Pediatr Surg Int (2007) 23:1139–1152
DOI 10.1007/s00383-007-2022-7

REVIEW ARTICLE

Interstitial cells of Cajal in the normal gut and in intestinal motility disorders of childhood

Udo Rolle · Anna Piaseczna-Piotrowska · Prem Puri

Published online: 30 October 2007

Abstract Interstitial cells of Cajal (ICCs) are pacemaker cells which are densely distributed throughout the whole gastrointestinal tract. ICCs have important functions in neurotransmission, generation of slow waves and regulation of mechanical activities in the gastrointestinal tract, especially for the coordinated gastrointestinal peristalsis. Therefore, a loss of ICCs could result in gastrointestinal motor dysfunction. In recent years c-kit labeling has been widely used to study pathological changes of ICCs in gastrointestinal motility disorders. Paediatric gastrointestinal motility disorders such as hypertrophic pyloric stenosis, Hirschsprung's disease, total colonic aganglionosis, hypoganglionosis, intestinal neuronal dysplasia, internal anal sphincter achalasia, megacystis microcolon intestinal hypoperistalsis syndrome have been reported to be associated with loss or deficiency of ICCs networks. This review describes the distribution of ICCs in the normal gastrointestinal tract and its altered distribution in intestinal motility disorders of childhood.

Keywords Interstitial cells of Cajal · Gastrointestinal motility · Intestinal motility disorders · Childhood

U. Rolle
Department of Paediatric Surgery, University of Leipzig, Leipzig, Germany

A. Piaseczna-Piotrowska · P. Puri (✉)
Children's Research Centre,
Our Lady's Hospital for Sick Children,
Dublin 12, Ireland
e-mail: prempuri@ucd.ie

Introduction

Interstitial cells of Cajal (ICCs) were firstly described by the Spanish neuro-histologist Ramon y Cajal in 1893. He discovered fibroblast like cells in the intestine both in the muscularis externa and the stroma of the villi. Furthermore they were found around the acini and in the blood vessels of the pancreas and in the myocardium [1–5]. Specific staining characteristics with methylene blue and silver chromate lead to the assumption that these cells were primitive neurones. However, later studies employed light and electron microscopy and demonstrated that they were neither neurons nor Schwann cells [6–8]. Electron microscopy identified these cells as a unique class of cells distinct from the enteric nervous system (ENS) and referred to them as interstitial cells of Cajal (ICCs) [9]. Subsequently, the development of antibodies to c-kit has allowed routine identification of ICCs in pathology specimens. C-kit is a transmembrane protein kinase receptor which is essential for the development and function of ICCs. In the gut, c-kit is expressed only in ICCs and mast cells. ICCs have been shown to be densely distributed throughout the whole gastrointestinal tract. ICCs have important functions in regulation of mechanical activities in the gastrointestinal tract, especially for the coordinated gastrointestinal peristalsis. Therefore a loss of ICCs could certainly result in gastrointestinal motor dysfunction. There is clear evidence that loss of ICCs impairs the gastrointestinal peristalsis in animal models and in human gastrointestinal motility disorders [10, 11].

Embryological development of ICCs

In contrast to Cajal's opinion, ICCs are not derived from the neural crest [12–14]. Developmental studies have

clearly demonstrated that ICCs and smooth muscle cells (SMCs) arise from common mesenchymal precursors [14, 15]. Furthermore it has been shown that the normal development of ICCs depends on the expression of the protooncogene c-kit that encodes the receptor tyrosine kinase Kit. Kit signaling is essential for development and maintenance of ICCs in the embryonic gastrointestinal tract [16]. During embryonic development ICCs precursors express Kit at day 11 in the mouse. Signaling via Kit becomes essential for the development of ICCs at embryonic day 15 in the mouse [14]. Kit signaling is necessary for the development of ICCs as well as for the onset of electrical rhythmicity [14].

Numerous studies investigating the fetal and postnatal development of ICCs in the human gastrointestinal tract showed that the distribution of ICCs varies with gestational age and the region within the gastrointestinal tract (GIT). Furthermore, there is clear evidence that the maturation of ICCs continues postnatally [17–19]. C-Kit positive ICCs were demonstrated within the human stomach from 9.5 weeks of gestation and in the small and large bowel from 12 to 13 weeks. With increasing age dense network of ICCs are found around the myenteric plexus. Numerous muscular ICCs are expressed within the muscle layers and particularly at the innermost part of the circular muscle. Furthermore, the myenteric ICCs show an increase in cell size and increased number of individual cytoplasmatic processes during early development [17–19].

Function of ICCs

That the ICCs may act as pacemaker cells of the intestinal motility was proposed in 1915 by Keith [20]. Later on, electron microscopical studies showed a close association of ICCs with nerve terminals and gap junctions within SMCs which further suggested that the ICCs may be pacemakers providing a connection between muscle and nerves [9, 21–22].

Extensive morphological and electrophysiological research has revealed following complex function of ICCs.

1. Interstitial cells of Cajal are pacemakers and actively propagate electrical slow waves in gastrointestinal (GI) muscles [23].
2. Interstitial cells of Cajal mediate both inhibitory and excitatory motor neurotransmission [24, 25].
3. Interstitial cells of Cajal serve as non-neural stretch-receptors in GI muscles, affecting both smooth muscle excitability and slow wave frequency [26].
4. Interstitial cells of Cajal form intimate associations with the intramuscular terminals of vagal afferents, thus they may also have a role in afferent signaling [27].

Organization and distribution of ICCs networks in the gastrointestinal tract

Various subtypes of ICCs have been described throughout the gastrointestinal tract in detail. ICCs have been classified according to the shape and arrangement of the ICCs as well as on the relationships to nerve plexuses, the relation to smooth muscles and the branching behavior with each other [28].

The largest density of ICCs occurs around the myenteric plexus. ICCs of the myenteric plexus (ICC-MP) comprises multipolar cells that are interconnected to each other and to neighboring structures (Fig. 1a). ICC-MPs form a dense network around the myenteric plexus in the small bowel and are less dense in the stomach and colon [28].

The ICCs are also present within the circular and longitudinal muscle intercalated between intramural neurons and SMCs. The ICCs of the intestinal muscle (ICC-IM) could be differentiated into two groups (Fig. 1b). ICCs of the circular muscle (ICC-CM) are bipolar cells which are orientated along the surrounding muscle cells. The distribution and density of ICC-CM varies considerable within the gastrointestinal tract. ICC-CMs are sparsely found in the small bowel not forming a network. In contrast ICC-CMs are densely distributed along nerve bundles in stomach and colon [28]. ICCs of the longitudinal muscle (ICC-LM) are bipolar cells similar to but less numerous than the ICC-CM [29]. ICCs of the deep muscular plexus (ICC-DMP) are multipolar cells which are found along the inner portion of the circular muscle layer, in a close relation to nerve bundles within the deep muscular plexus of the small intestine [30, 31].

ICCs of submucosa and submucosal plexus (ICC-SM and ICC-SMP) will be displayed at the interface between the submucosal layer and the innermost circular muscle layer within the stomach and the colon (Fig. 1c) [32–36]. Since these multipolar cells contain secondary processes they form a loose network [37,38].

Interstitial cells of Cajal of the subserosa (ICC-SS) comprises a group of stellate cells within the subserosal layer in mouse small bowel and mouse colon [38, 39].

Most clinical studies simply refer ICCs to myenteric ICCs (ICC-MP) and muscular ICCs (ICC-IM).

All different subtypes of ICCs have been demonstrated along the entire digestive tract from the esophagus to the internal anal sphincter (IAS) [40–42]. The distribution patterns and morphological features of ICCs depend on their anatomical location [43]. Despite the fact that the various classified subtypes of ICCs are generally almost uniformly distributed in the small bowel and colon, some differences are found in the mouse colon and human colon [44, 45]. The ICCs in the stomach have a different distribution in the proximal and distal regions [34, 46].

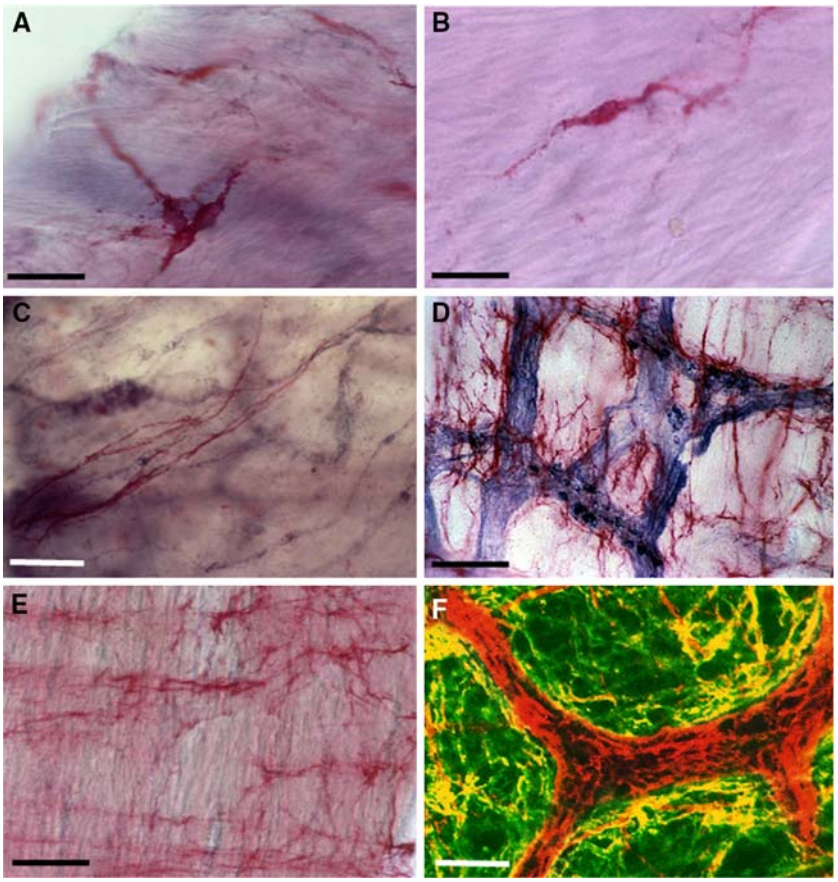

Fig. 1 **a** normal ICCs of myenteric plexus (ICC-MP), whole-mount preparation, c-kit immunohistochemistry, *scale bar* 20 μm. **b** Normal muscular ICCs (ICC-LM), whole-mount preparation, c-kit immunohistochemistry, *scale bar* 20 μm. **c** Normal submucosal ICCs (ICC-SM), whole-mount preparation, c-kit immunohistochemistry, *scale bar* 20 μm. **d** Normal ICC-MP and myenteric plexus, whole-mount preparation, c-kit immunohistochemistry/ NADPH-diaphorase histochemistry, *scale bar* 50 μm. **e** Normal ICC-CM, c-kit immunohistochemistry, whole-mount preparation, *scale bar* 50 μm. **f** Normal ICC-MP and myenteric plexus, whole-mount preparation, c-kit/PGP 9.5 immunohistochemistry, *scale bar* 50 μm

Identification of ICCs

In order to elucidate the distribution and morphology of ICCs various methods have been used in the last decades. ICCs show a commonly positive staining by methylene blue, silver staining, and Golgi impregnation. Their specific staining characteristics lead previously to the assumption that these cells were primitive neurones. The co-staining of neuronal structures made it difficult to discriminate between neurons and ICCs. Later with the use of electron microscopy it was possible to study the ultrastructure of the ICCs in greater details [47–50]. Electron microscopy is still considered the "gold standard" for the identification of ICCs and the demonstration of its characteristic ultrastructural features. Typical ultrastructural features of human ICCs are well-developed smooth endoplasmic reticulum, abundant intermediate filaments, no myosin filaments, numerous caveolae, dense bodies, dense bands and an oval indented nucleus [51–59]. Further morphological details of ICCs are gap junctions with SMCs and very close apposition with nerve terminals [60].

Despite the fact that electron microscopy enables the most detailed examination of cellular structures and cell-to-cell interactions it does not display the cellular distribution within different tissues. Furthermore electron microscopy is not applicable as a routine laboratory technique in research and diagnostic. Therefore a great advance in the research of ICCs biology occurred when it was recognized that ICCs express the gene product of c-kit. C-kit is a proto-oncogene that encodes the receptor tyrosine kinase, kit [23, 24, 61–63]. Labeling of the c-kit receptor or the measurement of the c-kit mRNA has provided reproducible methods for identifying ICCs throughout the gastrointestinal tract of different species, including human [45, 64–66], mice [67, 68], rats [69], and guinea pig [46, 70]. C-kit is expressed exclusively in ICCs and mast cells within the gastrointestinal tract [67].

Immunohistochemistry using c-kit labeling has now improved our understanding of the structure and distribution of ICCs networks and enhanced the perception of the anatomic relationships between ICCs, enteric neurons and SMCs in the gastrointestinal tract [34, 46, 64, 67, 70].

Whole-mount preparation technique

The complex relationship of the ICCs to the enteric nervous system (ENS) and other surrounding structures is difficult to appreciate on conventional thin sections. Whole mount preparation has been proven to be an elegant

technique for the visualization of the structure of the intrinsic networks (neurons, ICCs) and their relationship of branching and interconnecting to each other and to the neighboring tissues. It provides a method for the study of 3-dimensional morphology of the neuronal or ICCs networks [71–76]. Several investigators have used this technique in specimens from the human GIT with various staining methods, ranging from silver impregnation to enzyme histochemistry and immunohistochemistry [73–76]. The great advantages for the histological evaluation become obvious if whole-mount preparations were compared to conventional sections. Sections only partially show the morphology of the neurons, glial cells or ICCs being dependent on orientation and localization [73]. Whole-mount preparations of the longitudinal muscle layer with the adjacent myenteric plexus are made by separating the muscular layer from the submucosal layer, then removing the circular muscle layer from the longitudinal muscle. Subsequently, the mucosa is removed from the submucous layer to better visualize the submucous plexus.

Whole-mount preparation technique has been employed in previous studies to assess the normal and defective expression and three-dimensional topography of the ICCs [75]. Horisawa et al. [45] described the three-dimensional configuration of c-Kit positive cells as typical multipolar cells around the myenteric plexus and slender bipolar cells within the circular and longitudinal muscle layers. Another study used whole-mount preparations of the guinea pig small intestine and revealed closed relationships between muscular ICCs and nitric oxide synthase (NOS), vesicular acetylcholine transporter (vAChT), and substance P-like immunoreactivities [77]. Hence the enteric motoneurons, ICCs and SMCs form functional units that release transmitter and mediate and transduce neural inputs into mechanical responses in the gut [77, 78]. A recent study further showed clear colocalization of ICCs and nitrergic innervation in whole-mount preparations of the normal human gut [75]. Meshlike network of NADPH-diaphorase positive nerve fibers in the myenteric plexus was surrounded by a reticular network of c-kit positive ICCs (Fig. 1d–f) [75].

ICCs in intestinal motility disorders in childhood

Motility disorders of gastrointestinal tract occur frequently in various ages. These conditions can be congenital or acquired. In many cases the proper pathogenesis remains incompletely understood. Absent or deficient ICCs networks have been identified in a variety of motility disorders of childhood. In recent years c-kit labeling has been widely used to study pathological variations of ICCs in motility disorders [79].

Hypertrophic pyloric stenosis

Infantile hypertrophic pyloric stenosis is a common condition requiring surgery in the first month of life. The pathogenesis of the infantile hypertrophic pyloric stenosis, despite many hypotheses, remains poorly understood. Abnormalities of the pyloric innervation, extracellular matrix and SMCs have been reported [80–88]. Langer et al. [89] reported for the first time immature ultrastructural features of ICCs in infantile hypertrophic pyloric stenosis. Their findings were supported by studies, which provided substantial evidence that ICCs might have a role in the pathogenesis of hypertrophic pyloric stenosis [90–94]. Recently, Piotrowska et al. [95] showed lack of ICCs and heme oxygenase-2 (HO-2), which suggests impaired intracellular communication between ICCs and SMCs, contributing to the motility dysfunction in children with pyloric stenosis.

Only very few studies were focused on ultrastructural changes of the smooth muscle in hypertrophic pyloric stenosis [88, 96]. These studies suggested that the alteration of smooth muscle proteins seems to be an essential element of the pathophysiology in hypertrophic pyloric stenosis.

Hirschsprung's disease

Several investigators have studied the distribution of ICCs in the ganglionic and aganglionic bowel of patients with Hirschsprung's disease (HD) using c-kit-immunohistochemistry [45, 93, 97–104]. A reduced number of c-kit immunoreactive ICCs in aganglionic bowel and also in the transitional zone of HD patients was consis2tently reported (Fig. 2a, b) [97–104]. Vanderwinden et al. [101] described reduced number of ICCs with disrupted network in the aganglionic bowel. These ICCs did not form a network and showed no clear relation to the hypertrophic nerve trunks. Yamataka et al. [99, 100] found few c-Kit positive cells in the muscle layers in HD and a moderate number around the thick nerve bundles in the space between the two muscle layers in the aganglionic bowel. In contrast Horisawa et al. [45] reported no differences in c-Kit immunopositive cells in aganglionic segments compared with the corresponding area of ganglionic bowel. Nevertheless the latter study referred to the importance of regional differences in the distribution of c-Kit positive cells in the normal colon since different expression of c-kit positive cells was shown at different regions in the large bowel. This study did not differentiate between myenteric and muscular ICCs. Two further, recently published studies showed that ICCs were either normal or only slightly reduced in the affected bowel in HD compared to normal bowel [105, 106].

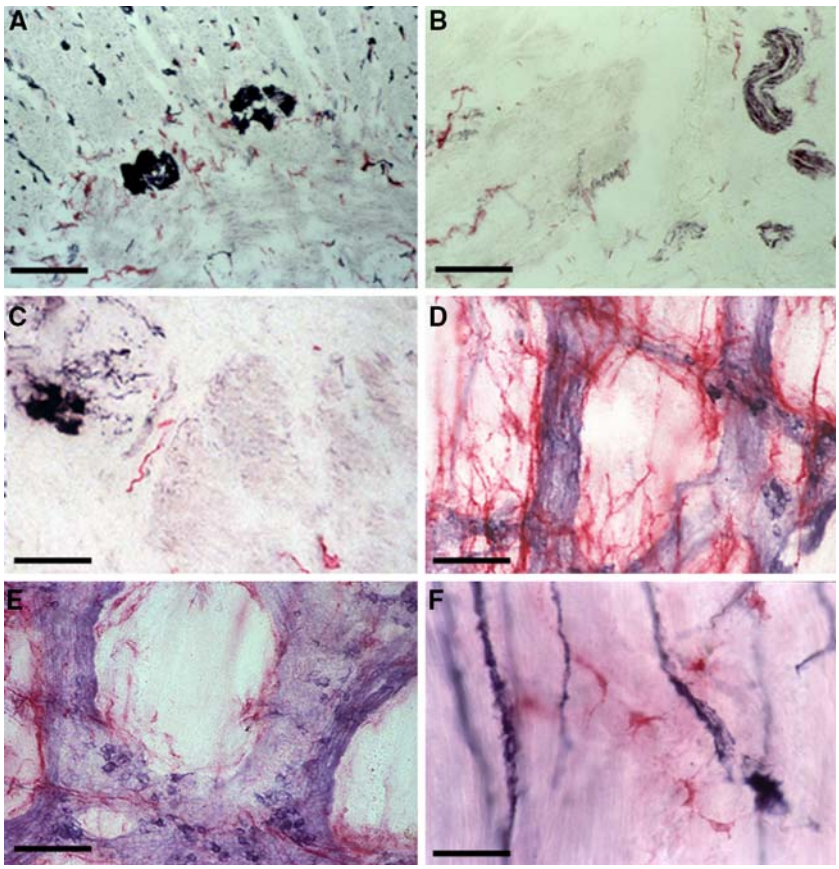

Fig. 2 **a** Normal ICC-MP and myenteric plexus, section preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 100 μm. **b** ICC-MP and hypertrophic nerve trunks in Hirschsprungs disease, section preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 100 μm. **c** ICC-MP and hypertrophic nerve trunks in hypoganglionosis, section preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 100 μm. **d** Normal ICC-MP and myenteric plexus, whole-mount preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 50 μm. **e** Reduced ICC-MP and myenteric plexus in intestinal neuronal dysplasia, whole-mount preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 50 μm. **f** Reduced ICC-MP and defective myenteric plexus in hypoganglionosis, whole-mount preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 50 μm

Advances in the management of Hirschsprung's disease (HD) afford most patients with HD a satisfactory outcome after definitive corrective surgery. However some patients continue to have persistent bowel dysfunction despite adequate resection of the aganglionic bowel. Postoperative motility problems after proper resection of aganglionic bowel have been attributed to intestinal neuronal dysplasia or altered distribution in ICCs. Rolle et al. in a detailed study examined the three-dimensional morphology of c-kit immunoreactive (IR) ICCs in the aganglionic, ganglionic bowel and transitional zone of HD bowel [102]. All types of myenteric ICCs were found to be markedly reduced in the aganglionic bowel and in the transitional zone. Furthermore, ICCs were also diminished in the ganglionic part of resected HD bowel. Muscular ICCs were markedly reduced in the aganglionic bowel, moderately reduced in the transitional zone and normal in the ganglionic bowel in HD. In detail, whole-mount preparations of aganglionic bowel showed only single c-kit immunoreactive ICCs at the level of the myenteric plexus. These cells appeared mainly as thin and bipolar cells closely related to the hypertrophic nerve trunks. Muscular ICCs were markedly reduced in number and were reduced expressed at the innermost layer of the circular muscle layer. Whole-mount preparations of the transitional zone of HD bowel that c-kit immunoreactive ICCs were evident as single cells or cell clusters closely related to the small myenteric ganglia without forming typical networks. Muscular c-kit immunoreactive ICCs were found between the smooth muscle fibers and were mainly expressed at the innermost layer of the circular muscle layer in reduced numbers. Whole-mount preparations of ganglionic HD bowel displayed numerous myenteric ICCs forming sparse networks around the ganglia of the myenteric plexus which were less developed as seen in normal bowel. Expression of the muscular ICCs was normal in the smooth muscle layers, particularly at the innermost part of the circular muscle layer [102]. The clear reduction of myenteric ICCs in the

normoganglionic sigmoid colon in HD is assumed to be the one cause for the dysmotility disturbances in some patients after pull-through operation [102]. Due to the developmental relationship between the ENS and the myenteric ICCs the defective expression of ICCs in the rectosigmoid HD may be a primary effect of the malformation. A further study confirmed that an abnormal distribution of C-kit positive cells in the normoganglionic segment can predict a poor clinical outcome in patients with HD [107]. Another recently published study introduced a new pathological protocol for the rapid assessment of enteric ICCs in order to improve the management of HD patients [108].

However, the provided data could not finally show if defective ICCs in aganglionic or ganglionic bowel of HD are the cause or the consequence of the neuropathy with subsequent chronic constipation.

Recently published results of animal studies on changes in ICCs in HD models are not conclusive as well. Sandgren et al. reported that changes in expression in the number of ICCs in ileum and colon proximal to the aganglionosis in lethal spotted mice [109]. In contrast, Ward et al. showed in the same model (lethal spotted ls/ls mice) that the distribution of ICCs in the ganglionic and aganglionic regions of the colons of ls/ls mutants were similar to wildtype controls [44]. Furthermore, this study revealed that the electrical activity and neural responses of the circular layer are significantly different in aganglionic segments [44]. Taniguchi et al. [110] showed that the ICCs had fewer processes and no attachments to the intermuscular nerves in the aganglionic bowel of ls/ls mice.

The currently accepted concept is that deficient expression of c-kit immunoreactive ICCs in the aganglionic bowel may contribute to motility dysfunction in HD by the defect of electrical pacemaker activity. Additional studies are necessary since the important question whether or not the lack of c-kit positive ICCs in HD is related to a true reduction of ICCs or to the absence of the c-kit receptor is not been answered yet.

Total colonic aganglionosis

Total colonic aganglionosis (TCA) is a severe form of ultra long HD with an incidence of 2–14% among all forms of intestinal aganglionosis.

Symptoms generally manifest in the neonatal age with intestinal obstruction, but unlike classical recto-sigmoid HD a normal passage of meconium in the first 24 h of life is present in a high percentage of infants [111]. It has also been reported that symptoms have a progressive development with worsening of constipation and a diagnosis made after the first year of life or in rare cases in adolescence or adulthood [111–115].

In the aganglionic segments of TCA, similar as in the aganglionic part of HD bowel there is the absence of ganglia cells in myenteric and submucous plexus. But contrary to HD bowel, where typical features include large nerves trunks and hyperplasia of nerve fibers in the intramuscular space and in the submucosal layer, in the aganglionic part of TCA bowel only few fibers and small hypertrophic nerve trunks were found [116]. This feature is similar to the results reported in the total intestinal aganglionosis [74]. Yamataka et al. described altered distribution of c-kit positive cells in the aganglionic bowel of HD and TCA patients [99]. This study showed absence of c-kit positive cells in the muscle layers in aganglionic part in TCA bowel while fewer c-kit positive cells there were found in the same part of HD bowel. Moreover, this study reported that abundant c-kit positive cells and an absence of stem cell factor (SCF) that is natural ligand of c-kit receptor were found in the intramuscular space of aganglionic bowel patients with TCA. The basis for this phenomenon remains unexplained. Altered expression of c-kit IR ICCs in the aganglionic part of TCA bowel was further reported by Solari et al. in whole mount preparation and conventional sections [116]. C-kit positive myenteric ICCs were lacking or markedly reduced in TCA compared to the normal bowel. Only single c-kit positive muscular ICCs were found in the circular and longitudinal muscle layer. In whole mount preparations, fine NADPH-positive nerve fibers ran parallel to longitudinal muscle fibers without crossing each other and sparse or lack of c-kit-positive ICCs were found between the muscle layers. These findings need careful interpretation since only a limited number of studies investigating expression of c-kit positive ICCs are available. Depletion of c-kit immunoreactive ICCs in the bowel through the whole aganglionic colonic segment in TCA may contribute to gut dysmotility in this disease which is already has marked abnormalities in the ENS.

Hypoganglionosis

Isolated hypoganglionosis is a rare condition, constituting 5% of all neuronal intestinal malformations. Its clinical presentation resembles HD [117–122]. Failure to pass meconium may be the first symptom in the neonatal period, whereas infants and older children present with chronic constipation. The diagnosis of hypoganglionosis by means of suction rectal biopsy is difficult. Rectal biopsies with absent or low level of acetylcholinesterase (AChE) activity in the lamina propria and absence or reduction of submucosal ganglia cells may raise the suspicion of hypoganglionosis. A full-thickness rectal biopsy allows the inspection of myenteric plexus and is essential for the diagnosis of hypoganglionosis. The characteristic histological features of

hypoganglionosis include sparse and small myenteric ganglia, absent or AChE activity in the lamina propria, and hypertrophy of muscularis mucosae and circular muscle. Meier-Ruge et al. [123, 124] performed morphological measurements in resected bowel specimens from patients with hypoganglionosis and found a dramatic decrease in plexus area and nerve cell number in the myenteric plexus and an increase in distance between ganglia.

Only very few investigators have examined ICCs in congenital and acquired hypoganglionosis [125–128].

Conventional sections and whole-mount preparations clearly revealed the marked reduction of c-kit immunoreactive ICCs in the hypoganglionic bowel (Fig. 2c, f) [125, 126]. Only a few c-kit immunoreactive myenteric ICCs were found around the myenteric plexus that contains only small ganglia. The defective myenteric ICCs were expressed mainly as single bi- or multipolar cells without forming a network. The c-kit immunoreactive muscular ICCs were also markedly reduced between the muscle bundles of the circular and longitudinal muscle layers and in the innermost part of circular muscle layer. These studies provide evidence that the marked reduction of ICCs-MY and ICCs-IM together with the reduced neuronal density may impair the generation of slow waves and neurotransmission causing motility dysfunction in hypoganglionosis.

Intestinal neuronal dysplasia

Intestinal neuronal dysplasia (IND), a clinical condition that resembles HD, was first described by Meier-Ruge in 1971 as a malformation of the enteric plexus [129–134]. Contrary to the classical aganglionosis, the typical histological finding in IND include hyperganglionosis and giant ganglia. The pathogenesis of IND is not known, but genetic factors could be involved in this disease [135, 136]. Defective innervation of the neuromuscular junction of affected bowel was described in patients with IND [137].

In 1983 Fadda et al. classified IND into two clinically and histologically distinct subtypes [129]. Type A occurs very rarely, is characterized by congenital aplasia or hypoplasia of the sympathetic innervation, and presents acutely in the neonatal period with episodes of intestinal obstruction, diarrhea, and bloody stools. Type B is characterized by malformation of parasympathetic submucous and myenteric plexus and accounts for over 95% of cases of isolated IND displaying a clinical picture that resembles HD and usually presenting with chronic constipation. The incidence of isolated IND has varied from 0.3 to 40% of all section rectal biopsies in different centers [130, 131, 138, 139]. In 1977, Puri et al. reported a case of IND immediately proximal to a segment of aganglionic colon [131]. Since then, there have been several reports of the combined occurrence of these disorders. IND associated with HD is usually type B. It has been reported that 25–35% of patients with HD have associated IND [142–143].

The characteristic histological features of IND B include hyperganglionosis of the submucous and myenteric plexuses, giant ganglia, ectopic ganglion cells, and increased AChE activity in the lamina propria and around submucosal blood vessels [144].

Results from our laboratory have demonstrated reduction of c-kit immunoreactive myenteric and muscular ICCs in the colon of patients with isolated IND and IND associated with HD. The whole-mount preparation clearly showed differences in the 3-dimensional architecture of myenteric and muscular ICCs and the myenteric plexus in IND compared to controls. Combined NADPH-d/c-kit staining in the whole mount preparation showed marked reduction of myenteric and muscular ICCs and hypertrophic ganglia cells in the myenteric plexus in the isolated IND and IND associated HD specimens compared to controls (Fig. 2d, e, 3a–c).

Internal sphincter achalasia

The internal anal sphincter (IAS), which is a specialized smooth muscle continuation of the circular muscle layer, plays a significant role in the maintenance of anorectal continence and in the pathophysiology of incontinence and constipation [145]. The IAS relaxes in response to rectal distension, a phenomenon called the rectosphincteric inhibitory reflex, which is mediated by intramural nerves descending from the rectum to the IAS [146]. Internal anal sphincter achalasia (IASA) is a clinical condition with presentation similar to HD but with the presence of ganglion cells on rectal biopsy [147–153]. The diagnosis of IASA is made on anorectal manometry, which shows the absence of rectosphincteric reflex on the balloon inflation. The exact pathogenesis and pathophysiology of IASA is not fully understood. Several authors have reported innervation abnormalities in the IASA [147–153]. Nitrergic nerve depletation, abnormal peptidergic innervation, defective innervation of the neuromuscular junctions has been reported in IASA [147–153].

Large number of ICCs are present among SMCs and between muscle bundles in the IAS [154]. Their pattern of distribution in association with the different proposed functions of ICCs could be responsible for normal physiological motility of the anorectum [42, 55]. It has been proposed that ICCs in certain regions of the gut may not act as pacemakers, but as stretch receptors [51]. A stretch receptor role has been proposed for certain ICC networks especially in the anorectum [42, 51]. The rectum can relax to accommodate an increase in volume of luminal contents

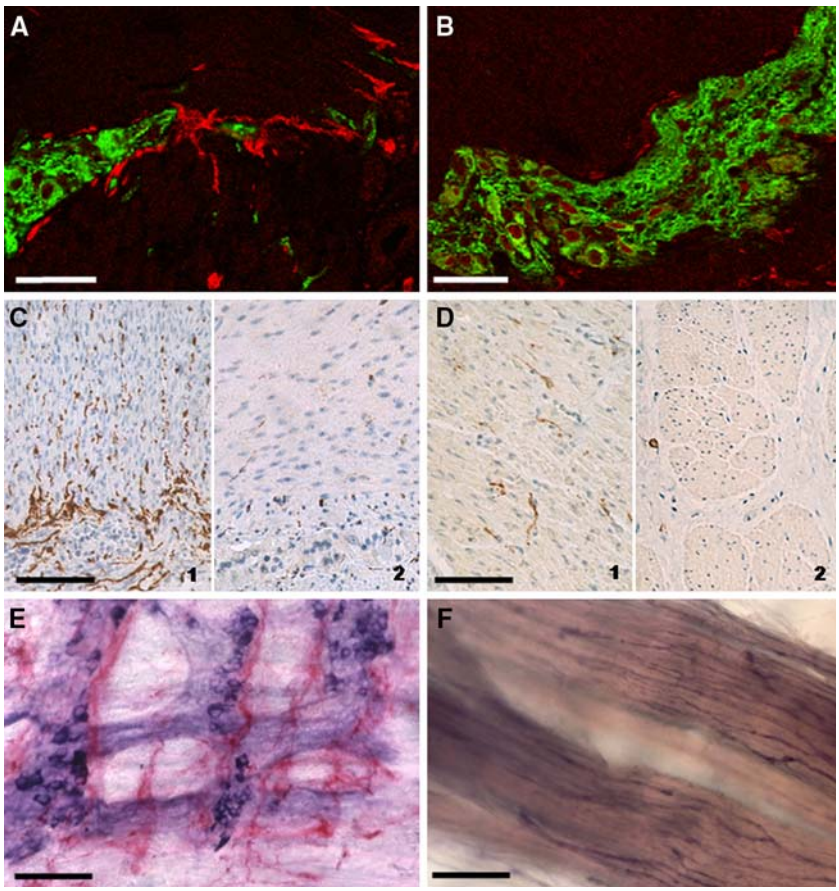

Fig. 3 **a** Normal ICC-MP and myenteric plexus, section preparation, c-kit/PGP 9.5 immunohistochemistry, laser scanning microscopy, *scale bar* 20 µm. **b** Reduced ICC-MP and myenteric plexus in intestinal neuronal dysplasia, section preparation, c-kit/PGP 9.5 immunohistochemistry, laser scanning microscopy, *scale bar* 20 µm. **c** *1* Normal ICC-MP and myenteric plexus, section preparation, c-kit/PGP 9.5 immunohistochemistry, *scale bar* 100 µm. *2* Reduced ICC-MP and myenteric plexus in intestinal neuronal dysplasia, section preparation, c-kit/PGP 9.5 immunohistochemistry, *scale bar* 100 µm. **d** *1* Normal ICC-CM, section preparation, c-kit/PGP 9.5 immunohistochemistry, *scale bar* 100 µm. *2* Marked reduced ICC-CM in internal anal sphincter achalasia, section preparation, c-kit/PGP 9.5 immunohistochemistry, *scale bar* 100 µm. **e** Normal ICC-MP and myenteric plexus in MMIHS, whole-mount preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 50 µm. **f** Absent ICC-CM in MMIHS, whole-mount preparation, c-kit immunohistochemistry/NADPH-diaphorase, *scale bar* 50 µm

meaning that the rectal wall is capable of detecting distending force, and then initiating relaxation of the SMCs. It is suggested that ICCs express neuronal nitric oxide synthase (nNOS) and heme oxygenase-2 (HO-2), two enzymes responsible for the production of nitric oxide [155, 156] and carbon monoxide [156–164] respectively, which play a role as a messenger molecule between ICCs and SMCs. Colocalization of both enzymes in the enteric plexus of anorectum and in ICCs suggests a modulatory role for the HO pathway in the nNOS-mediated non-adrenergic non-cholinergic (NANC) inhibitory neurotransmission of the IAS [159, 164]. The rectoanal inhibitory reflexes (RAIR) involve relaxation of the IAS in response to inhibitory neuronal inputs, involving a NANC pathway. After stimulation by NO, ICCs motility of the anorectum amplify the production of NO or CO in the positive feedback response [42, 55, 159, 165]. These findings suggest that ICCs may mediate the inhibitory innervation of the afferent limb of the rectoanal reflex. A recent published animal study supports the hypothesis that a normal RAIR requires an intact network of ICCs in the IAS. Therefore the loss of nitrergic innervation and/or deficiency of ICCs lead to impaired anal relaxation [165]. Conflicting results were provided by another animal study showing that c-kit expressing ICC do not have a critical role in the RAIR [166]. Moreover, these W/W^V mice showed a normal distribution of nNOS in the rectoanal region [166].

In the IASA patient the density of ICCs is markedly reduced in IAS specimens compared to normal IAS (Fig. 3d) [154]. These findings demonstrate that in the IAS, achalasia patients not only have a defective intramuscular innervation but also altered distribution of ICCs, which are

the coordinators of gastrointestinal motility. The lack or deficient expression of ICCs in the IASA may lead to defective generation of pacemaker activity, thus causing motility dysfunction.

Megacystis microcolon intestinal hypoperistalsis syndrome

Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is a rare congenital and generally fatal cause of functional intestinal obstruction in the newborn. This syndrome is characterised by abdominal distension caused by a distended non-obstructed urinary bladder, microcolon, and decreased or absent intestinal peristalsis. Usually incomplete intestinal rotation and shortened small bowel are associated [167–178].

Although about 180 cases have been reported in the literature, the etiology of this syndrome is not yet fully understood. Several hypotheses have been proposed to explain the pathogenesis of MMIHS: genetic [173], neurogenic [172, 175], myogenic [167, 169, 174] and hormonal origin [175, 176]. Histological studies of the myenteric and submucosal plexuses of the bowel of MMIHS patients have revealed normal ganglion cells in the majority of the patients, decreased in some, hyperganglionosis and giant ganglia in others [168–172]. Recently, an imbalance between several kinds of intestinal peptides was suggested as one of the possible causes of hypoperistalsis in MMIHS patients [176]. Puri et al. [168] demonstrated in 1983 vacuolar degenerative changes in the smooth muscles cells with abundant connective tissue between muscle cells in the bowel and bladder of patients with MMIHS and suggested that a degenerative disease of SMCs could be the cause of this syndrome. Marked reduction of contractile and cytoskeleton proteins in MMIHS bowel describing recently confirms myogenic hypothesis of etiopathogenesis of this disease [177]. Ciftci et al. [174] also reported a case without vacuolar degeneration but with excessive smooth muscle glycogen storage. They postulated that the pathogenesis involves a defect of glycogen-energy utilization. Srikanth et al. [176] have proposed that the initial event in the pathogenesis of MMIHS is an intramural inflammatory process affecting the gastrointestinal tract and urinary tract, resulting in fibrosis and destruction of the intestinal neuronal network, followed by hypoperistalsis.

Conventional sections and whole-mount preparations using NADPH-D histochemistry and c-kit immunohistochemistry revealed presence of c-kit IR ICCs in the region of the myenteric plexus (ICCs -MY) MMIHS bowel (Fig. 3e) [177]. These ICCs-MY formed a dense network around the myenteric plexus. In contrast only few single ICCs-IM were detected within the smooth muscle layers in MMIHS specimens (Fig. 3f). ICCs-IM were also absent at the innermost part of the circular muscle layer in MMIHS specimens.

Previous reports have showed vacuolic degeneration of SMCs and a marked reduction or lack of α-SMA and other contractile and cytoskeleton proteins in intestinal SMCs of MMIHS bowel [165–169]. Due to the possible common developmental mechanism these SMCs alterations may lead to a developmental failure for the precursor ICCs to proceed into ICCs-IM stimulated by nonneuronal KL. Reduced number of intramuscular ICCs in the gut wall may be partly responsible for the motility dysfunction in MMIHS.

Conclusion

Interstitial cells of Cajal have important functions in regulation of mechanical activity, so the loss of ICCs could certainly result motor dysfunction. Paediatric gastrointestinal motility disorders have been shown to be associated with loss or defects in ICCs networks. It is important to point out that all investigated human specimen were taken after clinical evident motility problems. Thus it is difficult to determine whether the loss of ICCs is a cause or a consequence of the disease process.

There is still an incomplete understanding of the cause-and-effect relationship between loss of ICCs and the development of motor symptoms in humans.

Further research needs to be focused on detailed and comparable animal models to study the consequences of losing ICCs. The correlation of pathological changes within visceral smooth muscles and ICCs might lead to a further understanding of a common underlying pathophysiological mechanism in motility disorders.

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