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Enteric ganglioneuritis and abnormal interstitial cells of Cajal; features of inflammatory bowel disease

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Abstract

Background/Aim. An increased prevalence of irritable bowel syndrome (IBS) and disturbances in cardiac and blood pressure reflexes have been described in patients with Crohn's disease (CD) and ulcerative colitis (UC). These features could be due to abnormalities in the gastrointestinal neurotransmission. The aims of this study were to examine whether histopathological changes in the enteric nervous system correlate to disturbances in cardiac and blood pressure reflexes, and the occurrence of IBS- and dyspepsia-like symptoms in these patients.

Methods. Thirty patients with CD and UC with bowel resection were examined by deep-breathing and orthostatic tests. The resection specimens were evaluated histologically regarding visceral neuro- or myopathy. All medical records were studied for treatment and clinical course.

Results. Ganglioneuritis was observed in 11 of 19 patients with CD and in 5 of 11 with UC. Only patients with CD had ganglioneuritis in the small intestine. Moreover, in CD the interstitial cells of Cajal (ICCs) in the small bowel showed atrophy and vacuolar degeneration, along with a reduced number of cells ($p=0.005$). In UC, the colonic ICCs were hyperplastic ($p=0.05$) without signs of degeneration. The indices of deep-breathing and orthostatic tests were impaired, except in CD with ganglioneuritis who showed normal test values. There were no correlations between histopathological alterations versus IBS and dyspepsia.

Conclusion. Visceral ganglioneuritis and pathologic ICCs were observed in patients with CD and UC. However, these histopathological abnormalities could not be related to the clinical or autonomic features of the disease.

Key words; autonomic nerve (AN) function tests, Crohn's disease (CD), dyspepsia, enteric nervous system, ganglioneuritis, interstitial cells of Cajal (ICCs), irritable bowel syndrome (IBS), ulcerative colitis (UC)

Introduction

Gastrointestinal motility and function require coordination between the intrinsic and extrinsic nervous systems, the interstitial cells of Cajal (ICCs), and smooth muscle cells, where the ICCs are mainly responsible for generation of the pacemaker activity regulating slow waves (1, 2).

The aetiology of functional gastrointestinal disorders, where irritable bowel syndrome (IBS) and dyspepsia are the most common, is controversial. Enteric ganglionitis has been found in some patients with severe IBS (3) and IBS symptoms are more frequent in patients with Crohn's disease (CD) and ulcerative colitis (UC) than in the general population (4). In CD and UC, proliferation and injurious changes have sporadically been described in enteric smooth muscles, enteric nerves and ICCs (5-9). These observations suggest that autonomic enteric neuropathy may be present in CD and UC and thus could be responsible for the IBS-typical symptoms. This hypothesis was supported by the finding that chronic intestinal pseudo-obstruction (CIPO), secondary to enteric neuropathy, has been observed in patients with CD or UC (10).

The aims of this study were to examine if there were (a) any pathological changes in the autonomic nervous system of the bowel in UC and CD, and if so, (b) any correlation between enteric neuropathy and disturbances in the autonomic nervous system regulating heart and blood pressure, or (c) the clinical development of IBS- and dyspepsia-like symptoms.

Material & Methods

Subjects

Patients from the Departments of Surgery and Medicine at the University Hospital of Malmö suffering from CD and UC, previously examined by autonomic nerve (AN) function tests (11, 12) were evaluated. All patients who had undergone surgical resections were included in the present study. Nineteen patients with the diagnosis of CD, mean age 44.5 ± 18.7 years, and 11 with UC, mean age 50.3 ± 13.0 years, were identified. Examination of medical records and personal interviews were performed 19.6 ± 10.6 years and 29.8 ± 7.4 years after the start of symptoms for patients with CD and UC, respectively. The patients with CD had undergone one or more of the following resections for clinical reasons; ileocecal resection (16), small intestinal resections (4) and/or partial colectomy (4). Both the small and large bowel were involved in 11 cases, only small intestine in 5 cases and only colon in 3 cases. Fourteen of the 19 patients were, or had been, treated with immunosuppressive drugs, 13 with 5-amino-salicylic acid (5-ASA), and 7 with metronidazole. All except one of the 11 patients with UC had undergone total proctocolectomy because of severe colitis refractory to medical treatment. One UC patient had died from adenocarcinoma in colon, and the colon was examined at autopsy. Of these 11 patients, 8 had used 5-ASA, 3 metronidazole and 1 azathioprine some time during the course.

The patients were classified as having CD and UC, and thus do not fulfil the Rome-II criteria for IBS and dyspepsia. Still, these criteria were used to classify patients with IBS- and dyspepsia-like symptoms (13), as these criteria are the only available for description of these symptoms. IBS was found in 9 of 19 patients with CD, and in 3 of 11 patients with UC. Dyspepsia was present in 9 of 19 patients with CD, and in 4 of 11 patients with UC. The symptoms, if present, had been present for several years.

Histological evaluation

The available tissues for conventional histopathology and immunohistochemistry is summarised in Table 1. The time lag between the start of symptoms and biopsy was 6.6 ± 7.2 years for CD and 14.5 ± 14.2 years for UC. Tissue pieces were cut from both the diseased and the macroscopically normal parts of the resected bowel specimens after the fixation in 4% formalin solution for 48 hours at room temperature and embedded into paraffin. Four μm thick sections were stained with haematoxylin and eosin.

Re-evaluation of the sections indicated that the initial diagnosis of UC was changed to CD in one patient, in all other patients the diagnoses were not changed. Representative sections were chosen for immunostaining with the T-cell marker of CD3 (Dako, Copenhagen, Denmark) and for the ICCs with *c-kit* (CD117; Dako). As negative controls, the sections were stained with non-immune serum instead of the antibodies. Only macro- and microscopically normal tissue components of the bowel, from the resection margins were used for evaluation of ganglioneuritis and ICCs.

The criteria for ganglionitis were previously described (3). Ganglionitis was diagnosed if the mean number of lymphocytes was equal or more than 2 lymphocytes / ganglion, or if there was a focus of lymphocytes present in connection with myenteric ganglion. The myenteric ganglionitis was always focal and graded as mild (score 1) when there were scattered peri- and intraganglionic lymphocytes, moderate (score 2) when there was microfocus present (a group of >10 - <20 T-cells) in the area of the myenteric plexus, and severe (score 3) when a macrofocus of T-cells (>21 lymphocytes) at a myenteric ganglion was observed. Neuritis was diagnosed when there was a "pearl-band" of three or more lymphocytes along small nerve fibres within either layer of the *t. muscularis*. Similarly to ganglionitis the neuritis was also always patchy. Due to the focal characteristics of the

inflammatory neuropathy, ganglionitis and neuritis were considered as one entity for statistical analysis, i.e. ganglioneuritis.

Visceral myopathy was diagnosed if there were signs of myositis or pathological myocytes, and visceral neuropathy was diagnosed if there were signs of altered neurons (vacuolisation, apoptotic necrosis, swollen or shrunken degeneration, inclusions).

The number of ICCs was determined by the counting of ICC-nuclei separately within the intermyenteric/periganglionic plexus between the circular and longitudinal muscle layers and around the ganglia/nerve fibres of the Auerbach plexus (ICC-MP) and within the whole circular muscle including the deep muscular plexus (ICC-CM). The results were given as the number of ICC-nuclei / 1 mm for the ICC-MP and the number of ICC-nuclei / 1 mm² of the circular muscle layer for the ICC-CM. The measures were taken by ocular micrometer. As controls, ten cases of small intestinal resections due to carcinoma and ten cases of colon resections due to diverticulosis were stained and counted similarly. Vacuolisation and cellular atrophy/hypertrophy are judged qualitatively based upon the examination of several hundred bowel-specimens after CD 177-staining from both controls and diseased bowels.

Evaluation of autonomic nerve (AN) function

The AN function tests were taken 9.0 ± 7.7 years after the debute of symptoms of CD and 18.6 ± 7.0 years after the debute of UC .

Deep-breathing test. The subjects performed six maximal expirations and inspirations in the supine position during recording of a continuous electrocardiogram (ECG). The expiratory/inspiratory (E/I) ratio was calculated from the mean value of the longest R-R interval during expiration and the shortest R-R interval during inspiration. This is an established test of vagal, parasympathetic nerve function (14).

Orthostatic test. The subject was tilted rapidly (2 sec) to the upright position (90°), and the immediate heart rate changes (an immediate acceleration followed by a

transient deceleration) were recorded on the ECG. These alterations in heart rate are determined by means of the acceleration index (AI) $[(A-B)/A] \times 100$; A =RR interval at rest, B = shortest RR interval before the transient deceleration; and by means of the brake index (BI) $[(C-B)/A] \times 100$; C = longest RR interval during the deceleration (15). The acceleration and brake indices evaluate not only parasympathetic nervous tone but also sympathetic nerve function (16).

Interpretation of autonomic test results. To match for age, all test results were expressed in age-related values-that is, SD-as previously described (17). An age-related value below -1.64 SD was considered abnormal and signified autonomic neuropathy (18).

Statistical analyses

Evaluation of the clinical course, histopathological findings, and AN function tests was performed blindly by 3 independent investigators, specialists in each area. Values are expressed as median [interquartile ranges]. Mann-Whitney U test was used for statistical calculations between groups, Spearman correlation for correlation and Fishers exact test to compare the frequencies of findings. $P < 0,05$ was considered statistically significant.

Ethical Consideration

This retrospective study was approved by the Ethics Committee at Lund University (LU 262-03). All patients gave informed consent before the data were analysed.

Results

Histopathology

Ganglioneuritis

Lymphocytic ganglioneuritis (Fig. 1) was observed in 11 of 19 cases of CD (58%). The inflammation was always patchy and did not contain granulomatous components or giant cells. Similar inflammatory neuropathy was seen in the large intestine in 5 of 11 patients with UC (42%). Ganglioneuritis was only seen in the small bowel in CD but not in UC ($p < 0.0001$). Eosinophilic leucocytes were part of the inflammatory infiltrate of the ganglioneuritis only in patients with UC (4 of 5 UC-cases with ganglioneuritis) but not in CD. No signs of visceral myopathy or degenerative neuropathy were observed in any of the patients.

Interstitial cells of Cajal

In 10 of 16 CD patients, the ICCs in the small bowel were atrophic, small and showed cytoplasmic vacuolisation (Fig 2). In 6 of these 10 patients there was also ganglioneuritis. The number of cells in the ICC-CM was decreased compared to controls (Fig 3). No signs of degeneration were seen in the colonic ICCs; the ICC-CM was hyperplastic in 5 of 7 patients compared to controls. In UC, no degeneration of the Cajal-cells occurred but hyperplasia was found in ICC-CM of the colon (Fig 4). The ICCs in the ileum were normal in all UC-patients. There was a positive correlation between hyperplasia of ICCs in colon and ganglioneuritis ($p = 0.03$).

Clinical and autonomic nervous follow up

The clinical expression of IBS- or dyspepsia-like symptoms did neither correlate with any histopathological findings nor autonomic neuropathy (data not shown). There was a positive correlation between BI and E/I ratio ($r_s = 0.54$, $p < 0.002$). Ganglioneuritis in CD, but not UC, correlated with higher values of BI ($r_s = 0.47$; $p < 0.01$) and E/I ($r_s = 0.385$; $p < 0.05$). The

indices of BI and EI were lower in patients with CD and UC than in healthy subjects, but the values were in the majority of cases not below the reference values. Patients with CD who also exhibited ganglioneuritis had more normal values than CD patients without ganglioneuritis (Fig 5).

Discussion

This is the first study describing ganglioneuritis of the autonomic enteric nervous system both in CD and UC, combined with pathological changes of ICCs in otherwise histologically normal segments. Damage to ICCs in the small intestine led to vacuolisation and a reduced amount of cells, whereas damage to colonic ICCs led to hyperplasia. There was no correlation between histopathology and the clinical signs and symptoms of IBS or dyspepsia. While patients with CD and UC had low autonomic BI and EI, inflammatory neuropathy in CD rendered more normal values.

Inflammatory neuropathy ultimately results in impaired gut motility and transit (19). Thus, the development of enteric neuropathy and damage to ICCs in patients suffering from CD and UC, may explain the earlier observations in these diseases with coexistence of CIPO (10) and gastrointestinal dysmotility not explained by an acute relapse of the disease (20, 21).

Our first important histopathological observation was the occurrence of ganglioneuritis in otherwise histologically normal parts of the bowel in both CD and UC. Previous studies described the histopathological abnormalities in otherwise inflamed areas (5, 7). The non-granulomatous inflammatory neuropathy in non-inflamed parts of the bowel in the present study in CD can be explained by the patchy and transmural nature of CD affecting various tissue components of the bowel. Our findings even suggest that similar phenomenon may occur in UC.

The second important histopathological finding of our study was the alteration of the ICCs in both CD and UC. These findings confirm what a few earlier studies have described about degenerative ultrastructural changes of ICCs both in humans with UC (6) and in inflamed canine bowel (22). In endothelin ETB receptor null rat, which is a model for inflammation in the bowel, Suzuki and coauthors (23) described the loss of ICCs in

connection with muscularis inflammation and increase of intramuscular and intermyenteric macrophages. In CD, Porcher and co-workers (8) described the almost complete abolition of ICCs within the longitudinal and circular muscle layers and a significant reduction in numbers within the myenteric and deep muscular plexuses. There is some discrepancy between our findings of the absence of degenerative ICC-changes in UC and the observations in the submucosal ICCs described by Rumessen (6). This may depend on that we did not examine our biopsies with electron microscopy. Another explanation can be that our analysis was concentrated on the intramuscular and intermyenteric ICCs. The vacuolisation seen in our CD patients can be the manifestation of either lipid-accumulation or hydrophic degeneration (6). The density-decrease of ICCs as described by Porcher and co-workers (8) can be explained by both the atrophy and loss of ICCs. It is tempting to speculate that in our patients the ICC-hyperplasia might be a compensatory process to restore normal bowel motility.

The enteric inflammatory neuropathy described by Törnblom *et al.* (3), supposed to be responsible for the symptoms in idiopathic IBS, was similar to the findings in our patients. As neither abnormal AN tests nor histopathology correlated with symptoms from IBS in the present study in contrast to our earlier findings in idiopathic IBS (3, 12), this infers that IBS is a heterogenous disease. We speculate if there is a different aetiology in idiopathic IBS compared with IBS in CD and UC. Further, the heterogenous histopathologic abnormalities found in different studies regarding functional gastrointestinal disorders suggest that the primary aetiology may differ between IBS patients (3, 24, 25, 26). The discrepancy between autonomic nervous tests and functional bowel symptoms implies that histopathological examination is necessary to diagnose enteric neuropathy. The relative small sample size in this study is a limitation. However, if there is no tendency to statistically significant correlation in a cohort of 30 cases, we can still not use these methods for clinical implications of which patients who suffer from enteric neuropathy or not, and the ethiology to

IBS and dyspepsia remains obscure. Further, the patchy localisation of the diseases may also be of importance for the results. Nevertheless, it is not possible to histopathologically examine the entire human gastrointestinal tract in each patient.

Increased firing of abnormally excitable nociceptive fibres has been discussed (27). The more normal values of BI and EI in CD with ganglioneuritis can be explained by abnormal afferent signalling from these areas that may lead to increased stress for the autonomic nervous system. The difference from UC in this aspect may depend on that these patients had no ganglioneuritis in the small intestine.

In conclusion, we describe the occurrence of lymphocytic inflammatory neuropathy in otherwise non-inflamed parts of the bowel together with pathological changes of ICCs in both CD and UC. These changes may explain the development of dysmotility in some of the patients, while it does not explain the symptoms of IBS and dyspepsia.

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Competing interests: The authors declare that they have no competing interests

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Table 1. Summarising the number of histopathological and immunohistochemical (IH) analyses performed *

Disease	Total number of patients	Histopathology and CD3 IH		Interstitial Cajal-cells <i>c-kit</i> (CD117) IH	
		Small intestine	Large intestine	Small intestine	Large intestine
Crohn's disease	19	18	17	16	7
Ulcerative colitis	11	10	11	5	10

* = In some cases there was no intestine available or the tissue was unsuitable for analysis

Legends to figures

Figure 1.A: Case 1800/81, Crohn's disease. Myenteric ganglion with heavy lymphocytic infiltrate both around and within the ganglion corresponding score 3 ganglionitis (haematoxylin and eosin; original magnification: x 150). B: Case 19132/98, Crohn's disease, myenteric ganglion. The inflammatory infiltrate of the ganglionitis is predominated by T-lymphocytes (CD3 immunoperoxidase, original magnification: x 150). C: Case 3258/95, Crohn's disease, part of the circular muscle. Heavy lymphocytic infiltrate with the formation of "pearl-band" along small nerve fibres representing neuritis (haematoxylin and eosin; original magnification: x 280).

Figure 2. Case 1429/03, Crohn's disease. Detail of the interstitial cells of Cajal within the intermyenteric/periganglionic plexus (ICC-MP) from tangentially embedded tissue. Many ICCs are vacuolised (long arrows). The short, thick arrow shows a "signet-ring-like" ICC due to the pressure of a large cytoplasmic vacuole (*c-kit* immunoperoxidase; original magnification x 360).

Figure 3. The number of interstitial cells of Cajal in the deep muscular plexus (ICC-CM)/ 1 mm² of the small intestine in controls (n=10) and in patients suffering from Crohn's disease (CD) (n=19). Mann Whitney U test.

Figure 4. The number of interstitial cells of Cajal in the deep muscular plexus (ICC-CM)/ 1 mm² of the colon in controls (n= 10) and in patients suffering from ulcerative colitis (UC) (n=11). Mann Whitney U test.

Figure 5. The correlation between ganglioneuritis and brake index (BI) values expressed as standard deviation (SD) in patients suffering from Crohn's disease. $R=0.553$; $P=0.019$. Score 1 = scattered lymphocytes, score 2 = >10 - <20 lymphocytes/ganglion and score 3 = >21 lymphocytes/ganglion