used. The chymase immunopositive cells were identified by immunohistochemical analysis using a monoclonal anti-human chymase antibody. In the normal colonic mucosa, a small number of chymase immunopositive mast cells were detected at the basal sites of the mucosa. There were no immunopositive cells in the submucosa. Chymase immunopositive mast cells were similarly observed in the inactive UC mucosa, but these cells decreased significantly in number in the active UC mucosa. In the inactive CD mucosa, the number of chymase immunopositive mast cells increased significantly ( $P<0.05$ ), and this was more clearly observed in the active CD mucosa. Furthermore, in the active CD mucosa, these cells were detected in the submucosa, propria muscularis, and surrounding fatty tissue. These observations suggest a crucial role for chymase immunopositive mast cells in the pathophysiology of CD. Since intestinal fibrotic changes such as stricture formation are a characteristic feature of CD, chymase immunopositive mast cells may act as a stimulus for the process of tissue fibrosis and tissue remodelling in the pathophysiology of CD.

## Introduction

Mast cells are recognized as a key cell type involved in hypersensitivity, and are also involved in a number of non-allergic

diseases (1). Upon activation, mast cells release their mediators to fulfil their biological functions. The neutral proteinases released by human mast cells include tryptase, chymase and carboxypeptidase, which has antigenic and enzymatic properties, similar to those of neutrophil cathepsin G (2,3). Mast cell tryptase, chymase and carboxypeptidase are reliable markers of mast cell degradation. Based on their content of proteinases, mast cells can be classified into two types; MC<sub>T</sub> cells which are defined as those containing tryptase but not chymase, and MC<sub>TC</sub> cells which are those containing both tryptase and chymase (4).

In recent years, considerable investigation has focused on the interactions between mast cells and fibroblasts. These studies have suggested that, in the presence of fibroblasts, secretion of chymase by mast cells promotes the differentiation and growth of interstitial connective tissue (5,8). Chymase generates angiotensin II by the conversion of angiotensin I (5), and in turn angiotensin II promotes the proliferation of fibroblasts and secretion of extracellular matrix (5). Thus, chymase has been reported to be an important factor involved in the pathogenesis of tissue fibrosis.

An increased number of mast cells was observed in the mucosa of the ileum and colon in patients with inflammatory bowel disease (IBD) (1,9-13). Tissue fibrosis is sometimes observed in IBD, especially in Crohn's disease (CD). Intestinal strictures, formed by collagen deposition and an increase in the number of fibroblasts, are commonly observed in CD patients (1,10). In the present study, to evaluate the role of chymase-immunopositive mast cells in the pathophysiology of IBD, we immunohistochemically investigated chymase immunopositive mast cells in the inflamed mucosa of IBD patients.

## Materials and methods

**Tissue samples.** The diagnoses of UC and CD were based on conventional clinical, endoscopic and histopathologic criteria. Surgically-obtained or biopsy specimens from 10 patients with UC (5 males and 5 females; mean age, 32 years) and 10 patients with CD (6 males and 4 females; mean age, 28 years) were used with informed consent. All patients were clinically and endoscopically active as defined by the colitis activity index for UC (14) and the Crohn's disease activity index (15). All patients were treated with salicylates, and 5/10 UC and 4/10

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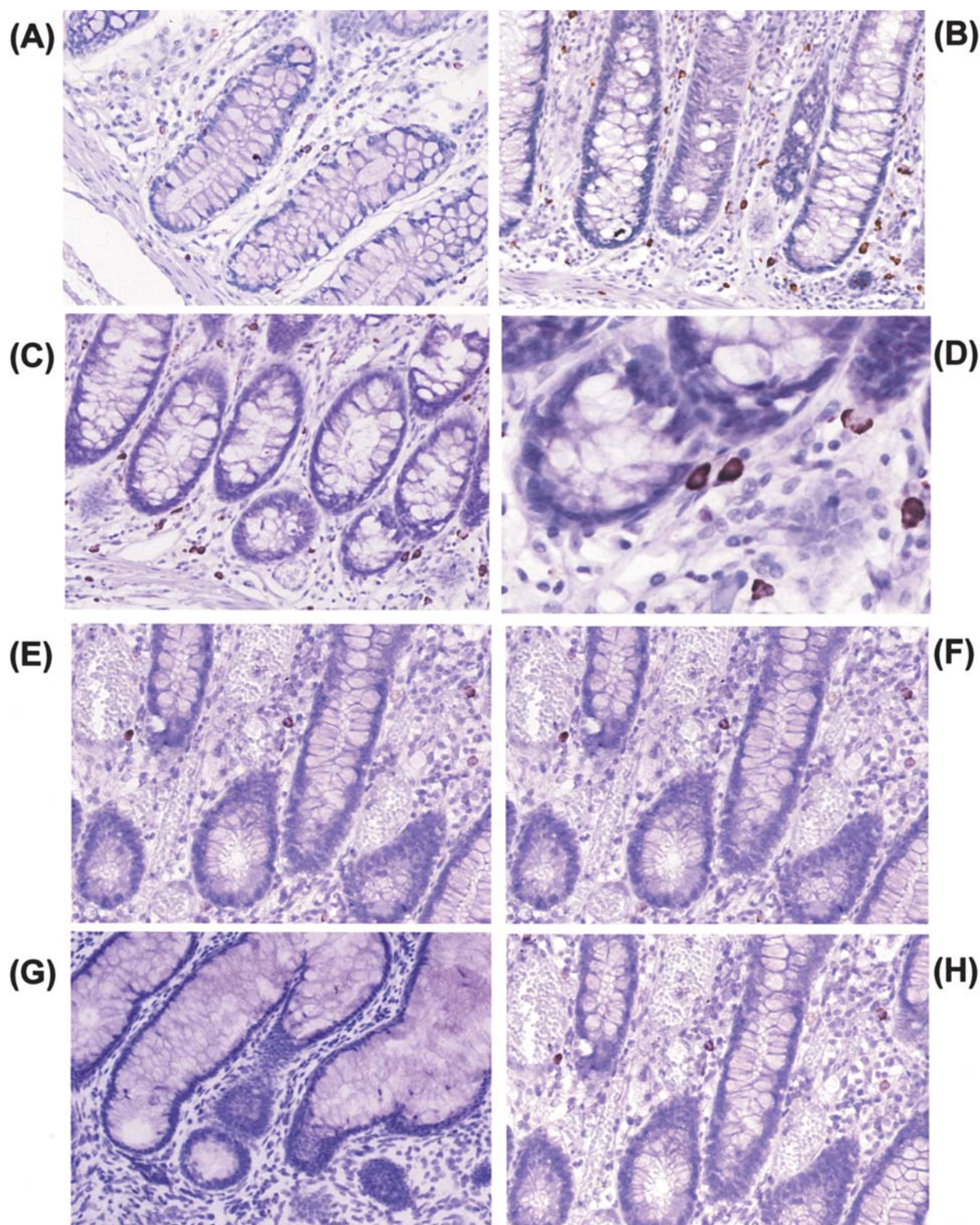


Figure 1. Immunostaining for chymase immunoreactive mast cells. Normal colonic mucosa (A to D), colonic mucosa of patients with inactive ulcerative colitis (UC) (E and F, and colonic mucosa of active UC patients (G and H). Magnification, x100; except D, which is x400.

CD patients received additional treatment with corticosteroids. Two UC patients were treated with azathioprine. Normal colorectal tissue was obtained by the surgical resection of colon cancer at sites distant from the tumors (n=10).

**Immunohistochemistry.** Immunohistochemical analyses were performed according to the method described in our previous

report. A monoclonal anti-human chymase antibody was used as the primary antibody (5). After incubation with the primary antibody, the sections were treated with a biotin-conjugated goat anti-rabbit IgG (Vector, Burlingame, CA) and avidin-biotin-peroxidase complexes (ABC, Vector).

According to the method described by Middle *et al* (16), an evaluation of chymase-like immunoreactivity was performed



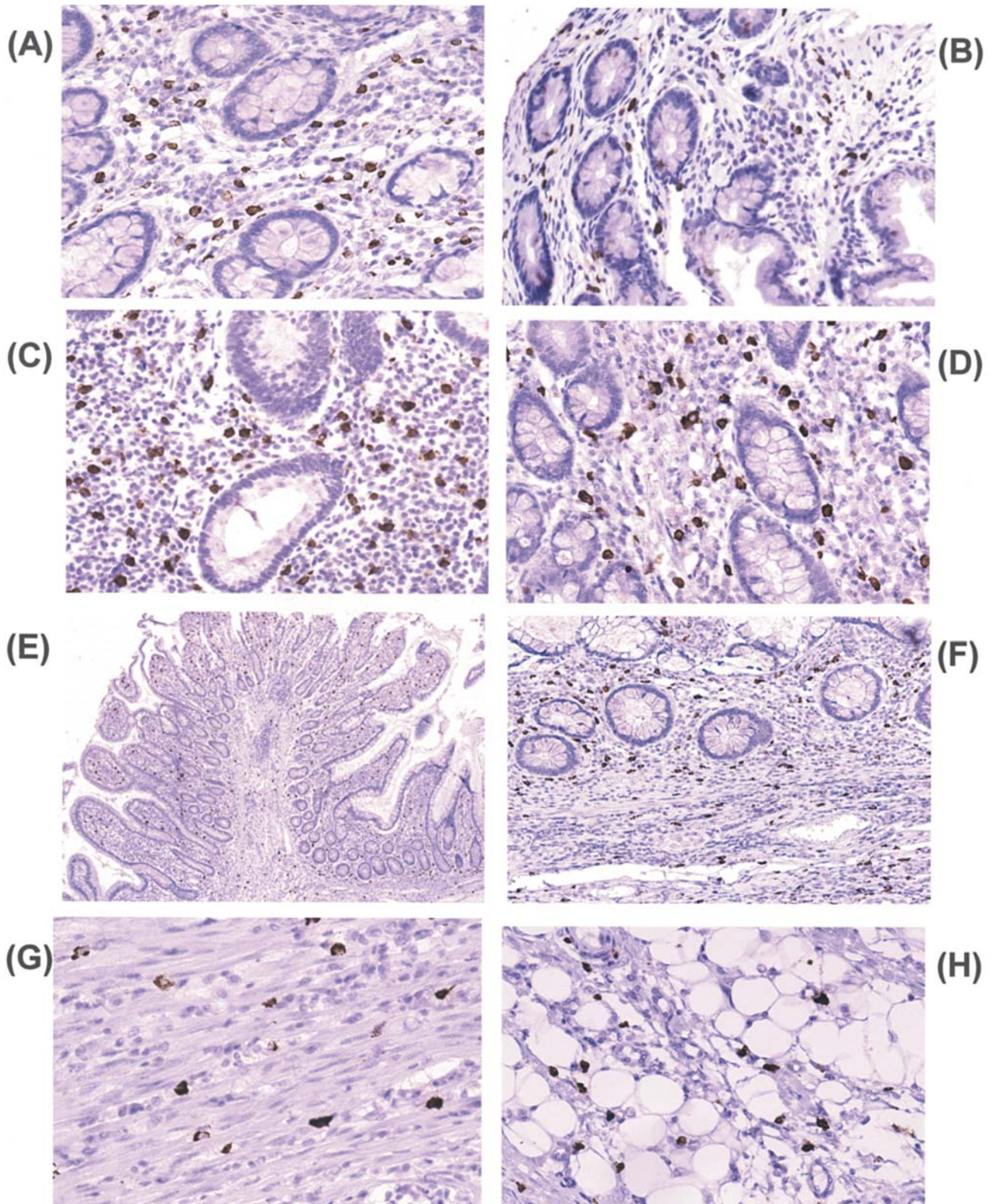


Figure 2. Immunostaining for chymase immunoreactive mast cells. Colonic mucosa of patients with inactive Crohn's disease (CD) (A and B), colonic mucosa of patients with active CD (C and D), ileum of active CD patients (E), colonic mucosa of active CD patient (F), propria muscularis of active CD patient (G), surrounding fatty tissue of active CD patients, (H). Magnification of x100 (A to D), x20 (E), x40 (F), and x200 (G and H).

on sections by two blinded evaluators. The corresponding areas of the sections were then marked, and high power fields were counted at a magnification of x400. The mean count from a total of five high power fields per slide was used.

**Statistical analysis.** The statistical significance of the differences was determined by the Mann-Whitney U-test (Statview version 4.5). Differences resulting in P-values less than 0.05 were considered to be statistically significant.



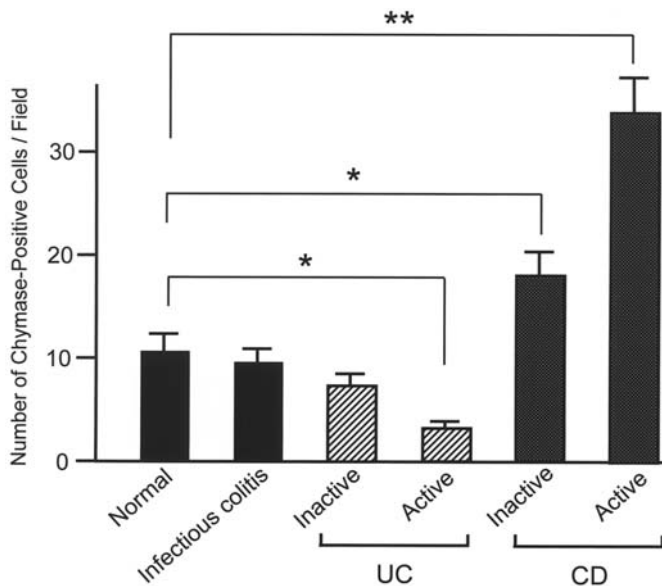


Figure 3. Number of chymase immunoreactive cells per field (magnification of  $\times 400$ ). Each data point represents the mean  $\pm$  SD ( $n=5$ ). \* $P < 0.05$ , \*\* $P < 0.01$ .

## Results

A small number of chymase immunopositive mast cells were scattered in the normal colonic mucosa (Fig. 1A-D). The majority of these cells were located at the basal sites of the mucosa. There were no immunopositive cells in the sub-mucosa. Similar staining patterns were observed in the mucosa of inactive UC patients (Fig. 1E and F). In contrast, the number of chymase immunopositive cells was decreased in the inactive UC mucosa as compared to the normal mucosa. This tendency was more clearly observed in the active UC mucosa. In the active UC mucosa, the number of chymase immunopositive cells was markedly decreased (Fig. 1G and H).

In the colonic mucosa of inactive CD patients, the number of chymase immunopositive mast cells was increased as compared to the normal mucosa (Fig. 2A and B). In the colonic mucosa of active CD patients, a marked increase in the number of chymase immunopositive mast cells was detected (Fig. 2C and D). A similar distribution of these cells was observed in the ileal mucosa of active CD patients (Fig. 2E). In the colonic mucosa of active CD patients, chymase immunopositive cells were detected in the submucosa (Fig. 2F), propria muscularis (Fig. 2G), and surrounding fatty tissue (Fig. 2H).

Fig. 3 shows that the number of chymase immunopositive mast cells was significantly decreased in the active UC mucosa and significantly increased in the CD mucosa. This tendency was also more evident in the active CD mucosa.

## Discussion

Mast cells are present in the connective tissue of nearly all organs. The major inflammatory and profibrogenic mediators of mast cells are stored in their cytoplasmic granules, and degranulation occurs in response to various stimuli. Chymase is a serine proteinase exclusively located in the granules of mast cells, and is released from the granules together with other preformed mediators. A large quantity of the active

form of chymase (10 pg per cell) in mast cells suggests that this mediator may play a role in mast cell related diseases (1). Indeed, chymase induces microvascular leakage in the skin, stimulates inflammatory cell accumulation, and modulates epithelial permeability (17-19). Furthermore, recent studies have shown a role for mast cell-derived chymase in the modulation of fibroblast functions. The released chymase converts angiotensin I to angiotensin II, and angiotensin II stimulates the proliferation, hypertrophy, and migration of smooth muscle cells and fibroblasts (5,8). Extracellularly, chymase is stabilized by binding to extra-cellular matrix constituents, and this extends the enzyme activity over a longer time (5). Chymase also initiates the degranulation of the surrounding mast cells by binding to them (5).

There are several reports concerning the increased number of mast cells in the IBD mucosa. Dvorak *et al* revealed that the number of mast cells was markedly increased in the inflamed mucosa of CD patients (9), and Nolte *et al* reported that the mucosal number of mast cells was significantly elevated in UC patients as compared to both CD patients and normal controls (13). In contrast, King *et al* demonstrated that the number of mast cells was significantly decreased in the active mucosa of UC patients as compared to the normal mucosa (11). Gelbmann *et al* reported that dramatically increased numbers of mast cells were observed in the hypertrophied and fibrotic muscularis propria of strictures in CD patients (10). However, there are no data regarding the number of chymase immunopositive mast cells in IBD.

To our knowledge, this is the first study on the distribution of chymase immunopositive mast cells in the intestinal mucosa of IBD patients. In the normal colonic mucosa, a small number of chymase immunopositive mast cells was observed in the lamina propria. These cells were juxtaposed to the muscularis mucosae, but no immuno-positive cells were detected in the submucosa. In the UC mucosa, a relative decrease in these cells was observed. This observation is compatible with a previous report of decrease in mast cell number in the UC mucosa (11). The precise reason for this phenomenon is unclear, but one possible explanation is a consequence of mast cell degranulation after activation. The most interesting observation in this study is that the number of chymase immunopositive mast cells was markedly increased in the CD mucosa. Although the number of mast cells has been reported to be increased in the CD mucosa (9,10), the changes in the number of chymase immunopositive cells have not been studied. In the inflamed mucosa of CD patients, chymase immunopositive cells are located not only in the lamina propria, but also in the submucosa. In particular, in the stricture lesions of CD patients, the location of chymase immunopositive cells was expanded into the propria muscularis. Furthermore, chymase immunopositive cells were detected in the fatty tissue surrounding the stricture lesion. Thus, these observations suggest a crucial role for chymase immunopositive mast cells in the pathophysiology of CD. Since stricture formation of the mucosa is closely related to the proliferation of fibroblasts and extracellular matrix deposition, chymase immunopositive mast cells may act as a stimulus of tissue remodeling through the chymase-angiotensin cascade.

In conclusion, we demonstrated that the number of chymase immunopositive mast cells was markedly increased in the active CD mucosa. Since released chymase stimulates tissue fibrosis, these increased chymase levels may play a crucial role in the pathological remodelling process of CD.

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