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VIP immunoreactive nerves and somatostatin and serotonin containing cells in Crohn's disease

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INTRODUCTION

With the progress of the studies on neuroendocrine and immunology in the gastrointestinal tract, it has been recognized that the intestinal neuroendocrine system and the immune system can influence and modulate each other and a neuroendocrine immunomodulation network in the intestine has been established[1]. Neuropeptides, such as vasoactive intestinal peptide (VIP), substance P (SP), somatostatin (SS) etc. are widely distributed in the gastrointestinal tract and they play an important role in the immunomodulation of the intestinal mucosa.

MATERIALS AND METHODS

Tissue specimens

Surgical specimens were obtained from ileum of 25 cases of CD (25 ileum) and 10 normal subjects (from sudden death and who received surgery for intestinal neoplasm). The specimens were fixed in 10% formalin and embedded in paraffin. Sections with thickness of 4ìm were made continuously.

Staining method

Immunohistochemical staining was carried out by ABC method. Antibodies included rabbit polyclonal antibodies to VIP (1:400), NSE (1:200), S-100 protein (1:400), SS (1:200), serotonin (1:600) (Dako Co.) and ABC Kit.

Histomorphometric analysis

Olympus CH light microscopy with occular linear micrometer and double quardral grid test system C64 (from the Academy of Military Medical Sciences) were used to measure immunoreactive neurons and nerve fibers in 10×40 high power field. The point count method was used[2]. Fifteen neuron cells (with nucleus) w ere examined at random, the maximal diameter of each was measured and the values were averaged. The total length of all immunoreactive nerve fibers was measured separately in the mucosa and the length was estimated per unit area (μm/μm) which was expressed as linear density (Lv%). In the submucosa and the muscle layers, the total volume of the immunoreactive neurons together with the nerve fibers was measured and the volume was estimated per unit which was expressed as volume density (Vv%). The numbers of SS, serotonin immunoreactive cells and a rgyrophil cells in the mucosa were counted at random in 10 high power fields of each case. The results averaged.

Statistical analysis

Results were analysed by *t* and t'test.

RESULTS

Immunohistochemistry of neurons and nerve fibers

In CD, the VIP-IR and NSE-IR nerve fibers in the mucosa of ileum were markedly increased. They were deeply stained, coarse, irregular (Figure 1) and the linear densities were significantly raised ($P<0.01$, $P<0.01$, respectively, Table 1). In the submucosa, the VIP-IR neurons were hypertrophial ($P<0.01$, Figure 2). VIP-IR, S-100 protein IR and NSE-IR nerve fibers were all remarkably increased. They were coarse, thickened and irregular (Figure 3). The neuron and nerve fiber volume density of each immunor eactive type stated above was significantly increased ($P < 0.01$, respectively). In the muscle layers the volume density of neurons and nerve fibers containing NSE or S-100 protein was also significantly increased ($P<0.01$, respectively, Figure 4), but the changes in VIP-IR neurons and nerve fibers were unremarkable.

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Immunoreactive cells of somatostatin and serotonin

In CD, argyrophil and serotonin-IR cells in the ileal mucosa around the lesions were significantly reduced (P<0.01, respectively, Figure 5). SS- IR cells were decreased (P<0.01, Figure 6), Table 2

DISCUSSION

In CD, there had been several reports about the morphological alterations in the enteric nervous system. Dvorak *et al*[3] described the changes in enteric nervous system in the surgical specimens from patients with CD including proliferation and focal necrosis of nerve fibers and hypertrophy of neurons in the myenteric plexus. Bishop *et al*[4] and Sj-lund *et al*[5] assessed the alterations of VIP-IR enteric nerve fibers in CD observation and counting. In the study, we used the histomorphometric analysis to obtain objective information about the changes in the immunoreactive enteric nervous system in CD. We found that in the submucosal plexus the VIP containing neurons were mar kedly hypertrophied, the linear density of VIP-IR nerve fibers in the mucosa an d the VIP-IR neuron nerve fiber volume density in submucosa were significantly increased. The results were consistent with that of Bishop *et al* and O'Morain *et al*[6] who measured by immunohistochemistry and radioimmuno assay, but different from that by Sj-lund *et al* and Koch. By immunohistoche mistry, Sj-lund *et al* found that in unaffected muscle layer of the ileum and the affected muscle layer of colon, the VIP-IR nerve fibers were reduced. The coarse VIP-IR nerve fibers were more frequently observed in the affected mucosa of ileum and in the affected muscle layers of the colon than those in the control. El-Sathy *et al*[7] reported that the areas of the argyrophil cells as well as those immunoreactive to chromogranin A and serotonin were significantly increased in both patients with UC and CD, compar ed with those in the controls. In patients with CD, the areas of polypeptide YY (PYY) and pancreatic polypeptide (PP) immunoreactive cells were significantly reduced. In this study, we also found that SS and serotonin containing cells and argyrophil ones were reduced in CD. These diversities among different investig ators may be due to the various locations selected the difference in degree of activity and the methods used.

This paper shows that VIP immunoreactive neurons and nerve fibers are increased whereas the immunoreactive cells containing somatostatin and serotonin are reduced, suggesting that there be abnormalities in neuroendocrine system in CD which may play an important role in the pathogenesis of CD.

Table 1 Lv and Vv of immunoreactive nerve fibers and neurons for V IP, S-100 protein and NSE in Crohn's disease

Item	CD		Control		P
	n	$\bar{x}\pm s$	n	$\bar{x}\pm s$	
VIP					
Mucosa Lv	25	3.6±1.2	10	2.0±0.8	<0.01
Submucosa Vv	25	2.0±1.1	10	0.9±0.8	<0.01
Submucosal neuron (diameter, μm)	25	21.1±5.6	10	13.1±2.0	<0.01
Myenteric plexus Vv	25	3.2±1.8	10	2.6±1.4	<0.01
NSE					
Mucosa Lv	25	3.4±0.7	10	1.9±1.4	<0.01
Submucosa Vv	25	5.1±2.2	10	1.3±0.5	<0.01
Myenteric plexus Vv	25	11.1±2.6	10	5.1±1.2	<0.01
S-100 protein					
Submucosa Vv	25	3.9±1.0	10	2.3±0.4	<0.01
Myenteric plexus Vv	25	9.4±2.3	10	5.7±1.0	<0.01

Table 2 Density of serotonin, SS containing cells and argyrophil cells in Crohn's disease

Group	Serotonin containing cell		SS containing cell		Argyrophil cell	
	n	$\bar{x}\pm s$	n	$\bar{x}\pm s$	n	$\bar{x}\pm s$
CD	25	4.2±1.7[b]	25	1.0±0.6[b]	25	3.2±1.5[b]
Control	10	8.9±2.9	10	2.0±1.2	10	7.7±2.5

[b]P<0.01, *vs* normal control.

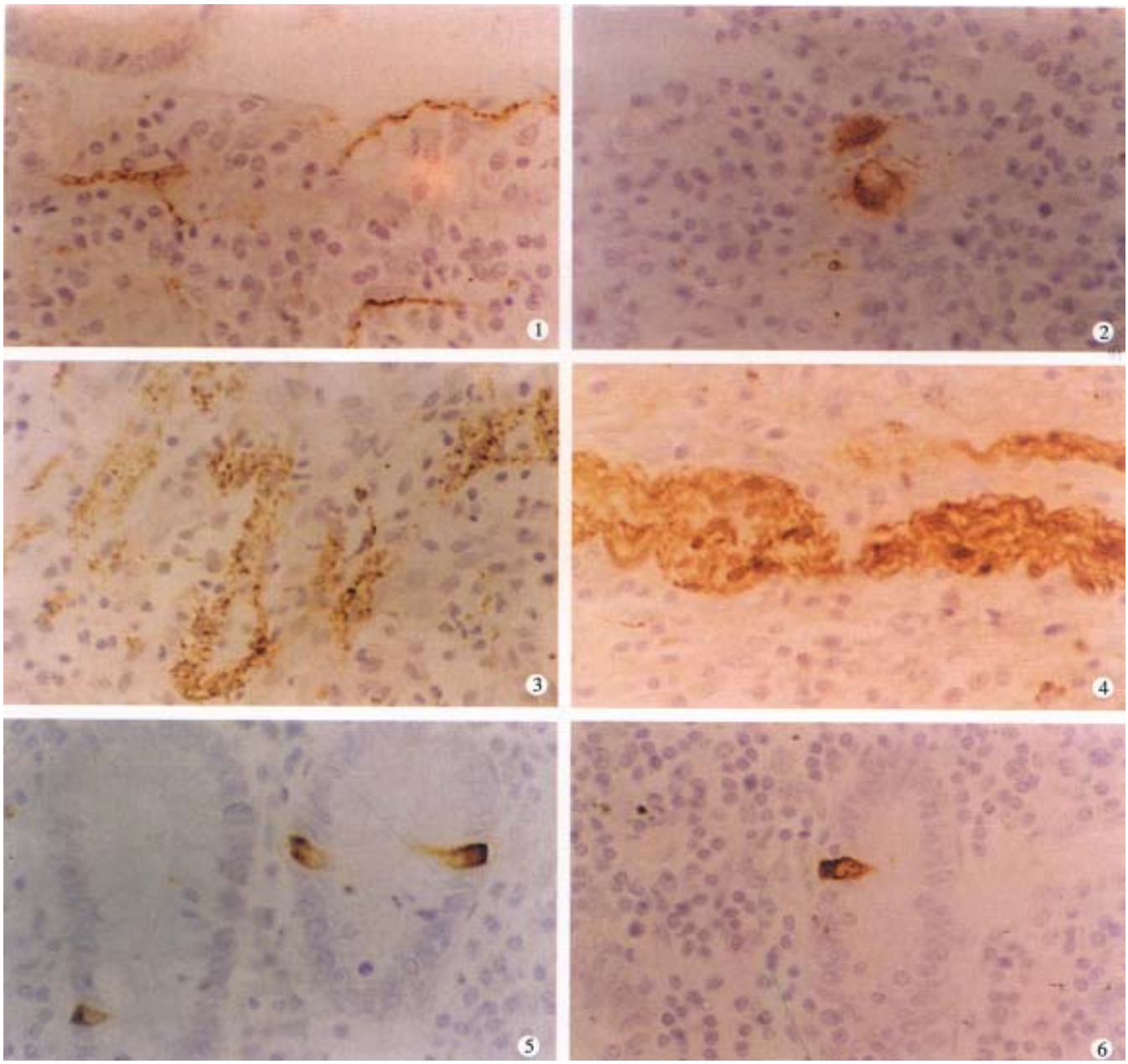

Figure 1 VIP immunoreactive nerve fibers were coarse in mucosa of CD. ABC method ×400
Figure 2 VIP immunoreactive neurons were hypertrophi al in submucosa of CD. ABC method ×400
Figure 3 VIP immunoreactive nerve fibers were irregu larly thickened in submucosa of CD. ABC method ×400
Figure 4 S-100 protein immunoreactive nerve fibers were increased in myenteric plexus of CD. ABC method ×400
Figure 5 Serotonin immunoreactive cells in mucosa of CD. ABC method ×400
Figure 6 Somatostatin immunoreactive cells in mucosa of CD. ABC method ×400

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