

## Activated Mast Cells Infiltrate in Close Proximity to Enteric Nerves in Diarrhea-predominant Irritable Bowel Syndrome

Mast cells (MC) may be one factor influencing the response of visceral afferent nerves to mechanical and chemical stimuli. The aim of this study was to evaluate the degree of infiltration and activity of colonic MC in irritable bowel syndrome (IBS). Biopsy specimens were obtained from the cecum and rectum of 14 diarrhea predominant IBS and 14 normal controls. Electron microscopy was used to determine the number of intact and degranulated colonic MC and to quantify these separately according to the distance between MC and enteric nerves. An increased number of MC in both cecum and rectum in the IBS group in comparison with the control group was demonstrated ( $p < 0.05$ ). Activated MC in close proximity to enteric nerves were significantly increased in both cecum and rectum of the IBS group compared to control group ( $p < 0.005$ ). In addition, activated MC were significantly increased in close proximity to the nerves compared to those in the remote area in both cecum and rectum of the IBS group ( $p < 0.0001$ ). MC were significantly increased and activated in both cecum and rectum of the IBS group compared to controls. MC may play a role in the gut sensory hypersensitivity of IBS.

**Key Words :** Adult; Functional Colonic Diseases; Colonoscopy; Intestinal Mucosa; Mast Cells

Chang Hwan Park, Young Eun Joo,  
Sung Kyu Choi, Jong Sun Rew,  
Sei Jong Kim, Min Cheul Lee\*

Department of Internal Medicine and Pathology\*,  
Chonnam National University Medical School,  
Gwangju, Korea

Received : 27 September 2002

Accepted : 3 December 2002

### Address for correspondence

Jong Sun Rew, M.D.

Division of Gastroenterology, Department of Internal  
Medicine, Chonnam National University Medical  
School, 8 Hak-dong, Dong-gu, Gwangju 501-757,  
Korea

Tel : +82.62-220-6296, Fax : +82.62-228-1330

E-mail : p1052ccy@hanmail.net

## INTRODUCTION

Irritable bowel syndrome (IBS), the most common disorder referred to gastroenterology clinics, is still one of the enigmas of the modern medicine, despite much research thereon it for nearly a century. From worldwide work over the last decades, it is now generally accepted that gut hypersensitivity to mechanical and chemical injury is most important in the pathophysiology of the disease entity (1). Due to its similarity with the airway sensitivity in asthma, some investigators regard IBS as 'the asthma of the gut' (2, 3). However, its underlying mechanisms and the exact causes are still poorly understood.

Mast cells (MC) may be one factor influencing the response of visceral afferents to mechanical and chemical stimuli, since MC are found in close proximity to gastrointestinal mucosal sensory nerve fibers containing neuropeptides and a bidirectional pathway among the central nervous system, gut, and MC has been demonstrated (4-7). The proximity of the MC to the enteric nerves offers an important pathophysiologic role for inducing changes in nerve function and the development of gut hypersensitivity. Therefore, the mast cell may be an important mediator in a complex interaction between pathological events (gut insult, inflammation, tissue or nerve injury) and psychological factors (stressful events, emotions).

In previous reports, an increased number of MC has been identified in the lamina propria of terminal ileum and cecum

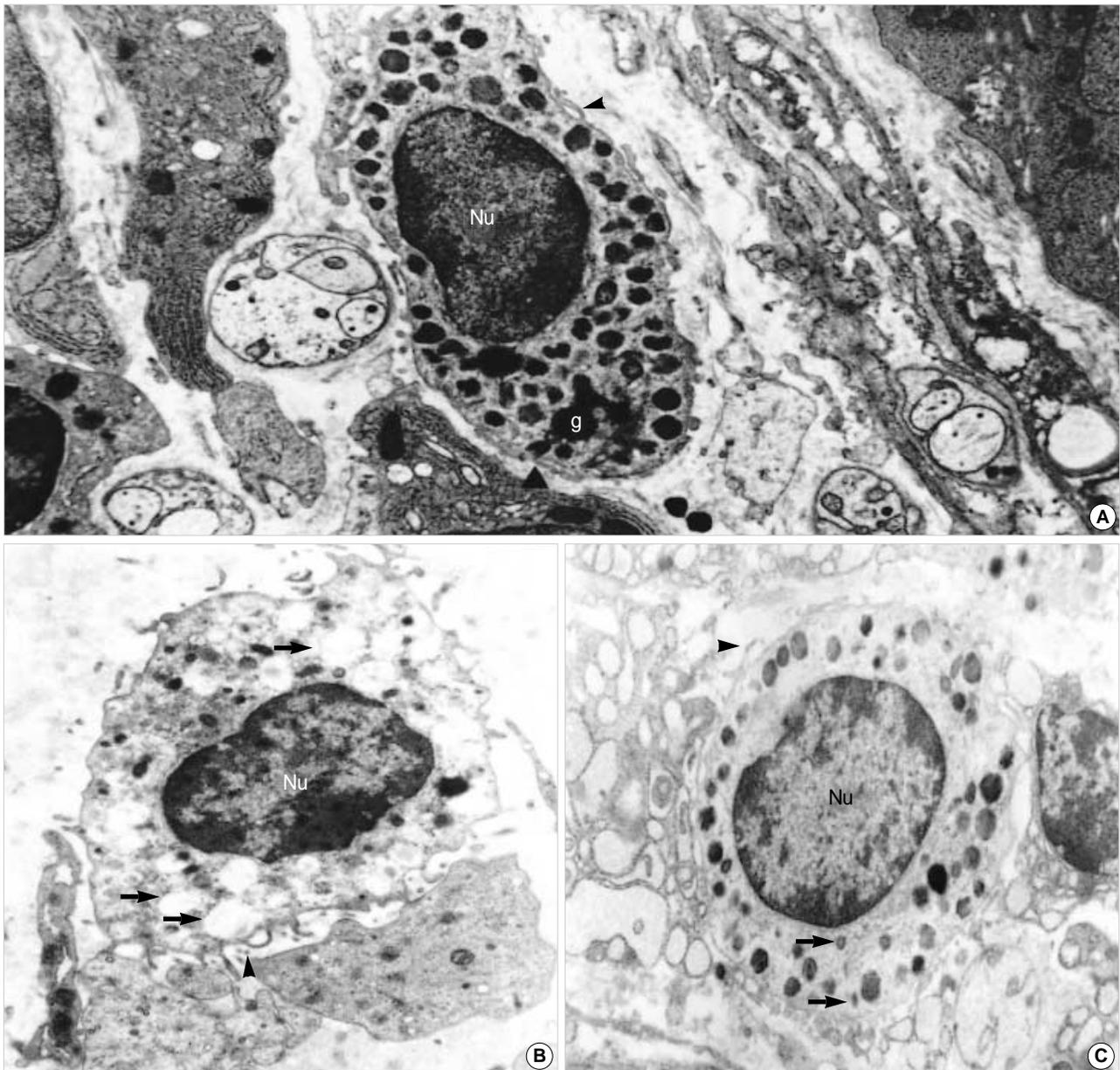
of the IBS patients (8, 9). However, other investigators found no increased MC infiltration in IBS (10, 11). It is possible that degranulated MC would not have been identified in these studies, because MC are not often recognizable by light microscopy. Therefore, the specific aim of this study was to evaluate the degree of infiltration and activity of MC according to the distance between MC and enteric nerves in the cecum and rectum of patients with IBS by electron microscopy.

## MATERIALS AND METHODS

### Patients

Twenty-eight adult patients undergoing colonoscopy were recruited. They were placed in two groups. In diarrhea-predominant IBS group, 14 patients fulfilled the Rome II criteria (12) with macroscopically and histologically normal colonic mucosa and no evidence of organic bowel disease. The Rome II criteria requires at least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of three features: 1) relieved with defecation and/or 2) onset associated with a change in frequency of stool and/or 3) onset associated with a change in form (appearance of stool).

In the normal control group, 14 individuals were included with macroscopically and histologically normal colonic mucosa



**Fig. 1.** Electron micrographs represent the different stages of mast cell (MC) cycle ( $\times 5,300$ ). All MC are characterized by a single nucleus with partially condensed chromatin (Nu) and by the numerous filopodia (arrowheads in all figures). (A) Resting MC filled with electron-dense unaltered secretory granules (g) in cytoplasm. (B) MC undergoing piecemeal degranulation evidenced by partially and completely empty granule chambers (arrows) in cytoplasm. (C) MC undergoing recovery from piecemeal degranulation evidenced by one or two layers of granules and focal densities (arrows) in granule chambers. (*Fig. 1. continued in next page*)

and neither persistent bowel symptoms neither organic nor functional bowel diseases. All of them were healthy volunteers. For all groups, patients with the following criteria by O'Sullivan et al. (9) were excluded: 1) history of atopy, food allergy, or asthma based on detailed medical history, 2) use of mast cell stabilizers or steroids in the month before the study, 3) active diverticulitis (incidental hemorrhoids or diverticula were not considered as exclusion criteria), and 4) patients presenting with gastrointestinal infection. All subjects gave their writ-

ten informed consent. The Human Ethics Review Committee of Chonnam University Hospital approved the study.

#### Colonoscopic biopsies and histologic evaluation

After overnight fasting and preparation with a polyethylene glycol, a colonoscopy (Olympus CF-230L) was performed, and more than 2 biopsy specimens were obtained from the cecum and rectum in both groups. The tissue specimens were

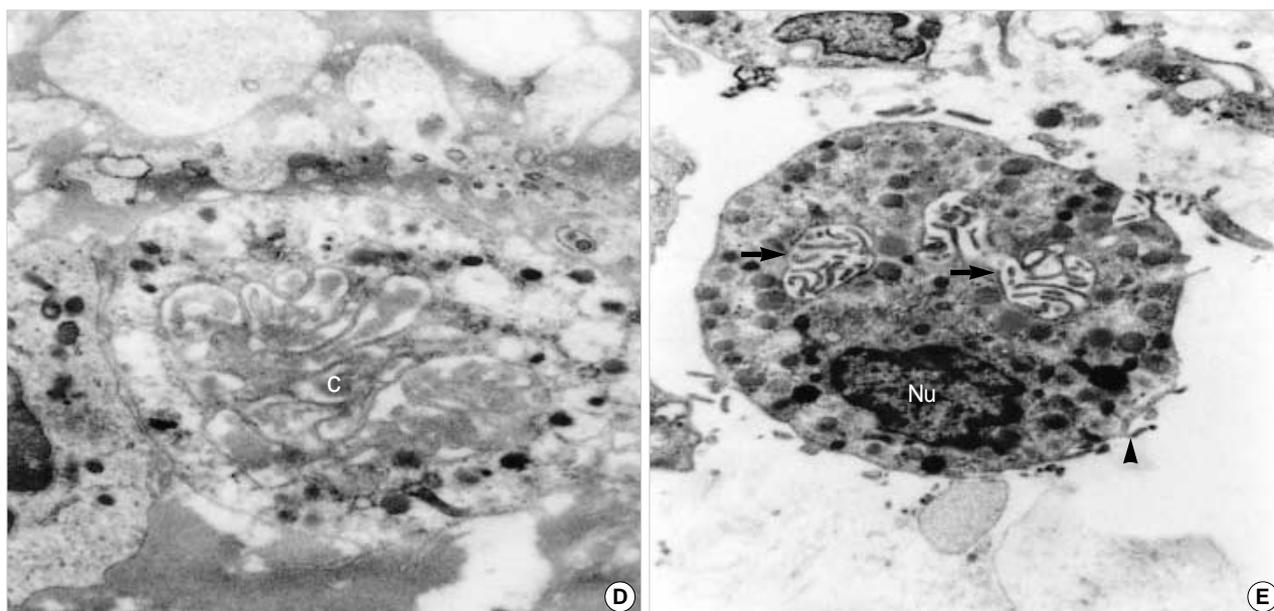


Fig. 1. (Continued from the previous page) Electron micrographs representing the different stages of mast cell (MC) cycle ( $\times 5,300$ ). (D) MC undergoing anaphylactic degranulation evidenced by channels (c) formed by the fusion of granules. (E) MC undergoing recovery from anaphylactic degranulation evidenced by canaliculi with internalized surface processes (arrows).

immediately fixed in 2.5% glutaraldehyde, post fixed in osmium tetroxide, and embedded in epon mixture. Thick sections, 1  $\mu\text{m}$  in thickness, were prepared, stained with toluidine blue, and used for light microscopic observation of MC and enteric nerves in mucosa. Ultrathin sections, 80 nm in thickness, were made by a LKB-V ultramicrotome with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a JEM 100CX type II electron microscope.

MC were identified by their ultrastructural morphology, and then quantitated on the ultrathin sections using a graticule  $150 \times 150 \mu\text{m}$  at  $\times 5,300$  magnification. All MC falling within the graticule and those touching the edges were counted in each field. A maximum of 20 consecutive such areas were counted. Intraepithelial mast cells, when found, were included in the total counts. MC had a single nucleus with partially condensed chromatin, electron-dense granules in cytoplasm, and narrow regular surface folds (Fig. 1A). The location, shape, granule size, presence of lipid bodies, and evidence of secretion and recovery were noted for each mast cell.

MC were divided into three types according to their ultrastructural characteristics; degranulation, rest, and recovery types (13, 14). Piecemeal type degranulation (PMD) was defined as the presence of partially or completely empty granule chambers in the absence of intergranular fusion (Fig. 1B). Recovery from piecemeal degranulation was recognized by the accumulation of dense material of varying morphology within the granules (Fig. 1C). Anaphylactic type degranulation (AND) was defined as secretion via degranulation channels or by the direct fusion of granule containers with the cell membrane (Fig. 1D). Recovery from anaphylactic

degranulation was recognized by the presence of marked exteriorization of fused granule chambers as surface processes or by the internalization of these processes as canaliculi (Fig. 1E) (14). Activated MC were scored as being in one of three categories including AND, PMD, or both (15).

Distance between MC and enteric nerve fibers was calculated with the index scale on the view box of electron microscope and then MC were classified into 3 groups (contact with nerve,  $\leq 2 \mu\text{m}$ , and  $\geq 2 \mu\text{m}$ ) according to the distance between MC and enteric nerve fibers.

### Statistical analysis

The Student *t*-test was used to compare MC counts between IBS and normal controls. The paired *t*-test was used to compare the activation rates of MC in neighboring area of enteric nerve with those in the remote area. The Pearson's chi-square test was used to compare non-ratio scale parameters between IBS and normal control. The statistical software program used was Statistical Package for the Social Sciences (SPSS/PC+10.0, Chicago, IL). All *p*-values calculated were two tailed: the  $\alpha$  level of significance was set at 0.05.

## RESULTS

### Demographic and clinical data

Age, gender, relevant medical history, and current medications were documented. Each group comprised 6 males and

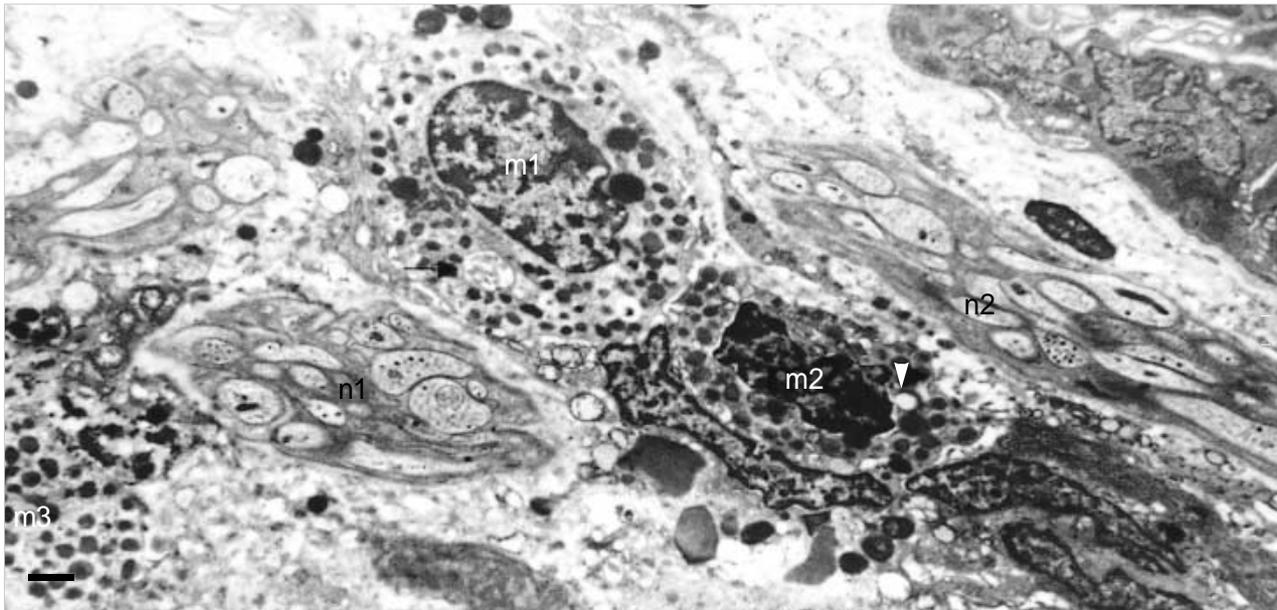


Fig. 2. The number of cecal mast cells (mc) in a patient with irritable bowel syndrome showing proximity to nerves in lamina propria ( $\times 2,800$ ). Bar= $1 \mu\text{m}$ . Upper one (m1) within  $1 \mu\text{m}$  to left enteric nerve (n1) shows recovery state from anaphylactic degranulation manifested by internalized surface processes (arrow). Middle one (m2) near contact with right enteric nerve (n2) shows piecemeal degranulation manifested by empty granule chambers (arrowhead). Low one (m3) relatively far away from neighboring nerves ( $>2 \mu\text{m}$ ) shows resting state manifested by electron dense granules.

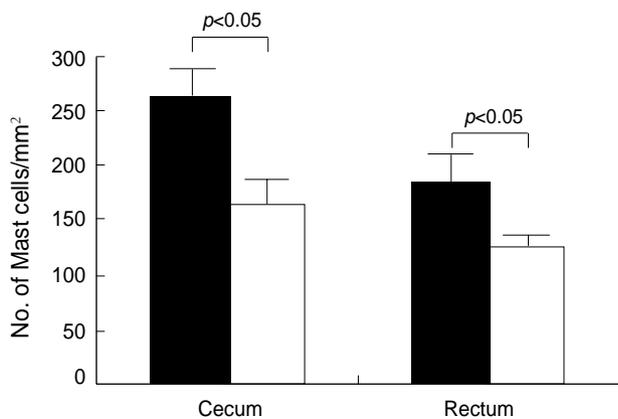


Fig. 3. Mean numbers of mast cells (MC) per square millimeter of lamina propria in colonic mucosa. The number was significantly higher in both cecum and rectum of patients with diarrhea-predominant IBS (black box) than in normal controls (blank box).

8 females. The mean age in normal controls was  $48.8 \pm 3.9$  yr. The mean age in diarrhea-predominant IBS was  $47.6 \pm 3.8$  yr. No significant differences in the ages and sex ratio were present between diarrhea-predominant IBS and normal control groups.

#### Mast cell identification and quantification

MC were predominantly round or oval shape, but spindle-shaped MC were also seen in both groups. Piecemeal-type

degranulation and recovery were the common morphologic changes. While, in control group, no or one cecal mucosal mast cell was detected in close proximity to enteric nerves in high-power field ( $\times 5,300$ ), cecal mast cells showing degranulation were frequently gathered in close proximity to the nerves in lamina propria of patients with diarrhea-predominant IBS (Fig. 2). The number of MC (as represented by the mean number of MC per square millimeter of lamina propria) was significantly higher in the cecal mucosa of patients with diarrhea-predominant IBS ( $262.7 \pm 35.5/\text{mm}^2$ ) than in normal controls ( $165.1 \pm 25.3/\text{mm}^2$ , Fig. 3,  $p < 0.05$ ). The number of MC was also significantly higher in the rectal mucosa of patients with diarrhea-predominant IBS ( $184.1 \pm 27.0/\text{mm}^2$ ) than in normal controls ( $124.6 \pm 10.7/\text{mm}^2$ , Fig. 3,  $p < 0.05$ ).

#### Activity of MC according to the distance between MC and enteric nerves

The activity of MC in close proximity to enteric nerves ( $\leq 2 \mu\text{m}$  in distance) was significantly higher in the cecum of diarrhea-predominant IBS group ( $180.9 \pm 23.7/\text{mm}^2$ ) than in normal controls ( $100.2 \pm 15.2/\text{mm}^2$ , Fig. 4,  $p = 0.0083$ ). In addition, the activity of MC in close proximity to the nerves was also significantly higher in the rectum of diarrhea-predominant IBS group ( $127.9 \pm 25.7/\text{mm}^2$ ) than in normal controls ( $70.1 \pm 7.0/\text{mm}^2$ , Fig. 4,  $p = 0.0343$ ). Activated MC in close proximity to enteric nerves ( $\leq 2 \mu\text{m}$  in distance) were significantly more frequent in the cecum of diarrhea-predominant

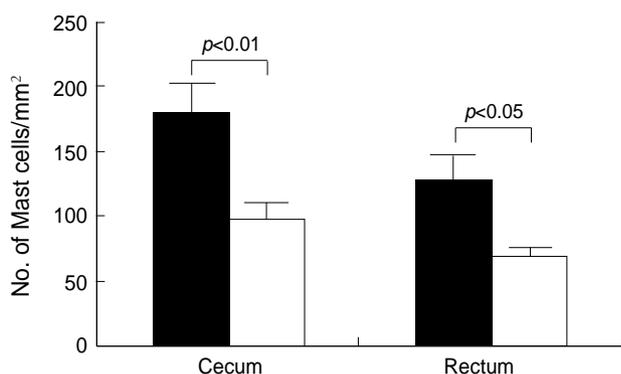


Fig. 4. Mean numbers of mast cells (MC) per square millimeter of lamina propria in colonic mucosa according to the distance between MC and enteric nerves. The number of MC in close proximity to enteric nerves ( $\leq 2 \mu\text{m}$ ) was significantly higher in both cecum and rectum of patients with diarrhea-predominant IBS (black box) than in normal controls (blank box).

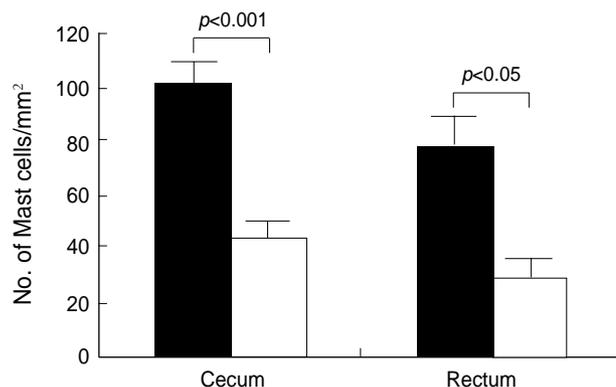


Fig. 5. Mean numbers of activated mast cells (MC) per square millimeter of lamina propria in colonic mucosa according to the distance between MC and enteric nerves. Activated MC in close proximity to enteric nerves ( $\leq 2 \mu\text{m}$ ) were significantly more frequent in both cecum and rectum of patients with diarrhea-predominant IBS (black box) than in normal controls (blank box).

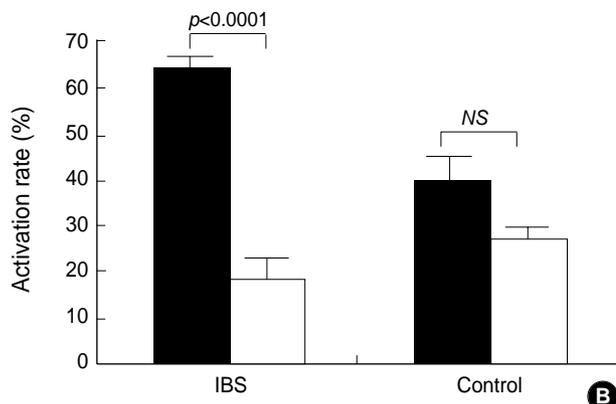
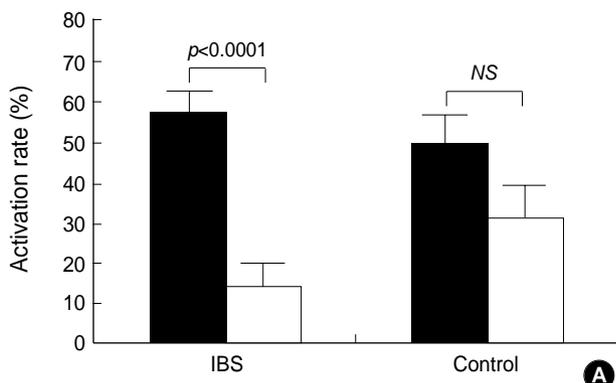


Fig. 6. Activation rates of mast cells (MC) according to the distance between MC and enteric nerves show a significant regional difference in patients with diarrhea-predominant irritable bowel syndrome (IBS). The activation rate was calculated by the percent of activated MC (showing anaphylactic degranulation, piecemeal degranulation, or both). (A) The activation rate of MC in close proximity to nerves (black box,  $\leq 2 \mu\text{m}$  in distance) is significantly higher than that of MC away from nerves (blank box,  $>2 \mu\text{m}$  in distance) in the cecum of patients with diarrhea-predominant IBS. (B) The activation rate of MC in close proximity to nerves (black box,  $\leq 2 \mu\text{m}$  in distance) is significantly higher than that of MC away from nerves (blank box,  $>2 \mu\text{m}$  in distance) in the rectum of patients with diarrhea-predominant IBS.

IBS group ( $101.0 \pm 10.7/\text{mm}^2$ ) than in normal controls ( $44.6 \pm 6.6/\text{mm}^2$ , Fig. 5,  $p=0.0001$ ). Also, activated MC in close proximity to the nerves were significantly more frequent in the rectum of diarrheapredominant IBS group ( $78.3 \pm 12.2/\text{mm}^2$ ) than in controls ( $29.6 \pm 5.3/\text{mm}^2$ , Fig. 5,  $p=0.0036$ ).

Activation rate of MC was significantly higher in close proximity to enteric nerves (57.3%) than away from enteric nerves (14.6%) in the cecum of the diarrhea-predominant IBS group (Fig. 6A,  $p<0.0001$ ). In addition, the activation rate of MC was significantly higher in close proximity to the nerves (64.1%) than away from nerves (18.6%) in the rectum of the diarrhea-predominant IBS group (Fig. 6B,  $p<0.0001$ ). However, there was no significant difference in the activation rate between MC in close proximity to nerves and those away from nerves in both cecum and rectum of the control group (Fig. 6).

## DISCUSSION

This study provides the first electron microscopic evidence of an increase in mast cells in both cecum and rectum of IBS patients compared to controls. In previous reports, an increase in mast cells has been identified in the lamina propria of terminal ileum and the cecal mucosa of the IBS patients by light microscopy (8, 9). However, other investigators found no differences in the degree of mast cells infiltration in IBS compared to controls by light microscopic examination (10, 11). It is possible that degranulated mast cells would not have been identified in these studies, because some of degranulated mast cells are not often recognizable by light microscopy. In this study, the electron microscopic technique not only allowed accurate identification of both intact and degranulated mast cells, but also allowed these to be quantified separately. In addi-

tion, the electron microscopic technique allowed quantification of mast cells according to the distance between mast cells and enteric nerves.

From this electron microscopic examination, 69.7% of cecal MC and 61.7% of rectal MC were within 2  $\mu\text{m}$  from the enteric nerves in lamina propria in IBS patients, and the activation rate of MC in close proximity to enteric nerves was significantly higher in IBS patients than in controls. This increase of activated MC in close proximity to enteric nerves in the IBS group strongly suggests that potent chemicals derived from MC, including histamine, 5-HT, platelet activating factor, prostaglandins, cytokines, and leukotrienes (16, 17), may be implicated in the generation of gastrointestinal symptoms via their effects on enteric nervous system. These mast cell products have the potential of activating and/or sensitizing visceral afferent fibers (18). In animal models, degranulation of intestinal mast cells results in a reduced threshold for pain responses to balloon distension (19) that was prevented by treatment with mast cell stabilizing drugs. The enteric nervous system may respond to the mast cell products by initiating a program of coordinated secretion and propulsive motility that could result in symptoms of IBS (20).

A series of observations support bi-directional interactions between the central nervous system and the immune system (21, 22). Evidence from ultrastructural and light microscopic studies suggests that enteric mast cells are innervated by projections from the central nervous system (23-25). In analogy to neuroimmune interactions in other parts of the immune system (26, 27), altered outputs of central stress circuits in terms of the hypothalamus-pituitary-adrenal axis and sympathetic and sympathoadrenal responses may have profound influences on the gut immune system, including mast cell numbers and cytokine profiles (26-28). Therefore, mast cell degranulation in the gut occurs in response to psychological stress (29, 30), and can even result from Pavlovian conditioning (29).

Release of mast cell protease into the systemic circulation is a marker for degranulation of enteric mucosal mast cells. This can be demonstrated as a conditioned response in laboratory animals to either light or auditory stimuli and in humans as a conditioned response to stress (30), indicative of a brain to enteric mast cell connection. Findings that stimulation of neurons in the brain stem by thyrotrophin-releasing hormone (TRH) evokes degranulation of mucosal mast cells in the rat small intestine are additional evidence for brain mast cell interactions (31). In the upper gastrointestinal tract of the rat, intracerebroventricular injection of TRH evokes the same kinds of gastric inflammation and erosions as cold restraint stress. In the large bowel, restraint stress exacerbates nociceptive responses and these effects are associated with increased release of histamine (32). Intracerebroventricular injection of corticotrophin-releasing factor (CRF) mimics the responses to stress. Intracerebroventricular injection of a CRF antagonist or pretreatment with mast cell stabilizing drugs suppresses

stress-induced responses. Alternatively, mast cell products including different cytokines and chemokines released in the gut may have profound influences on the responsiveness of central stress circuits including gene expression of CRF and vasopressin (33, 34), and these products may play a major role in the pathogenesis of the symptom complex including hyperalgesia, fever, anorexia, and taste aversion via the vagus nerve (21, 22).

The study of mast cells in IBS may be confounded by many factors, so that comparison with the few published studies is hampered. Additionally, the few reports of quantitative changes in human gastrointestinal mucosal mast cells show striking differences in the prevalence of mast cells in the different layers of the gut wall and in the different regions (35-37). Although Weston et al. and O'Sullivan et al. reported increases in MC in ileo-cecal region and suggested that raised MC in IBS may be a feature specific to the ileo-cecal region (8, 9), increases of MC in both cecum and rectum were documented in our study. Interestingly, Weston et al. did not include colonic specimens and O'Sullivan et al. documented that MC were also elevated in the ascending colon and descending colon but that the difference was not statistically significant (8, 9). Methodological differences exist in such as patient selection, sample size, biopsy site, and the method of mast cell identification. Our study was based on cecal and rectal samples from diarrhea-predominant IBS, and electron microscopic examination allowed precise identification of both intact and degranulated mast cells.

In conclusion, MC were significantly increased in both cecum and rectum of the patients with IBS compared to controls. The increased infiltration and activation rate of MC in close proximity to enteric nerves may play a role in the gut sensory hypersensitivity of IBS. More work is clearly needed to further elucidate the role of immune cells in IBS.

## REFERENCES

1. Mayer EA, Gebhart GF. *Basic and clinical aspects of visceral hyperalgesia*. *Gastroenterology* 1994; 107: 271-93
2. Read NW. *Irritable bowel syndrome (IBS) - definition and pathophysiology*. *Scand J Gastroenterol Suppl* 1987; 130: 7-13.
3. White A, Upton A, Collins SM. *Is irritable bowel syndrome the asthma of the gut?* *Gastroenterology* 1988; 94: A494.
4. Baird AW, Cuthbert AW. *Neuronal involvement in type 1 hypersensitivity reactions in gut epithelia*. *Br J Pharmacol* 1987; 92: 647-55.
5. Castro GA, Harari Y, Russell D. *Mediators of anaphylaxis-induced ion transport changes in small intestine*. *Am J Physiol* 1987; 253: G540-8.
6. Perdue MH, Gall DG. *Intestinal anaphylaxis in the rat: jejunal response to in vitro antigen exposure*. *Am J Physiol* 1986; 250: G427-31.
7. Yang Y, Zhou D, Zhang W. *Mast cells of ileocecal junction in irritable bowel syndrome*. *Zhonghua Nei Ke Za Zhi* 1997; 36: 231-3.
8. Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. *Terminal ileal mu-*

- cosal mast cells in irritable bowel syndrome. *Dig Dis Sci* 1993; 38: 1590-5.
9. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, O'Morain CA. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000; 12: 449-57.
  10. Talley NJ, Butterfield JH. Mast cell infiltration and degranulation in colonic mucosa in the irritable bowel syndrome. *Am J Gastroenterol* 1996; 91: 1675-6.
  11. Irvine EJ, Gaebel K, Driman D, Riddell RH, Collins SM. Mucosal mast cells (MMC) numbers are normal in biopsies of patients with irritable bowel syndrome (IBS). *Gastroenterology* 1995; 108 (Suppl): A860.
  12. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; 45 (Suppl 2): II43-7.
  13. Dvorak AM. Ultrastructural analysis of anaphylactic and piecemeal degranulation of human mast cells and basophils. In: Foreman JC, ed. *Immunopharmacology of Mast Cells and Basophils*. London, Academic Press. 1993: 89-113.
  14. Dvorak AM. Ultrastructural analysis of human basophil and mast cell recovery after secretion. *Semin Clin Immunol* 1994; 8: 5-16.
  15. Wilhelm M, King B, Silverman AJ, Silver R. Gonadal steroids regulate the number and activational state of mast cells in the medial habenula. *Endocrinology* 2000; 141: 1178-86.
  16. McKay DM, Perdue MH. Intestinal epithelial function: the case for immunophysiological regulation. *Cells and mediators (1)*. *Dig Dis Sci* 1993; 38: 1377-87.
  17. Metcalfe DD. Mast cell mediators with emphasis on intestinal mast cells. *Ann Allergy* 1984; 53: 563-75.
  18. Vergnolle N, Bunnet NW, Sharkey KA, Brussee V, Compton SJ, Grady EF, Cirino G, Gerard N, Basbaum AI, Andrade-Gordon P, Hollenberg MD, Wallace JL. Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat Med* 2001; 7: 821-6.
  19. Coelho AM, Fioramonti J, Bueno L. Mast cell degranulation induces delayed rectal allodynia in rats: role of histamine and 5-HT. *Dig Dis Sci* 1998; 43: 727-37.
  20. Wood JD, Alpers DH, Andrews PL. Fundamentals of neurogastroenterology. *Gut* 1999; 45 (Suppl II): II6-16.
  21. Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. *Auton Neurosci* 2000; 85: 49-59.
  22. Hansen MK, O'Connor KA, Goehler LE, Watkins LR, Maier SF. The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. *Am J Physiol Regul Integr Comp Physiol* 2001; 280: R929-34.
  23. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 1987; 84: 2975-9.
  24. Williams RM, Berthoud HR, Stead RH. Vagal afferent nerve fibers and mast cells in rat small intestinal mucosa. *Neuroimmunomodulation* 1997; 4: 266-70.
  25. Gottwald T, Lhotak S, Stead RH. Effect of truncal vagotomy and capsaicin on mast cells and IgA-positive plasma cells in rat jejunal mucosa. *Neurogastroenterol Motil* 1997; 9: 25-40.
  26. Chrousos GP. Stress, chronic inflammation, and emotional and physical well-being: concurrent effects and chronic sequelae. *J Allergy Clin Immunol* 2000; 106: S275-91.
  27. Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol Metab* 1999; 10: 359-68.
  28. Mayer EA, Collins SM. Evolving pathophysiologic models of functional gastrointestinal disorders. *Gastroenterology* 2002; 122: 2032-48.
  29. MacQueen G, Marshall J, Perdue M, Siegel S, Bienenstock J. Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. *Science* 1989; 243: 83-5.
  30. Santos J, Saperas E, Nogueiras C, Mourelle M, Antolin M, Cadahia A, Malagelada JR. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* 1998; 114: 640-8.
  31. Santos J, Saperas E, Mourelle M, Antolin M, Malagelada JR. Regulation of intestinal mast cells and luminal protein release by cerebral thyrotrophin-releasing hormone in rats. *Gastroenterology* 1996; 111: 1465-73.
  32. Gue M, Del Rio-Lacheze C, Eutamene H, Theodorou V, Fioramonti J, Bueno L. Stress-induced visceral hypersensitivity in rats; role of CRF and mast cells. *Neurogastroenterol Motil* 1997; 9: 271-9.
  33. Watkins LR, Wiertelak EP, Goehler LE, Smith KP, Martin D, Maier SF. Characterization of cytokine-induced hyperalgesia. *Brain Res* 1994; 654: 15-26.
  34. Kresse AE, Million M, Saperas E, Tache Y. Colitis induces CRF expression in hypothalamic magnocellular neurons and blunts CRF gene response to stress in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; 281: G1203-13.
  35. Pulimood AB, Mathan MM, Mathan VI. Quantitative and ultrastructural analysis of rectal mucosal mast cells in acute infectious diarrhea. *Dig Dis Sci* 1998; 43: 2111-6.
  36. Norris HT, Zamchek N, Gottlier LS. The presence and distribution of mast cells in the human gastrointestinal tract at autopsy. *Gastroenterology* 1963; 44: 448-55.
  37. Bischoff SC, Wedemeyer J, Herrmann A, Meier PN, Trautwein C, Cetin Y, Maschek H, Stolte M, Gebel M, Manns MP. Quantitative assessment of intestinal mast cells and eosinophils in inflammatory bowel disease. *Histopathology* 1996; 28: 1-13.