Increased Populations of Neuroendocrine Cells in the Colon of Patients with Hirschprung's Disease

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The distribution of mucosal neuroendocrine (NE) cells in the colon from 21 patients with Hirschprung's disease (HD) and from 4 controls were studied. Immunohistochemical stains were done out using antibodies against chromogranin A and neuron-specific enolase (NSE). Immunostaining with both antibodies gave similar results: immunoreactive cells significantly increased in the aganglionic bowel compared with ganglionic bowel and controls ($p<0.05$). The increase in NE cells was found over the entire length of the aganglionic segments. These results demonstrating the increased levels of NE cells in the mucosa of aganglionic colon suggest that the NE cells may have a role in regulating the sustained contraction of the aganglionic intestine. [Journal of Turgut Özal Medical Center 1997;4(3):270-273]

***Key Words** : Hirschprung's disease, neuroendocrine cells*

Hirschprung'lu hastaların kalın barsaklarında artmış nöroendokrin hücreler

Hirschprunglu 21 hastanın kolon materyallerinde ve 4 kontrol biyopsisinde mukozal nöroendokrin hücrelerin dağılımı Chromogranin A ve NSE'ye karşı antikorlar kullanılarak immunohistokimyasal olarak incelendi. Her iki antikorla boyamada benzer sonuçlar elde edildi ve aganglionik bölgelerde immunoreaktif hücrelerde artış bulunduğu ($p<0.05$) görüldü.. Nöroendokrin hücrelerdeki bu artışın tüm aganglionik kolon segmentinde bulunduğu görülmüştür. Aganglionik bölgelerde bu hücrelerdeki artış, nöroendokrin hücrelerin sürekli kontraksiyonla ilişkili olabileceklerini düşündürmektedir. [Turgut Özal Tıp Merkezi Dergisi 1997;4(3):270-273]

***Anahtar Kelimeler** : Hirschprung hastalığı, nöroendokrin hücreler*

The gastrointestinal tract, from the esophagus to the anal canal, is extensively populated by a heterogenous collection of peptide- and amine-producing neurons and neuroendocrine (NE) cells (1). The gut NE cells are responsible for the production of more than 20 hormones that act as a chemical messenger in orchestrating the various secretory, motor, and absorbtive functions of the gut (2).

Altered numbers of NE cells have been associated with several diseases of the gastrointestinal tract, often those affecting the mucosa (3). Such diseases include coeliac disease, graft versus host disease, ulcerative colitis and Crohn's ileitis. In the case of latter condition, an increased level of innervation, or so-called neuromatous hyperplasia, has been known for some time to be present in affected areas of bowel.

Many of the neurons of the enteric nervous system are peptidergic. In recent years several authors have reported abnormal peptidergic patterns of innervation in the aganglionic segment of bowel in Hirschprung's

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disease (HD) (4,5). So one might expect an interaction between peptidergic neurons and peptide containing NE cells in HD. As in the case of Crohn's ileitis, there are only a handful studies of the mucosal NE cells in HD (6-10). NE cell markers such as argyrophilia, chromogranin, neuron-specific enolase, synaptophysin and Leu-7 can be used to study whole populations of NE cells. Thus, the aim of the present study was to establish whether any alteration in gut NE cell numbers occurs in typical cases of HD by immunostaining for NSE and chromogranin.

MATERIALS AND METHODS

The entire resected specimens of colon were obtained from 21 patients (aged four months to ten years) with HD. For control staining, rectal biopsy tissue was obtained from four patients without HD. The control group was of pediatric population. From the operation specimens, representative samples, measuring approximately 1x0.5 cm were taken from various localizations, including involved and uninvolved areas. Then the specimens were fixed in 10% formaldehyde solution and embedded in paraffin in the usual manner. 6 micrometer-thick sections were cut serially and used for routine hematoxylin and eosin (H.E) staining and immunostaining. Conventional H.E staining was performed for te diagnosis of HD and identification of ganglionic, transitional zones, and aganglionic segments.

For immunohistochemistry, all sections were deparaffinized in xylene, hydrated and immersed in hydrogen peroxyde solution. In every case, three specimens from three different localizations were used. Immunohistochemical studies were carried out using streptavidin-peroxidase method (Maxitags, Immunon). The following primary antisera were used: NSE (Immunon) and antichromogranin A (Immunon). Development of peroxidase achieved by freshly prepared diaminobenzidine solution.

To quantitate the endocrine cell populations in the sections, the number of cells overlying a uniform length (0.4 mm) of muscularis mucosa was counted each section. Only portions of mucosa with continuous epithelium were counted. For each immunostaining, a minimum of 10 units was counted in perpendicularly-orientated section using a x20 objective magnification. Statistical significance was tested by the two-sample t test.

RESULTS

Histology: Aganglionic, transitional zone, and ganglionic segments were confirmed in all of the 21 HD cases after serial examination of resection specimens. Normal morphology was confirmed in the control cases.

Immunohistochemistry: Immunostaining of mucosal NE cells was demonstrated with both antibodies in all cases and controls. Chromogranin A and NSE immunoreactive cells were mainly situated toward the base of crypts (each a similar distribution) (Figures 1 and 2). There was no significant staining difference between chromogranin A and NSE. The number of immunoreactive cells significantly increased in the aganglionic segment compared with the ganglionic segment ($p<0.05$) and controls ($p<0.01$). Immunostaining for NE cell markers also increased in the transitional zone compared with ganglionic segment of bowel in HD.

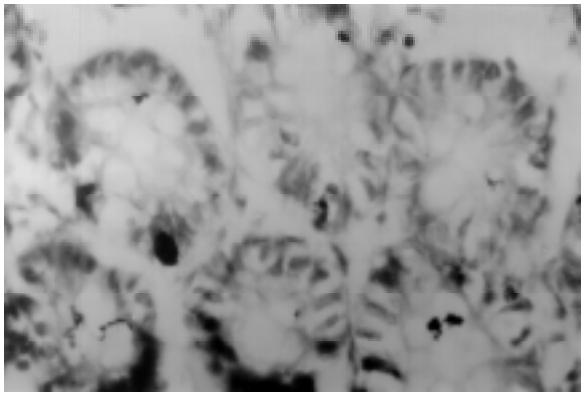

Figure 1. Chromogranin A immunoreactive endocrine cells in base of crypts of aganglionic large bowel (Streptavidin-biotin, DAB, x200)

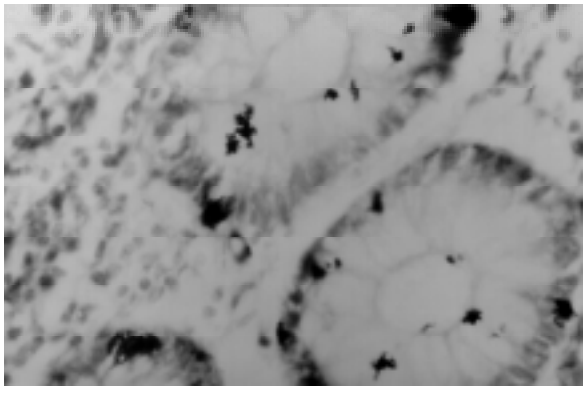

Figure 2. NSE immunoreactive cells in the base of a crypt (Streptavidin-biotin, DAB, x200)

DISCUSSION

With the use of immunohistochemistry and serial sectioning, it has been shown that the number of NE cells in the aganglionic segment of colon in patients with HD was significantly increased when compared with the number in the ganglionic segment (7,8,10). The hypoganglionic (transitional) segment of bowel showed an intermediate number of NE cells. These results were seen with both generic endocrine cell markers chromogranin A and NSE.

Earlier studies have suggested that mucosal NE cells decreased in HD corresponding to the absence or decrease in peptidergic nerve and ganglion cells (5,9,11). However, some recent studies that compared the aganglionic segment with the ganglionic segment found all endocrine cell types significantly increased in the aganglionic tissue (10-12). In the normal large intestine there is a marked increase in NE cells in the rectum compared with other areas of colon. Thus, one must be selective when obtaining control tissues to compare with HD. In the present study the increase in NE cells was found over the entire length of the aganglionic segment.

Relevant to the number of NE cells in HD, a recent study showed that there was a significant reduction in NE cells in HD patients with enterocolitis (However, in our study in no case a prominent enterocolitis pattern was observed) (13). These findings may be partially responsible for previous conflicting reports of NE cell distribution.

The origin of endocrine cells in the intestinal mucosa has been a matter of some debate. The unitarian theory, which claims that all gut mucosal cells, including NE cells originate from endoderm, is favored by most recent embryological studies (14). NE cells have been proposed to be end stage and non-proliferating. In Crohn's ileitis and graft-versus-host disease, the NE cells were often found clumped where the colonic epithelium had been destroyed possibly being spared by the disease process, just as it fails to affect other non-proliferating tissues like nerve and muscle (3,15). But, in contrast to these diseases, in HD there is no epithelial destruction.. So that the above hypothesis cannot explain the situation in HD. It should be borne in mind, however, that large numbers of NE cells seen in HD may be due to a functional change with increased content of product within cells allowing more to be visualized by immunohistochemistry.

The relevance of NE cell population to the histogenesis of HD cannot be defined from the results of this study. This study indicates that in contrast to the absence of ganglion cells and many peptidergic nerves in HD, the mucosal NE cells increased in the aganglionic compared with the ganglionic colon of HD. This suggests that the NE cells are not directly associated with these nerve fibers and probably have an independent derivation. The increase in NE cells may have a role in the sustained contraction of the aganglionic segment mediated by the release of neuropeptide and biogenic amines.

Enolase (NSE), immunostaining with both antibodies gave similar results: immunoreactive cells significantly increased in the aganglionic bowel compared with ganglionic bowel and controls ($p<0.05$). The increase in NE cells was found over the entire length of the aganglionic segments. These results demonstrating the increased levels of NE cells in the mucosa of aganglionic colon suggest that the NE cells may have a role in regulating the sustained contraction of the aganglionic intestine.

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