Enteric Nerves and Interstitial Cells of Cajal Are Altered in Patients With Slow-Transit Constipation and Megacolon

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Background & Aims: **A variety of gastrointestinal motility disorders have been attributed to alterations of interstitial cells of Cajal and malformations of the enteric nervous system. This study evaluates both the distribution of interstitial cells of Cajal and the pathohistology of the enteric nervous system in 2 severe human colorectal motility disorders.** ***Methods:*** **Colonic specimens obtained from patients with slow-transit constipation (n = 11), patients with megacolon (n = 6), and a control group (n = 13, nonobstructing neoplasia) were stained with antibodies against c-kit (marker for interstitial cells of Cajal) and protein gene product 9.5 (neuronal marker). The morphometric analysis of interstitial cells of Cajal included the separate registration of the number and process length within the different regions of the muscularis propria. The structural architecture of the enteric nervous system was assessed on microdissected whole-mount preparations.** ***Results:*** **In patients with slow-transit constipation, the number of interstitial cells of Cajal was significantly decreased in all layers except the outer longitudinal muscle layer. The myenteric plexus showed a reduced ganglionic density and size (moderate hypoganglionosis) compared with the control group. Patients with megacolon were characterized by a substantial decrease in both the number and the process length of interstitial cells of Cajal. The myenteric plexus exhibited either complete aganglionosis or severe hypoganglionosis.** ***Conclusions:*** **The enteric nervous system and interstitial cells of Cajal are altered concomitantly in slow-transit constipation and megacolon and may play a crucial role in the pathophysiology of colorectal motility disorders.**

Chronic constipation is a common and costly complaint. Although most cases can be managed with increased fiber intake and laxatives, a subgroup of patients has severe intractable constipation due to colonic inertia (slow-transit constipation [STC]). Surgical intervention is considered a therapeutic option for patients with STC who do not respond to aggressive medical therapy.[1] The underlying pathogenetic mechanisms of STC are poorly understood and believed to be multifactorial.[2] Among the various etiologic concepts discussed, alterations of 2 cell systems have been suggested to cause the disturbed intestinal motor functions: the enteric nervous system (ENS) and, more recently, the interstitial cells of Cajal (ICC).

The crucial role of a morphologically intact ENS for the mediation of normal motility patterns is best shown if enteric ganglia are completely missing. After the first description of intestinal aganglionosis in Hirschsprung's disease,[3] there is also convincing evidence that nonaganglionic alterations of the ENS such as hypoganglionosis or hyperganglionosis may lead to severe disturbances of colonic motor functions, including the development of megacolon.[4,5] In regard to patients with STC, several qualitative, as well as quantitative, changes of the ENS have been described. The neuropathologies included an abnormal enteric neurochemistry,[6–9] a decrease in argyrophilic neurons,[10] a decrease in intraganglionic neurofilaments,[11] and hypoganglionosis of the myenteric plexus.[12]

In identifying a further histopathologic correlate for STC apart from neuronal malformations, the ICC have more recently been considered a promising candidate. ICC are regarded as intestinal pacemakers due to their ability to generate slow-wave activity.[13,14] In addition, ICC have been considered to function as intermediaries in neural control of the gut by transducing inhibitory and excitatory signals from the ENS.[15,16] The recognition of these physiologic properties has led to the study of ICC in several gastrointestinal motility disorders. As for the human colon, first evidence of a relative loss of ICC was reported by Yamataka et al.[17] and Vanderwinden et al.,[18] who studied colonic specimens from children with congenital aganglionosis. However, few data are available

Abbreviations used in this paper: ENS, enteric nervous system; ICC, interstitial cells of Cajal; PGP, protein gene product; SCF, stem cell factor; STC, slow-transit constipation.

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concerning alterations of ICC in adult patients with colorectal motor dysfunctions. Although the development of megacolon due to intestinal innervation disorders also occurs in adolescence and adulthood,[19] systematic studies for ICC have not been performed in these patients. In a case report, Faussone-Pelligrini et al.[20] described an adult patient with megacolon due to acquired intestinal hypoganglionosis associated with a relative loss of ICC. The only study in adult patients with STC was performed by He et al.,[21] who addressed the distribution of ICC by means of a volumetric analysis. The data showed a significantly decreased volume of ICC in all layers of the colonic muscularis propria compared with controls. However, the assessment of neuronal structures was confined to nerve fibers within the circular muscle layer and did not include the ganglia of the nerve plexus layers.

The aim of the present study was to determine quantitative changes of ICC in adult patients with severe colorectal motility disorders. According to their clinical presentation, the patients were subdivided into a group with STC and a group with megacolon. In addition to the morphometric analysis of ICC, both groups were subjected to a detailed histopathologic assessment of the ENS to characterize the extent and severity of concomitant innervation disorders.

Materials and Methods

Patients

Control group. Patients in the control group (n = 13; mean age, 61.1 years; range, 23–83 years; 7 women and 6 men) underwent partial colectomy for nonobstructive carcinoma (T1–T2) or adenoma not suitable for endoscopic resection. All control patients reported normal bowel habits with stool frequencies at regular intervals. Radiographic studies showed normal anatomy of the colon and rectum. Rectal manometry did not show any abnormalities.

Patients with STC. All patients (n = 11, mean age, 49.8 years; range, 19–70 years; all women) had a history of long-standing intractable constipation with defecatory frequencies ranging between once per 10 and 20 days. Medical therapy did not improve their bowel habits, and defecation frequently had to be achieved by manual emptying or the application of enemas. The colonic transit time studied by radiopaque markers was markedly increased and ranged between 140 and 220 hours. Secondary causes of constipation, as well as motor dysfunctions of the upper gastrointestinal tract, had been previously excluded. Rectal manometry did not show any abnormalities. On defecography analysis, the patients showed neither an outlet obstruction nor megacolon. The patients underwent subtotal colectomy with ileorectostomy performed by either laparotomy (n = 6) or laparoscopy (n = 5).

Patients with megacolon. All patients (n = 6; mean age, 44.6 years; range, 17–67 years; 3 women and 3 men) had chronic constipation and abdominal distention dating back to childhood. However, a definitive diagnosis had not been made until adolescence or adulthood. Barium enema examinations showed megacolon that extended from the upper rectum to the sigmoid and descending colon. At rectal manometry, the inhibitory rectoanal reflex was absent in 4 patients. These patients had shown an absence of nerve cells and a positive acetylcholinesterase reaction in submucosal rectal biopsy specimens highly suggestive of Hirschsprung's disease. Submucous biopsy specimens obtained from the other 2 patients had shown normal histology. The dilated colonic segments were removed by deep anterior resection and transversorectostomy.

For all groups, specimens were obtained immediately after resection from corresponding sites of the rectosigmoid colon and processed for immunohistochemical visualization of ICC and the ENS. The study of human tissue was approved by the local ethical committee of the Medical University of Luebeck.

ICC

Immunocytochemistry. The specimens were cut into blocks (20-mm length) and fixed in a solution containing 2% paraformaldehyde in 0.1 mol/L phosphate-buffered saline supplemented with 0.2% picric acid. After dehydration and cryoprotection with 10% sucrose, serial cryosections (10 μm thickness) were cut perpendicular to the intestinal axis. ICC were visualized by indirect immunocytochemical demonstration of the c-kit receptor. The intensity of the immunoreaction was enhanced by the application of a tyramide signal amplification system (TSA Indirect; NEN Life Science Products, Boston, MA) according to the manufacturer's instructions. The sections were pretreated with 0.5% blocking reagent and incubated in polyclonal rabbit antiserum (1:500 dilution) directed against the c-kit receptor (C-19; Santa Cruz Biotechnology, Santa Cruz, CA). After incubation in biotinylated goat anti-rabbit immunoglobulin G (1:100 dilution) and streptavidin-peroxidase (1:100 dilution), the biotinyl tyramide amplification reagent was applied. Before chromogenic visualization with aminoethylcarbazole, the slides were treated again with streptavidin-peroxidase (1:100 dilution). To identify the nuclei of the red-stained ICC, the sections were counterstained with Meyer's hematoxylin. No staining of ICC was observed when the antibody had been omitted.

Morphometric analysis. Morphometric analysis was performed to determine both the number and the process length of ICC per area. The determination of the process length was achieved by means of an intersection counting method. The measuring grid (350 μm × 350 μm) consisted of 8 horizontal and 8 vertical lines with the lines 50 μm apart. The following equation was used to calculate the process length of ICC per area:

$$\text{Process Length/Area (mm/mm}^2\text{)} = \text{Distance Between Grid Lines} \times \text{Counted Intersections} \times \text{Grid Area}^{-1}.$$

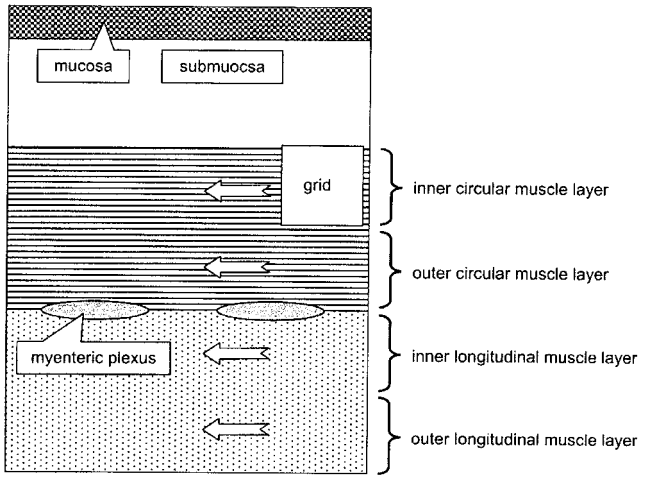

Figure 1. For the morphometric analysis of ICC, the muscularis propria was subdivided into 4 layers. Each layer was analyzed with the measuring grid to determine the number and process length of ICC per area (see text for details).

To determine the number of ICC per area, the nuclei observed within the grid were counted according to the following equation:

Number of ICC/Area (No./mm^2) = Counted Nuclei × Grid Area^{-1}.

The different subpopulations of ICC described within the human colon[22] were taken into account by dividing the muscularis propria into 4 layers: (1) inner circular muscle layer including the submucosal border, (2) outer circular muscle layer adjacent to the myenteric plexus, (3) inner longitudinal muscle layer adjacent to the myenteric plexus, and (4) outer longitudinal muscle layer adjacent to the serosa (Figure 1).

In each of these layers, 20 areas were examined with a 25× objective using the previously described measuring grid (total area measured = 7000 μm × 350 μm). A 63× objective was used to identify those ICC processes that were less readily discernible at lower magnification. The data obtained for ICC processes and nuclei were averaged and expressed as mean values ± SD for each group. Statistical comparison between the different groups was performed using the Mann–Whitney U test with $P \leq 0.05$ considered an indicator of significance.

ENS

To comprehensively visualize the structural architecture of the intramural nerve plexus, the colonic wall was dissected into whole mounts as described previously.[23] Briefly, rectangular segments (40 mm length, 20 mm width) were excised from the resected colonic specimens, stretched (60 mm length, 30 mm width), and fixed in a solution containing 2% paraformaldehyde in phosphate-buffered saline supplemented with 0.2% picric acid for 24 hours. After treatment with 50% ethanol (1 hour), the specimens were treated with 0.05% thimerosal (24 hours) and 0.1% $NaCNBH_3$ (30 minutes). Under stereomicroscopic control, the intestinal wall was separated into its different layers to expose the myenteric and submucosal plexus compartments. To dissolve connective tissue components, the whole mounts were subjected to a maceration procedure with 30% KOH for 20 minutes. After pretreatment with 10% normal goat serum, the samples were incubated in a polyclonal rabbit antiserum (1:800 dilution) directed against protein gene product 9.5 (PGP 9.5; Ultraclone, Isle of Wight, England). Incubation in goat anti-rabbit immunoglobulin G (1:100 dilution) was followed by application of an anti-rabbit peroxidase/antiperoxidase complex (1:100 dilution). The immunoreaction was visualized with the chromogen chloronaphtol.

Results

ICC

Control group. ICC were readily discernible throughout the entire smooth muscle coat. ICC processes running within the circular and longitudinal muscle layer were orientated preferably parallel to the axis of the smooth muscle cells (Figure 2*A*). At the submucosal border, ICC formed a specialized network that extended from the inner circular muscle layer into the submucosa. The myenteric ganglia were surrounded on both sides by ICC that frequently contacted the ganglionic borders. The highest density of ICC was found within the outer circular muscle layer (32.1 per mm^2) followed by the inner circular muscle layer (24.6 per mm^2). Within the longitudinal muscle layer, the density of ICC decreased toward the serosa, yielding 15.1 per mm^2 in the inner portion and 3.6 per mm^2 in the outer portion (Table 1). The total process length of ICC per mm^2 measured 9.7 mm in the inner circular muscle layer, 13.4 mm in the outer circular muscle layer, 9.5 mm in the inner longitudinal muscle layer, and 2.2 mm in the outer longitudinal muscle layer (Table 2).

Patients with STC. The topographic organization of ICC in patients with STC basically resembled the structural features of ICC observed in the control group (Figure 2*B*). All ICC subpopulations (located at the submucosal border, adjacent to the myenteric plexus, and within the muscularis propria) were present. However, the morphometric assessment showed a significant numerical decrease of ICC in all layers examined except for the outer longitudinal muscle layer. The mean number of ICC per mm^2 was reduced to 11.7 in the inner circular muscle layer, 14.1 in the outer circular muscle layer, and 8.4 in the inner longitudinal muscle layer (Table 1). Although, in general, the mean values of the process length were lower, they did not differ significantly from the control group in any of the 4 layers examined (Table 2). On histologic examination, some ICC exhibited a

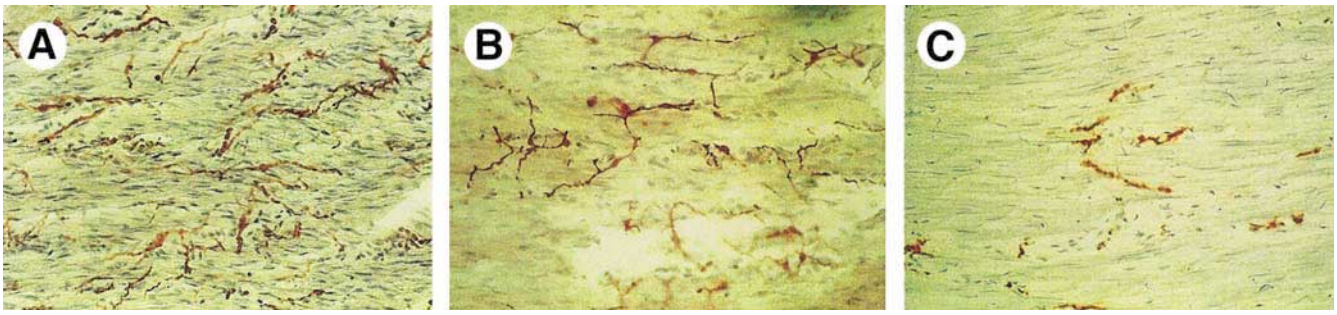

Figure 2. Distribution of ICC. (*A*) Control group: ICC are densely distributed within the muscularis propria. Intramuscular ICC are shaped ovally and have bipolar processes that are orientated preferably parallel to the axis of the smooth muscle cells. (*B*) STC: Compared with the control group, ICC are decreased in number. Although most ICC processes are readily discernible, some ICC exhibit blunted and shortened ramifications. (*C*) Megacolon: Both the number and the process length of ICC are remarkably decreased. However, the smooth muscle coat is not completely bare of pacemaker cells but contains ICC within all layers examined. C-kit immunohistochemistry, circular muscle layer; original magnifications 20×.

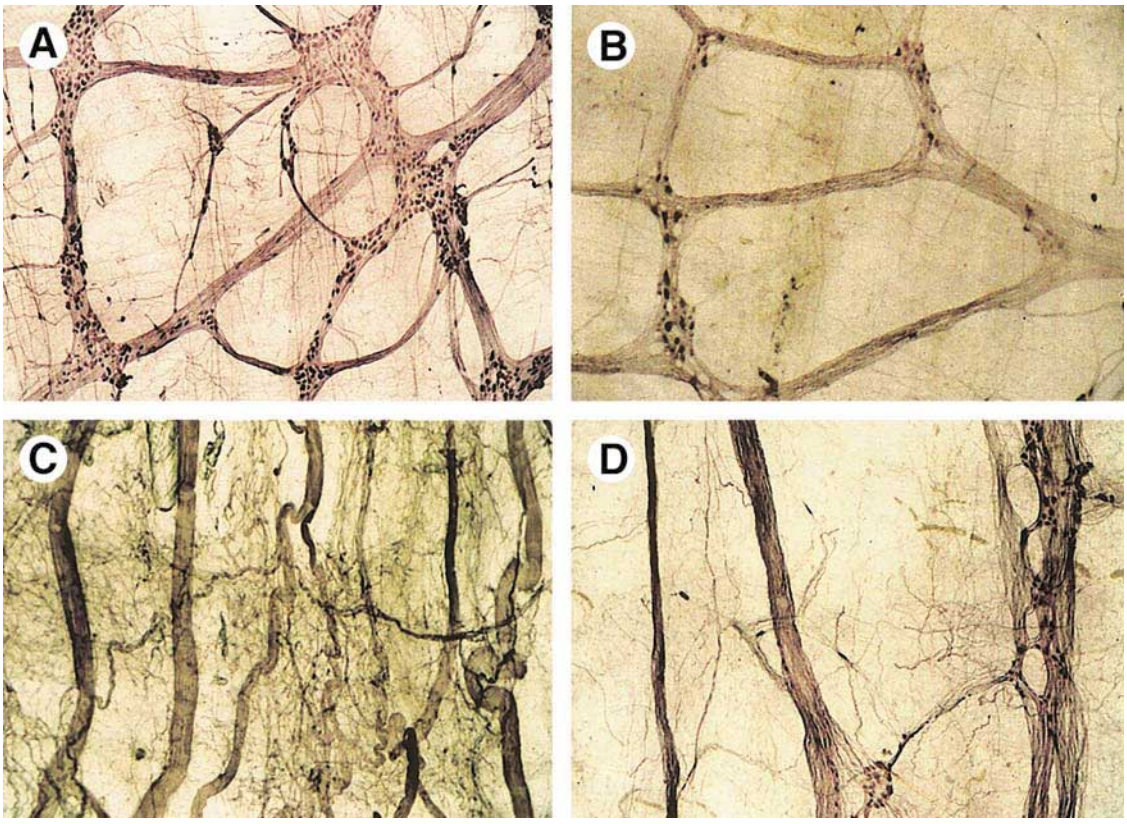

Figure 3. Architecture of the ENS (myenteric plexus). (*A*) Control group: The nerve network is composed of ganglia and interconnecting nerve fiber strands. Smaller branches (tertiary nerve fiber strands) ramify from primary and secondary nerve fiber strands and extend into the adjacent muscle layers. Although most of the neurons are located within the ganglia, some nerve cell bodies are also observed within the nerve fiber strands. (*B*) STC: Compared with controls, the meshes of the nerve network are widened and the ganglia are reduced in size, containing a decreased amount of nerve cells. (*C*) Megacolon: Thickened nerve fiber strands run within the intermuscular plane and extend parallel to each other in a caudocranial direction. The affected bowel wall is completely bare of ganglia (aganglionosis). (*D*) Megacolon: Two of 6 patients with megacolon did not show complete aganglionosis but exhibited severe oligoneuronal hypoganglionosis. Few nerve cells are encountered within the hypertrophied nerve fiber strands and form small intrafascicular ganglia. However, the structural features are similar to those observed in specimens with complete aganglionosis (e.g., parallel-orientated nerve fiber strands; compare with *C*). PGP 9.5 immunohistochemistry applied to whole-mount preparations; original magnification 5×.

Table 1. Density of ICC

ICC nuclei (no. mm^2)	Control (mean ± SD)	STC		Megacolon	
		Mean ± SD	*P*	Mean ± SD	*P*
Circular muscle					
Inner portion	24.6 ± 11.9	11.6 ± 4.5	<0.05	10.0 ± 6.8	<0.05
Outer portion	32.1 ± 13.7	14.0 ± 6.7	<0.05	8.4 ± 7.5	<0.05
Longitudinal muscle					
Inner portion	15.1 ± 7.9	8.4 ± 3.4	<0.05	4.8 ± 2.8	<0.05
Outer portion	3.6 ± 2.4	2.4 ± 1.0	NS	1.1 ± 1.0	<0.05
Entire tunica muscularis	18.8 ± 3.3	9.2 ± 2.8	<0.05	6.1 ± 3.2	<0.05

blunting of their processes and the network appeared disrupted.

Patients with megacolon. ICC were present in all layers of the muscularis propria, including the regions at the submucosal border and adjacent to the myenteric plexus. Nevertheless, histologic assessment showed an obvious decrease in both the number and the process length of ICC compared with the control group (Figure 2*C*). This observation was confirmed at morphometric analysis; whereas the numerical reduction was statistically significant within all layers examined (Table 1), the process length was decreased significantly except for the inner circular muscle layer (Table 2).

ENS

Control group. All patients had a normally configured ENS. The most prominent intramural nerve network was the myenteric plexus, composed of densely distributed ganglia and interganglionic nerve fiber strands delimited by the circular and longitudinal muscle layer (Figure 3*A*). Whereas primary and secondary nerve fiber strands established the connections between the ganglia, tertiary nerve fiber strands constituted a fine structured network ramifying into the adjacent muscle layers. The submucosal plexus comprised 3 separate nerve networks that could be distinguished by its different topography and architecture (not shown): the plexus submucosus extremus with thin nerve fiber strands extending at the outermost submucosal border, the plexus submucosus externus composed of elongated ganglia and interconnecting nerve fiber strands, and the plexus submucosus internus located adjacent to the mucosa and composed of small round ganglia and rather thin nerve fiber strands.

Patients with STC. The general features of the ENS in patients with STC were similar to those observed in the control group. Both the myenteric and the submucosal plexus layers showed the typical network character and were equipped with ganglia located at the intersections of nerve fiber strands. However, compared with the control group, the myenteric plexus of patients with STC showed a notable decrease in ganglionic density and size. The meshes of the nerve network were widened, and the ganglia contained a reduced amount of nerve cells (Figure 3*B*). According to a consensus conference on innervation disorders of the colon,[24] these alterations were classified as moderate oligoneuronal hypoganglionosis. The structural architecture of the submucosal plexus layers did not differ substantially from the control group. Only one patient had a hypoganglionic submucosal nerve network with a decreased number of ganglia. None of the patients exhibited hyperplastic changes (e.g., giant ganglia) of the submucosal plexus, thereby excluding the diagnosis of intestinal neuronal dysplasia.

Table 2. Process Length of ICC

ICC processes (total length, mm/mm^2)	Control (mean ± SD)	STC		Megacolon	
		Mean ± SD	*P*	Mean ± SD	*P*
Circular muscle					
Inner portion	9.7 ± 3.7	7.9 ± 2.5	NS	5.3 ± 4.2	NS
Outer portion	13.4 ± 5.0	10.8 ± 4.3	NS	4.7 ± 5.5	<0.05
Longitudinal muscle					
Inner portion	9.5 ± 1.9	8.5 ± 3.3	NS	2.8 ± 3.1	<0.05
Outer portion	2.2 ± 1.4	2.7 ± 2.4	NS	0.6 ± 0.5	<0.05
Entire tunica muscularis	8.7 ± 2.4	7.5 ± 2.7	NS	3.4 ± 3.2	<0.05

Patients with megacolon. Patients with megacolon had severe malformations of the ENS. In those patients suspected of having Hirschsprung's disease on preoperative biopsy specimens (n = 4), the diagnosis of complete intramural aganglionosis was confirmed. The affected colonic segments were bare of intramural ganglia and characterized by thickened nerve fiber strands extending between the longitudinal and circular muscle layer and throughout the submucosa. In the intermuscular plane, the hypertrophied nerve fiber strands were oriented in a caudocranial direction (Figure 3*C*); in the submucosa, they formed an irregular network (not shown). A similar spatial arrangement of the ENS was found in the other 2 patients; myenteric nerve fiber strands extended in a caudocranial direction and did not form a normal network. However, in contrast to the cases with complete aganglionosis, the specimens contained disseminated nerve cells within the thickened nerve fiber strands (intrafascicular ganglia) (Figure 3*D*). Because the number of nerve cells was highly decreased compared with both the control group and patients with STC, this innervation disorder was classified as severe oligoneuronal hypoganglionosis.

Discussion

Alterations of ICC

It has been established that, apart from the ENS, ICC are actively involved in the mediation of intestinal motility. The scientific "renaissance" of ICC, originally described about 100 years ago,[25] is based on the observations that ICC generate electrical slow-wave activity[13,14] and are capable of mediating neuronal input.[15,16,22] Evidence of these 2 main roles has mostly been derived from animal experiments in which ICC have been removed mechanically[26] or damaged chemically[27] or in which the kit tyrosine kinase receptor has been inactivated either by blocking antibodies[28] or loss of function due to spontaneous mutation of the c-kit gene.[13,14,29] The loss of ICC function abolishes electrical slow waves and leads to decreased contractile response, resulting in delayed intestinal transit. Alterations of ICC, either structural or quantitative in nature, have been reported in a variety of human gastrointestinal motility disorders, such as in achalasia, pyloric stenosis, chronic intestinal pseudo-obstruction, or Hirschsprung's disease, suggesting a functional impact of ICC on the maintenance of normal gastrointestinal motility patterns.[30–32]

STC. The morphometric analysis in patients with STC (n = 11) yielded a significantly decreased density of ICC. Similar findings have been reported in a recent study performed in 6 patients with STC.[21] The distribution of ICC has been assessed by volumetric techniques calculating the relative volume of immunoreactive ICC within the surrounding nonimmunoreactive tissue (measured area per specimen, 1.6 mm^2). Although this method offers several advantages over conventional cell counting techniques (e.g., 3-dimensional visualization of ICC, identification of single cells in areas of high cellular density), the morphometric approach applied in the present study allowed a reliable stereologic determination of both the number and the process length of ICC (measured area per specimen, 9.8 mm^2). Because the measurements are based on conventional light microscopy and widely available morphometric devices, they can easily be reproduced.

Despite the different morphometric approaches, both the present study and the study by He et al.[21] have shown statistically significant alterations of ICC in patients with STC. The numerical decrease of ICC also included those ICC located at the submucosal border, which have been considered to represent the pacemaker region within the human colon.[33] The relative loss of intestinal pacemaker cells may represent an underlying morphologic substrate for the decreased smooth muscle contractile activity, thereby contributing to the prolonged colonic transit time. Nevertheless, in both studies, there is no clear cutoff between controls and patients with STC; the values of the control group overlap with those obtained from patients with STC, suggesting that a lowered number of ICC per se does not necessarily lead to symptoms of chronic constipation. The exact amount of ICC required for a clinically satisfactory propelling function is not known, and a certain degree of redundancy seems to be likely.

The overall process length of ICC per intestinal area (9.8 mm^2) as measured by the intersection counting method did not differ significantly between patients with STC and the control group. However, at histologic evaluation, it was apparent that ICC of patients with STC exhibited considerably shortened but more numerous processes (multiple blunted ramifications), an observation that was obviously missed by the intersection counting method (registration of overall and not individual ICC process length) but had been described previously in the study by He et al.[21] using a volumetric approach to analyze the altered ICC morphology. Based on these findings, it may be suggested that ICC have compensated their numerical decline by developing more processes, however, of smaller size.

Megacolon. One of the first bits of evidence that ICC are altered in human gastrointestinal motility disorders was derived from studies examining the distribu-

tion of ICC in congenital megacolon.[17,18,34,35] Adult patients with megacolon have not been systematically studied so far. To our knowledge, there is only one case report of acquired megacolon[20] and a series of 6 adult patients with chagasic megacolon[36] in which ICC have been examined. Moreover, all studies used either a mere descriptive or semiquantitative approach to determine quantitative alterations of ICC, which might explain the contradictory findings regarding the distribution of ICC in patients with megacolon. In contrast to patients with STC, both the number and the process length of ICC were significantly reduced in patients with megacolon. The substantial decrease of ICC shown in the present study is in accordance with previous reports[17,18,35] but not with the study by Horisawa et al.,[34] who found unchanged ICC populations within the aganglionic bowel segment. Surprisingly, the highest density of remaining ICC was found at the submucosal border of the circular muscle layer, the area in which pacemaker activity has been mapped for the human colon.[33] This observation may question whether the pacemaker region is exclusively confined to ICC located at the submucosal border. However, on histologic examination, the network of ICC at the submucosal border seemed to be disrupted and discontinuous (more than 50% decrease of ICC) resembling similar features as reported by Vanderwinden et al.[18]

In both groups (patients with megacolon and with STC), the ICC populations were not totally depleted, suggesting either a progressive decline of ICC or a single event that caused the numerical reduction. Clinically, all patients had long-standing severe constipation dating back to childhood. Conservative treatment relieved the symptoms only temporarily and failed on long-term observation. Based on the clinical picture, the disease has to be considered a static rather than a progressive process, suggesting that the reduced amount of ICC was caused by a congenital developmental disorder. However, because the present data only reflect the actual distribution of c-kit immunoreactive ICC at the time of operation, it cannot be excluded that ICC may have redifferentiated into non–c-kit expressing cells or cells of smooth muscle phenotype before the histologic assessment. Monitoring the fate of ICC over a long period (e.g., alterations of their cytoarchitecture and their immunocytochemical and electrophysiologic properties) seems to be important but requires a sophisticated study design (e.g., collection of material at different time intervals, subtle histomorphologic and functional assessment of ICC, close clinical follow-up).

Malformations of the ENS

STC. The neuronal decrease shown in the present study is in accordance with previous studies reporting a loss of argyrophilic myenteric neurons,[10] a reduction in intraganglionic neurofilaments,[11] and a reduced number of PGP 9.5 immunoreactive neurons within myenteric ganglia.[12] Although the meshes of the myenteric plexus were widened and exhibited a reduced ganglionic density, the network was not disrupted and basically displayed similar architectural features as observed in controls. This might explain why a recent study using PGP 9.5 immunohistochemistry for the determination of the myenteric fraction on conventional cross sections yielded no significant differences between patients with STC and controls.[37] Alterations of the submucosal plexus previously reported in patients with STC (intestinal neuronal dysplasia with hyperplasia of ganglia and nerve fiber strands)[38] could not be confirmed in the present study.

Megacolon. In patients with megacolon, the intramural nerve networks were completely disintegrated and replaced by hypertrophic nerve fiber strands. The fact that 2 of the 6 patients did not have a complete lack of intramural neurons clearly shows that, apart from aganglionosis (classic Hirschsprung's disease), nonaganglionic innervation disorders (e.g., severe hypoganglionosis) are capable of causing megacolon. This concept is supported by an early contribution of Howard et al.[4] and 2 more recently published case reports of an infant[39] and an adult patient[20] with myenteric hypoganglionosis who developed megacolon. The neuronal malformations described in these 2 cases were accompanied by a complete[39] and a relative[20] loss of ICC, respectively.

Concomitant Alterations of ENS and ICC

There is increasing evidence that many gastrointestinal motility disorders caused by neuronal malformations are associated with concomitant alterations of ICC (e.g., pyloric stenosis, achalasia, Hirschsprung's disease, chagasic megacolon, chronic intestinal pseudo-obstruction).[31,32] Furthermore, it can be deduced from the present data that the severity of neuronal malformations correlates with the extent of ICC alterations; the loss of nerve cells seems to parallel the loss of ICC. This observation may suggest mutual influences between the ENS and ICC during development. Animal experiments have provided clear evidence that, unlike enteric neurons, ICC are of mesenchymal origin and do not derive from neural crest cells.[40,41] However, although ICC and the progenitor cells of the ENS are of different origin, during embryogenesis, enteric neurons provide a source of the natural ligand for the c-kit receptor: the stem cell factor

(SCF).[42] SCF/kit signaling is required for the maintenance of the ICC phenotype,[41] and withdrawal of SCF leads to redifferentiation of ICC into a mesenchymal smooth muscle phenotype.[43] Thus, a complete or a relative lack of enteric neurons (aganglionic and hypoganglionic conditions, respectively) would abolish or reduce the neuronal source of SCF and may lead to a decreased population of phenotypically differentiated ICC.

Although this model fits well into the present findings, it remains speculative whether a deficient colonization of the bowel wall by neural crest–derived neurons causes a depletion of the ICC population. It has to be acknowledged that, in addition to neurons, the gut provides other sources of SCF. Smooth muscle cell lineages also express SCF; hence, enteric nerve cells are not necessarily required for the development of ICC.[41,44] Experimental studies have shown that, in aneural explants of murine[40] and chick[45] gut, ICC develop normal morphology. It is not yet clear if their function is fully maintained in the absence of enteric neurons which, under normal conditions, are intimately associated with ICC and considered to be their "physiologic partners." Even if the development of both cell systems can take place independently, the topographic and functional refinement between ICC and neuronal structures might be impaired during postnatal maturation.[32,41]

Conclusions

In summary, the present study suggests an etiologic link between severe disorders of colorectal motility and alterations of both the intestinal pacemaker cell system and the ENS. Our own and previous data[21] would speak in favor of a combined immunologic assessment of c-kit and PGP 9.5 expression in patients with idiopathic STC and megacolon. A meaningful histopathologic examination requires a standardized morphometric analysis and can be performed either postoperatively on resected colonic specimens or preoperatively on full-thickness biopsy specimens. This approach, if applied to a larger number of carefully selected patients, may further elucidate the underlying pathophysiology of functional gastrointestinal motility disorders still considered to be "idiopathic" in nature.

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