

Abnormalities in endocrine and immune cells are correlated in dextran-sulfate-sodium-induced colitis in rats

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Abstract. The interaction between the gut hormones and the immune system has been suggested to serve an important role in the pathophysiology of inflammatory bowel disease. The aims of the present study were to elucidate the possible abnormalities in the colonic endocrine cells in rats with dextran sodium sulfate (DSS)-induced colitis, and to determine whether they are correlated with alterations in the immune cells. A total of 24 male Wistar rats were divided into two groups: Control and DSS-induced colitis. Colonic tissues were harvested via postmortem laparotomy from all of the animals at the end of the experimental period, and fixed and sectioned for histology. The colonic endocrine and immune cells in those tissue samples were immunostained and their densities quantified by computerized image analysis. The densities of chromogranin A, serotonin, peptide YY and oxyntomodulin cells were significantly higher, and those of pancreatic peptide and somatostatin cells were lower in rats with DSS-induced colitis than in the controls. The densities of mucosal leukocytes, T and B lymphocytes, macrophages/monocytes, and mast cells were significantly higher than in the controls, and these changes were closely associated with the aforementioned changes in all endocrine cell types. These observations indicate an interaction between intestinal hormones and the immune system as represented by immune cells.

Introduction

The etiology of inflammatory bowel disease (IBD) remains unclear (1-3). There are three main forms of IBD, ulcerative colitis (UC), Crohn's disease (CD) and microscopic colitis

(MC), which exhibit differences in prevalence, clinical course and prognosis (3-6). While the onset of UC and CD occurs predominantly in individuals <40 years, the onset of MC occurs in those >60 years (3,7). In addition to the morbidity caused by IBD, it considerably reduces the quality of life of patients (3,5,6).

The gastrointestinal endocrine cells are a component of the local regulatory system of the gut, the neuroendocrine system (NES) (8), which also includes the enteric nervous system (8). The NES regulates gastrointestinal motility, secretion, absorption, cell proliferation, visceral sensitivity, local immune defense and appetite (8,9). Abnormalities in several intestinal endocrine cells have been reported in IBD (10-29). It is believed that the interaction between the hormones secreted by the gut endocrine cells and the immune system serve a major role in the pathophysiology of the IBD (30,31).

The primary aim of the present study was to establish the presence of abnormalities in the colonic endocrine cells following dextran sulfate sodium (DSS)-induced colitis in rats, which closely mimics human UC (32). Furthermore, the existence of a correlation between any colonic endocrine cell abnormalities and changes in the densities of various types of immune cells was determined.

Materials and methods

Rats. A total 24 male Wistar rats (age, 12 weeks; Hannover GALAS; Taconic Biosciences, Lille Skensved, Denmark) with a mean body weight of 280 g (range, 231-380 g) were housed in Macrolon III cages with water and food available *ad libitum*. They were fed a standard diet (B & K Universal, Nittedal, Norway) consisting of cereal products (88.5%), soy protein (6%), animal protein (2.5%), soy oil (0.5%), and vitamin, mineral and amino-acid supplements (2.5%). The animals were maintained at a temperature of 21±1°C and a relative humidity of 55±5%, and under a 12/12-h light/dark cycle.

The animals were left to acclimatize in the animal house for 7 days prior to the experiment, and were then divided into 2 groups of 12 animals each: Control and DDS-induced colitis (DSS group). Animals in the control group were provided with normal drinking water for 7 days, while those in the

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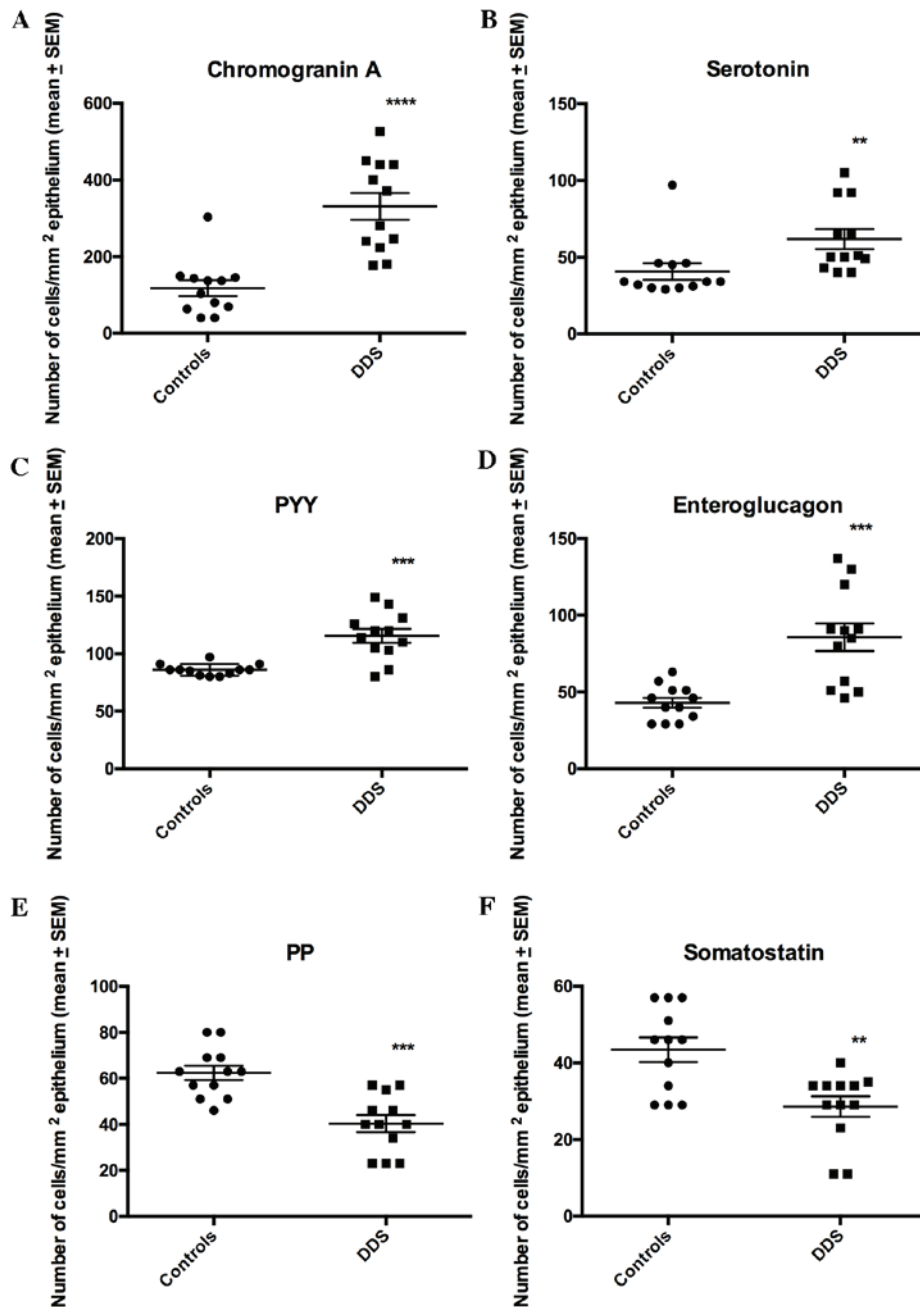


Figure 1. Densities of various endocrine cell types: (A) Chromogranin A; (B) serotonin; (C) PYY; (D) enteroglucagon; (E) PP and (F) somatostatin, in the controls and in rats with DSS-induced colitis. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. controls. DSS, dextran-sulfate-sodium; SEM, standard error of the mean; PP, pancreatic peptide; PYY, peptide YY.

DDS-colitis group were instead provided with distilled water containing 5% DSS (molecular weight, 40 kDa; prepared daily; TdB Consultancy AB, Uppsala, Sweden) for 7 days, according to a previously described protocol (33,34). All of the animals were monitored twice daily and were weighed once daily. Animals that showed any signs of pain were given a subcutaneous, 1 ml injection of Temgesic solution (containing 0.3 g/ml Temgesic; Merck Millipore, Darmstadt, Germany).

At the end of the 7-day period, the animals were sacrificed by CO₂ inhalation, and the colon was dissected out via a postmortem laparotomy. Tissue samples were collected from the lower part of the colon for further, histopathological and immunohistochemical examinations.

The local ethical committee for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes approved the study protocols (project no. 20124629).

Histopathology and immunohistochemistry. The tissue samples were fixed overnight in 4% buffered paraformaldehyde, embedded in paraffin and then sectioned at a thickness of 5 μm. The sections were deparaffinized and then stained with hematoxylin-eosin, or immunostained using the ultraView Universal DAB Detection kit (version 1.02.0018, Ventana Medical Systems, Inc., Basel, Switzerland) and the BenchMark Ultra IHC/ISH staining module (Ventana Medical Systems, Inc.).

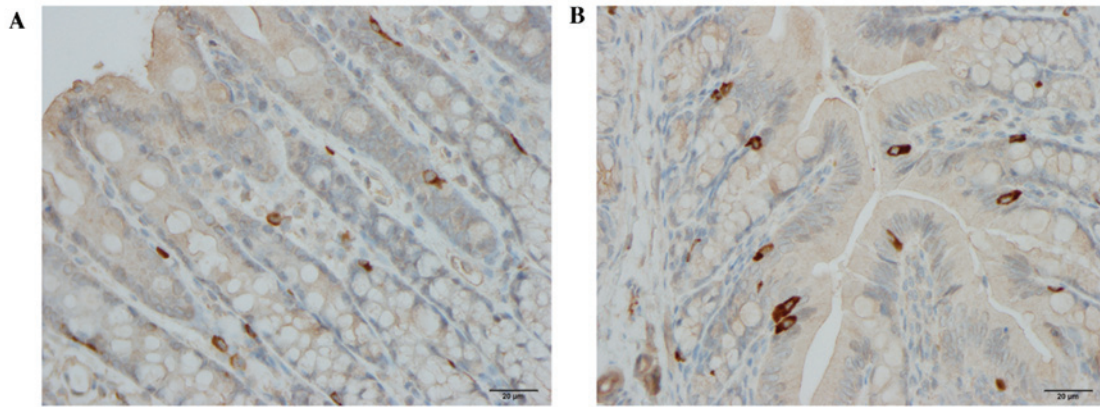


Figure 2. Chromogranin A (CgA)-immunoreactive-cells in (A) a control and in (B) a rat with DSS-induced colitis.

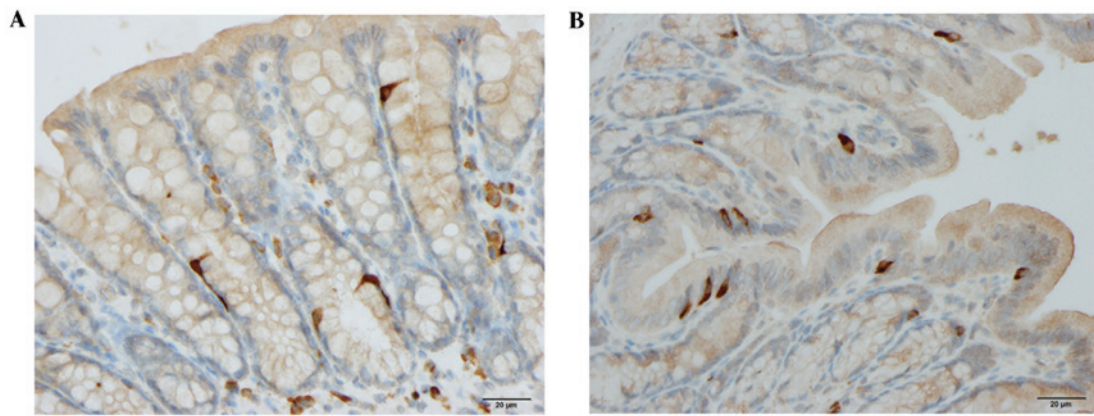


Figure 3. Peptide YY cells in (A) a control rat and (B) in a rat with DSS-induced colitis.

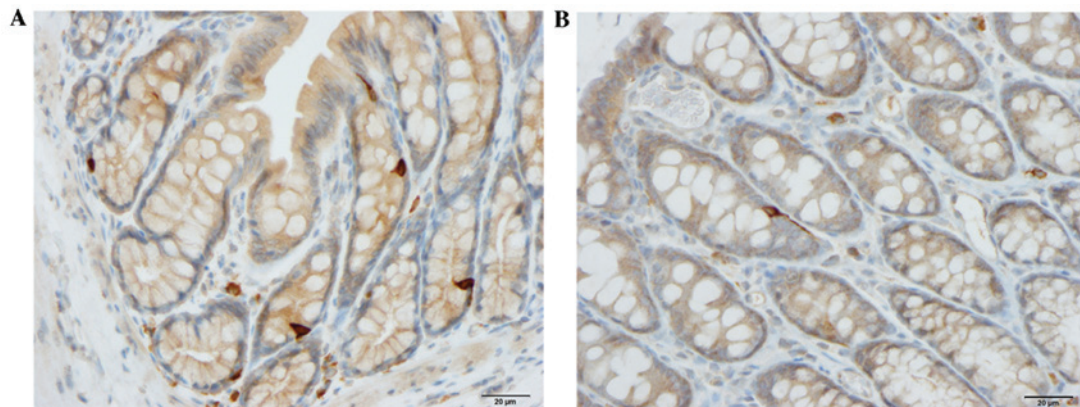


Figure 4. Somatostatin cells in the colon of (A) a control rat, and (B) in a rat with DSS-induced colitis.

For immunostaining, the sections were incubated with one of the following primary antibodies for 32 min at 37°C: Monoclonal mouse anti-N-terminal of purified chromogranin A (CgA; cat. no. M869; Dako, Glostrup, Denmark) diluted 1:1,000, monoclonal mouse antiserotonin (cat. no. 5HT-209; Dako) diluted 1:1,200, polyclonal antiporcine peptide YY (PYY; cat. no. PYY 11A; Alpha Diagnostic International, San Antonio, TX, USA) diluted 1:1,400, polyclonal rabbit antisynthetic human pancreatic polypeptide (PP; cat. no. #114; Diagnostic BioSystems, Pleasanton, CA, USA)

diluted 1:800, polyclonal rabbit antiporcine oxyntomodulin 'glicentin/enteroglucagon' (cat. no. BP508; Acris Antibodies GmbH, Herford, Germany) diluted 1:400, polyclonal rabbit antisynthetic human somatostatin (cat. no. A566; Dako) diluted 1:200, monoclonal mouse antihuman CD45 (cat. no. M0701; Dako) diluted 1:100, monoclonal mouse antihuman CD5 (cat. no. IS082; Dako) diluted 1:200, monoclonal mouse antihuman CD57 (cat. no. IS647; Dako) diluted 1:100, monoclonal mouse antihuman CD23 (cat. no. IS781; Dako) diluted 1:100, monoclonal mouse antihuman CD68 (cat. no. M0814; Dako) diluted

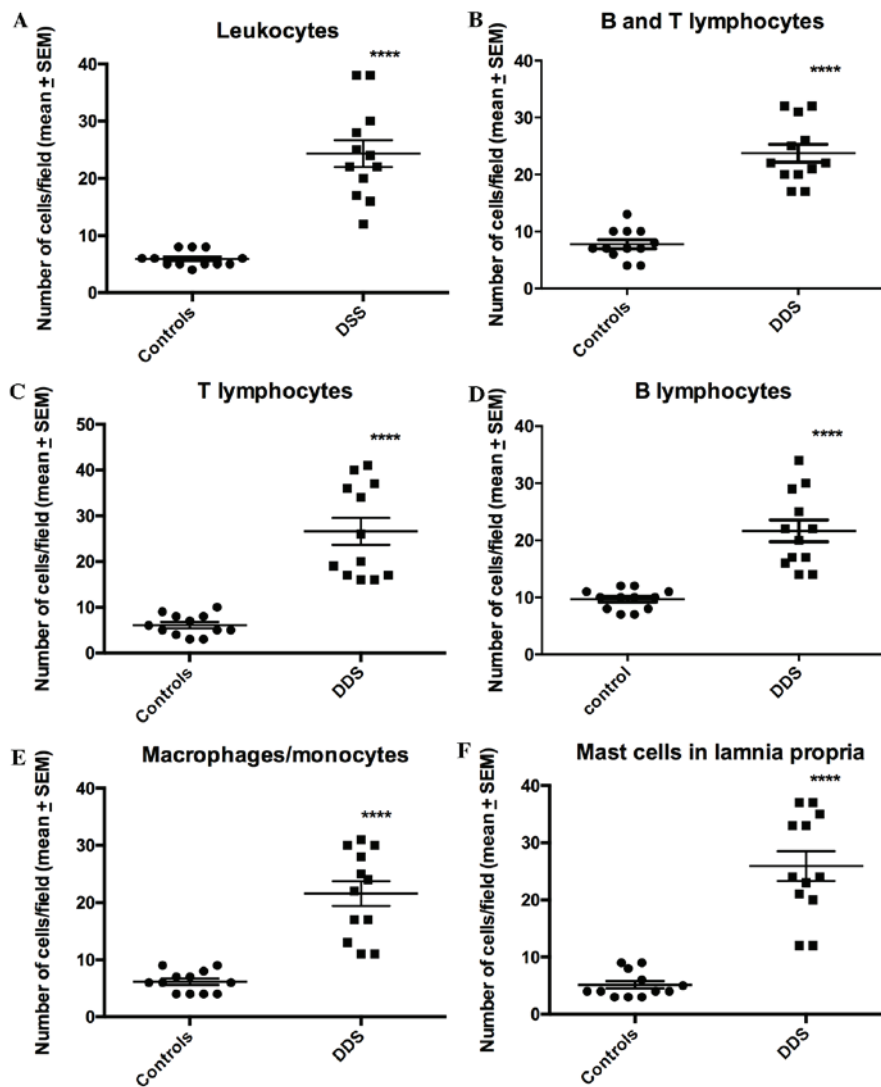


Figure 5. Densities of different types of immune cells: (A) Leukocytes; (B) B and T lymphocytes; (C) T lymphocytes; (D) B lymphocytes; (E) macrophages/monocytes and (F) mast cells in the lamina propria of control rats and rats with DSS-induced colitis. **** $P < 0.0001$ vs. controls. DSS, dextran-sulfate-sodium.

1:100 and monoclonal mouse antihuman mast-cell tryptase (cat. no. M7052; Dako) diluted 1:100. CD45 is considered a common leukocyte antigen and is expressed exclusively on cells of the hematopoietic system and their progenitors. CD5 is expressed on B and T lymphocytes, CD57 is expressed by subsets of natural killer cells and CD8⁺ lymphocytes, and by a small proportion of CD4⁺/CD45R0⁺ T lymphocytes, CD23 is expressed on B lymphocytes, CD68 labels human monocytes, macrophages, and myeloid cells, and mast-cell tryptase is expressed predominantly in mast cells (35).

Quantification of endocrine and immune cells. The endocrine and immune cells were quantified by counting each cell type in 10 randomly chosen microscopic fields. Measurements were performed on a computer linked to a microscope (BX43; Olympus Corporation, Tokyo, Japan) that was equipped with a digital camera (DP26; Olympus Corporation), and using cellSens imaging software (version 1.7; Olympus Corporation). The number of endocrine cells in the epithelial lining of the intestinal lumen and immune cells in the lamina propria of each field were counted on a computer screen, and the area of the epithelial cells

was determined by manual drawing using the computer mouse. A 40X objective was used, for which each frame (field) on the monitor represented a tissue area of 0.035 mm². The data are presented as density measurements (i.e., the number of endocrine cells/mm² epithelium, and the number of immune cells per field). Immunostained sections were coded and mixed, and measurements were made by the same person (Professor Magdy El-Salhy), who was blind to the identity of the sections.

Statistical analysis. Differences between the control and DSS groups were tested using the Mann-Whitney nonparametric test. The existence of a correlation between abnormalities/alterations in the densities of endocrine cells and immune cells was determined using the nonparametric Spearman's correlation test. The data are presented as the mean \pm standard error, and $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The histopathological examinations of the colonic tissues produced normal results in the control group, whereas the DSS

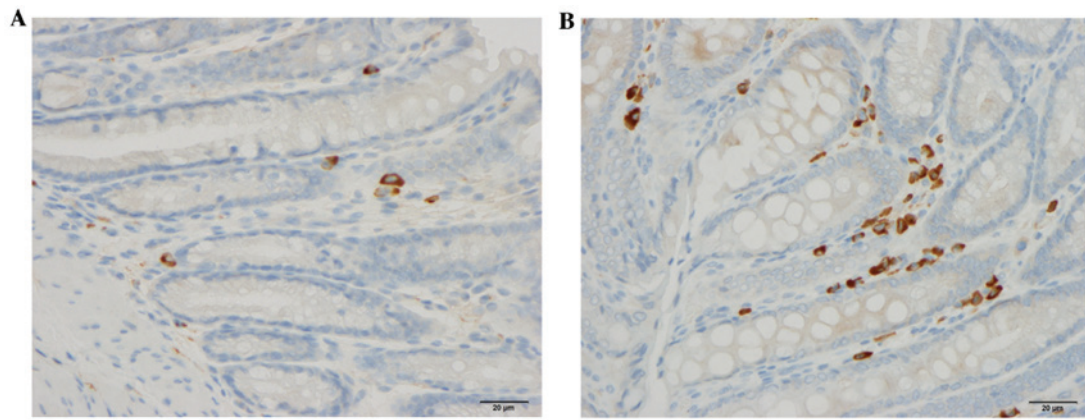


Figure 6. Submucosal leukocytes in (A) a control rat and (B) in a rat with DSS-induced colitis.

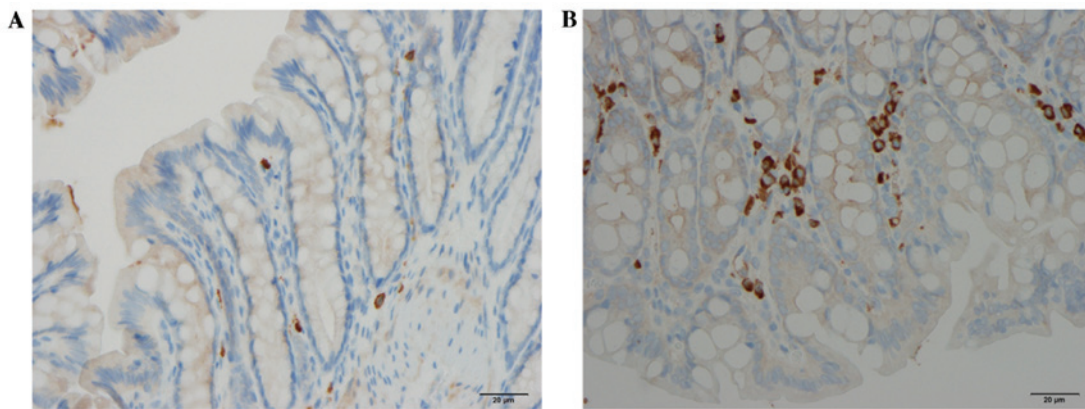


Figure 7. B/T lymphocytes in the submucosa of (A) a control rat and in (B) a rat with DSS-induced colitis.

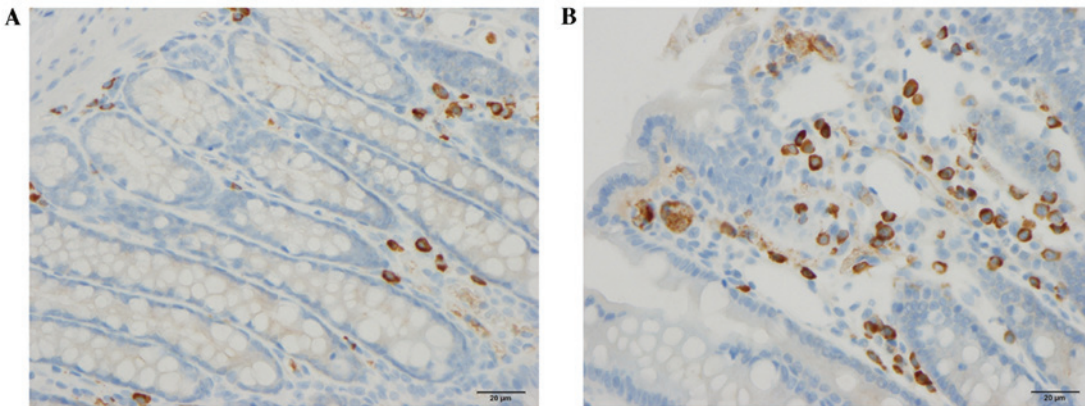


Figure 8. Submucosal mast cells in (A) a control rat and in (B) a rat with DSS-induced colitis.

group had severe-to-moderate inflammation with disturbed mucosal architecture, crypt abscesses, edema, bleeding and infiltration of immune cells into the mucosa and submucosa.

Endocrine cells. The densities of CgA, serotonin, PYY and enteroglucagon cells were all significantly higher in the DDS group (333.1 ± 34.7 , 61.8 ± 6.5 , 115.6 ± 5.9 and 85.7 ± 9.0 cells/mm² epithelium, respectively) than in the control group (117.4 ± 20.7 , 40.7 ± 5.5 , 86.0 ± 1.5 and 42.9 ± 3.3 cells/mm² epithelium;

$P < 0.0001$, $P = 0.0006$, $P = 0.002$ and $P = 0.0003$, respectively; Figs. 1-3). Conversely, the densities of PP and somatostatin cells were significantly higher in the control group (62.4 ± 3.1 and 43.4 ± 3.2 cells/mm² epithelium, respectively) than in the DSS group (40.3 ± 3.7 and 28.6 ± 2.7 cells/mm² epithelium, respectively; $P = 0.0002$ and 0.007 , respectively; Figs. 1 and 4).

Immune cells. The densities of all of the immune cell types were significantly higher in the DDS group than

Table I. Summary of the Spearman correlation coefficient (*r*) and P values between different endocrine cell types and various immune cells.

Endocrine cell type	Immune cell type					
	Leukocytes	B/T lymphocytes	T lymphocytes	B lymphocytes	Macrophages/ monocytes	Mast cells
Chromogranin A	r=0.8 P=0.006	r=0.7 P=0.009	r=0.6 P=0.03	r=0.7 P=0.008	r=0.8 P=0.0009	r=0.7 P=0.008
Serotonin	r=0.7 P=0.007	r=0.7 P=0.004	r=0.8 P=0.004	r=0.8 P=0.006	r=0.8 P=0.004	r=0.7 P=0.008
Peptide YY	r=0.6 P=0.03	r=0.6 P=0.04	r=0.7 P=0.02	r=0.6 P=0.03	r=0.7 P=0.02	r=0.7 P=0.02
Enteroglucagon	r=0.6 P=0.04	r=0.8 P=0.006	r=0.8 P=0.006	r=0.7 P=0.02	r=0.7 P=0.02	r=0.9 P=0.0005
Pancreatic peptide	r=-0.7 P=0.006	r=-0.7 P=0.007	r=-0.8 P=0.001	r=0.7 P=0.01	r=-0.7 P=0.006	r=0.7 P=0.006
Somatostatin	r=-0.7 P=0.01	r=-0.6 P=0.02	r=-0.6 P=0.02	r=-0.8 P=0.0009	r=-0.5 P=0.006	r=-0.8 P=0.0007

in the control group (Figs. 5-8): Leukocytes, 5.9 ± 0.4 vs. 23.3 ± 2.2 cells/field ($P < 0.0001$); B/T lymphocytes, 7.8 ± 0.8 vs. 23.8 ± 1.6 cells/field ($P < 0.0001$); T lymphocytes, 6.8 ± 0.7 vs. 26.6 ± 2.9 cells/field ($P < 0.0001$); and B lymphocytes, 9.8 ± 0.6 vs. 22.1 ± 2.3 cells/field ($P < 0.0001$).

Correlation between endocrine and immune cells. The Spearman correlation coefficients and P-values between different endocrine cell types and various immune cells are summarized in Table I. The abnormalities in CgA, serotonin, PYY, and enteroglucagon cells were identified to be positively correlated with the alterations of all types of immune cells, while a negative correlation was observed for PP and somatostatin cells.

Discussion

Animal models of IBD are either those with chemically induced colitis or mutant (knockout) mice (7,32,36-39). Although neither of these models accurately mimic human IBD, they are useful tools towards understanding the pathophysiological mechanisms underlying IBD (32). DSS-induced colitis is a mixed Th₁/Th₂ cytokine-mediated colitis (40,41) and is considered to be a model for UC with clinical and morphological features similar to that of human UC (32,42,43). However, DSS-induced colitis lacks the chronicity seen in human UC (32).

The present study identified that the densities of all of the colonic endocrine cell types were affected in rats with DSS-induced colitis. Furthermore, the abnormalities in the colonic endocrine cells were closely correlated with the alterations in several immune-cell types following the induction of colitis. These observations lend support to the hypothesized role of gut hormones in immune activation and inflammation (30,31,44).

CgA belongs to the family of granins (45,46), and is localized to the stomach and small and large intestines (47-50). It is

commonly used as a marker for gastrointestinal and endocrine tumor cells (51,52). The increase in CgA-immunoreactive cells observed in the present study could reflect an increase in the cell density of the total colonic endocrine cells following the induction of colitis. This observation is in line with the previously reported increases in CgA cells in UC and CD (10,23). However, CgA itself inhibits the vascular leakage caused by tumor necrosis factor α (53). Furthermore, CgA-derived peptides reduce the release of interleukin (IL)-16 and IL-5, hence reducing the number of lymphocytes at inflammatory sites and thus the proinflammatory action of lymphocytes and monocytes (54-56). The increase in the density of CgA cells reported herein was closely associated with the increase in immune cells. Taking into consideration the known interaction between CgA and immune cells, this increase in CgA density is likely a response defense mechanism against inflammation.

Serotonin is a potent hormone that exerts several effects at its numerous receptor types. Thus, it stimulates gastric and intestinal motility, modulates visceral sensitivity, and stimulates intestinal secretion (8,57). The present observation of an increased density of colonic serotonin cells in DSS-induced colitis relative to healthy controls is in agreement with previously published observations in patients with UC, CD and MC, and in animal models of colitis (10,12,58-60). However, additional studies identified that the serotonin cell density reduced in UC and remained unchanged in CD (61,62). It has been reported that serotonin serves an important role in intestinal inflammation (30,54). Thus, the number of serotonin cells has been reported to be reduced in mice lacking the T-lymphocyte receptors (54), IL-13 receptors have been localized on serotonin cells (63), and serotonin receptors have been observed in lymphocytes, monocytes, macrophages and dendritic cells (64). In addition, serotonin affects the proliferation of lymphocytes, protects natural killer cells, inhibits the apoptosis of immune cells, and promotes the recruitment of T cells (65-68). Thus, the fact that the increase in serotonin-cell

density in DSS-induced colitis was to be closely associated with the increased densities of the immune-cell types was expected.

PYY and oxyntomodulin are colocalized in the same endocrine cell type (69,70). However, the degree of that colocalization differs according to the animal species (70). PYY delays gastric emptying, and is a key mediator of the ileal brake. It also inhibits gastric and pancreatic secretion, and stimulates the absorption of water and electrolytes (71). Oxyntomodulin has an incretin effect, inhibits gastric and pancreatic secretions, and reduces gastric motility (8). PYY and oxyntomodulin have been previously observed to exhibit anorexigenic effects (72), and the present observation of increased PYY and oxyntomodulin cell densities is in agreement with previous observations in UC and IL-2 gene knockout mice (10,59). Whereas the increase in the oxyntomodulin cell density identified is in line with previous observations in IL-2 knockout mice, it disagrees with observations in UC, where oxyntomodulin cell density was unchanged (10,59). The close correlation between the increase in PYY and oxyntomodulin cell densities with the increase in the densities of the immune cells identified in the current study indicates an interaction between the endocrine and immune systems.

PP stimulates gastric acid secretion and the motility of the stomach and small intestine, and relaxes the gallbladder (8). Somatostatin inhibits intestinal contraction, and gut exocrine and neuroendocrine secretions (8). In addition, somatostatin inhibits lymphocyte proliferation, immunoglobulin synthesis and neutrophil elastase release (73-77). The reduction in PP cell density observed in the present investigation is in line with what has been reported in UC and CD (10). Although the reduction in somatostatin cell density in DSS-induced colitis observed in the current study is also in line with previous publications on UC and CD (28,29), it is in disagreement with a study in which the density of somatostatin cells was observed to be unchanged in these conditions (10). As for the other endocrine cell types assessed in the current study, the correlation between the alterations in the PP and somatostatin cell densities points to their involvement in the inflammatory process.

A potential interaction is suggested between inflammation as indicated by the increase in immune cells and the colonic endocrine cells. It is possible that the increase in serotonin and the reduction in somatostatin cell densities results from inflammation, and that the changes in CgA, PYY, oxyntomodulin and PP cells are secondary responses to the changes in serotonin and somatostatin. Cytokines appear to serve a significant role in the proliferation and differentiation of intestinal stem cells (78-80). It is suggested that inflammation with increased cytokine production increases the serotonin and reduces the somatostatin cell densities by affecting their early progenitors, and that these alterations would result in increased gastrointestinal motility and secretion in addition to visceral hypersensitivity. As a compensatory defense, an increase in PYY and oxyntomodulin, and a reduction in PP would slow gastrointestinal motility and reduce gastrointestinal secretions. The increase in CgA, which appears to have anti-inflammatory effects, may simply reflect the total increase in colonic endocrine cells or another defensive action against inflammation (30).

The induction of colitis by DSS in rats affects all of the colonic endocrine cells. Given the available data on the interactions between hormones and the immune system, it can be hypothesized that inflammation induces the proliferation of serotonin cells and inhibits that of somatostatin cells, in response to which there is a secondary change in the densities of CgA, PYY, oxyntomodulin and PP cells. The close correlation between the changes in all endocrine cell types and immune cells emphasizes the importance of the role of interactions between the intestinal hormone and immune systems in the pathophysiology of intestinal inflammation.

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