Regional and transmural density of interstitial cells of Cajal in human colon and rectum

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Hagger, Robert, Sussan Gharaie, Caroline Finlayson, and Devinder Kumar. Regional and transmural density of interstitial cells of Cajal in human colon and rectum. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1309–G1316, 1998.—The interstitial cells of Cajal (ICC) are thought to play an important role in the control of gut motility. The regional and transmural pattern of distribution of ICC in the normal human colon and rectum was evaluated with immunohistochemistry using an anti-c-kit antibody. The transmural distribution of ICC was constant throughout the whole colon, the density of ICC was significantly greater at the myenteric plexus than at either the longitudinal or circular muscle layers, and in the rectum the transmural distribution was more even. Regionally, at the myenteric plexus, the transverse colon had a significantly greater density of ICC compared with the right colon (P = 0.038), left colon (P = 0.006), and rectum (P = 0.008). The pattern of distribution of ICC identified in this study is consistent with the proposed roles of ICC as colorectal pacemakers, intermediaries of the neural control of muscle activity, and coordinators of colorectal muscle activity. The highest density of ICC was at the myenteric plexus of the transverse colon, which is the proposed region of pacemaking activity.

gut motility; mast cells; myenteric plexus

The interstitial cells of Cajal (ICC) are a group of cells found throughout the gut from the esophagus to the anus. ICC are thought to play an important role in the control of gut motor activity, acting as pacemakers (31), intermediaries in the neural control of gut muscular activity (5), spatial coordinators of gut motility (7), and as stretch receptors (8, 9). These putative roles are not mutually exclusive; the particular function that ICC fulfill may be dependent on the location within the gastrointestinal tract.

The study of ICC in gut tissue has been helped by immunohistochemical methods using anti-c-kit antibody. ICC express the protooncogene c-kit (15, 36), which encodes a tyrosine kinase cell surface receptor (38). Immunohistochemistry using an anti-c-kit antibody is now providing a more selective technique for identifying ICC in animal (15, 32) and human tissues (33, 37) at the level of light microscopy; previously reliable identification of ICC has relied on ultrastructural characteristics seen through electron microscopy (10, 11, 23–26).

In the human colon, reports of ICC distribution have been mainly based on electron microscopy studies. ICC are found within the myenteric muscle sheaths in the intermuscular plane (11). At the submucosal border of the circular muscle, ICC are found in association with a morphologically distinct inner layer of smooth muscle cells (10, 25). ICC are also seen in the main bulk of the circular muscle layer and the main intermuscular septa (25). An immunohistochemical study of the colon using an anti-c-kit antibody in a small number of patients showed ICC to be present in the longitudinal and circular muscle layers and the intermuscular plane and in association with the submucosal plexus at the inner margin of the circular muscle layer (34).

The functions of the colorectum include absorption of water and sodium from the chyme that enters from the ileum and also the fatty acid products of bacterial fermentation and then removal of stool from the body during defecation. The motor activity and gross morphology of the colon and rectum are adapted so that these processes may proceed efficiently. Segmental differences are apparent: the right colon acts as a storage region for ileal effluent so that absorption may occur, the left colon acts as a conduit for the passage of feces, and the rectum can act as a temporary storage region for stool before defecation.

The aim of this study was to evaluate the transmural and regional pattern of distribution of ICC in the normal human colorectum, using immunohistochemistry with an anti-c-kit antibody.

MATERIALS AND METHODS

Subjects

Colorectal tissue was obtained from surgical resections performed for carcinoma of the colon or rectum, from patients whose previous bowel habit had been normal. Tissue was defined as normal when the carcinoma was nonobstructing and the section was not invaded by the tumor. The regions of interest studied were the right, transverse, and left colon and the rectum. The specimens were fixed in phosphate-buffered 10% Formalin (pH 7.0) and then processed into paraffin wax. Thirty-one right colonic, 31 transverse colonic, 53 left colonic, and 28 rectal tissue sections were obtained from 108 surgical specimens. There were 52 male and 56 female patients (median age 74 yr, range 44–91 yr). The mean age of patients from which specimens were obtained did not differ significantly among the regions.

Immunohistological Staining

Immunohistochemistry was performed using the avidin-biotin-peroxidase method. The primary antibody used was a commercial rabbit affinity-purified polyclonal anti-c-kit antibody (Oncogene Science, Uniondale, NY). Sections were cut at 4 µm from paraffin-embedded tissue blocks and mounted on aminoalkylsilane-coated slides (Sigma Chemical, St. Louis, MO). The sections were deparaffinized in xylene and methanol. Endogenous peroxidase activity was blocked by a 30-min immersion period in 0.3% hydrogen peroxide in absolute methanol at room temperature. To unmask antigenic sites,
sections were boiled in a microwave for 10 min in citrate buffer (0.21%, pH 6.0) and then allowed to cool for 20 min. To prevent nonspecific absorption of immunoglobulin, we incubated the sections for 20 min in 3% BSA (Sigma Chemical) in Tris buffer (0.05 M, pH 7.6). The sections were then incubated with the primary antibody, rabbit anti-c-kit antibody 0.1 µg/ml in 3% BSA-Tris for 24 h at 4°C. Sections were then incubated at room temperature for 30 min with a biotin-conjugated swine anti-rabbit immunoglobulin (DAKO, Glostrup, Denmark) diluted 1:250 in Tris buffer, followed by a 30-min incubation period in avidin-biotin-peroxidase complex (DAKO). Each incubation period was followed by gentle washing in Tris buffer. The bound complex was visualized by using the 3,3'-diaminobenzidine (DAB)-hydrogen peroxide reaction according to the method of Graham and Karnovsky (12). The sections were immersed in DAB (0.5 mg/ml) and 0.03% hydrogen peroxide in Tris for exactly 10 min. The sections were then washed well in tap water. Counterstaining was performed with hematoxylin. The sections were dehydrated in methanol and mounted using an automatic Tissue Tek coverslipping device (Sakura Finetek Europe, Zoeterwoude, The Netherlands).

Distribution Analysis

The density of distribution of ICC was assessed in the longitudinal and circular muscle layers and in the intermuscular plane of the colorectum. The density of ICC was graded after the evaluation of 10 well-stained and well-orientated high-power fields (×400 magnification), with the field having an area of 0.152 mm². The longitudinal muscle layer, intermuscular layer, and circular muscular layer were each assessed by 10 different fields. The grades of “sparse,” “few,” “moderate,” and “many” reflected an average count of 0–1, 2–3, 4–8, and >8 cell bodies per high-power field, respectively. Only ICC with nuclei were counted. The assessment was made by two independent observers. If there was disagreement in the grade assigned, a consensus was reached after joint review. Interrater agreement gave a χ² statistic of 0.45, with a weighted χ² of 0.59.

Statistical Analysis

The χ² test was used to compare the transmural variations of ICC density observed among the circular muscle layer, myenteric plexus, and longitudinal muscle layer within a segment of the colorectum. The density of ICC was also compared among the different segments of the colorectum for each layer of the muscularis propria.

RESULTS

The immunohistochemical method clearly identified c-kit-positive cells. ICC and mast cells showed c-kit immunoreactivity in the colon and rectum. ICC had a fusiform cell body with a large oval nucleus and two or more dendritic processes (Fig. 1), and mast cells were round with a round central nucleus. These characteristics allowed the two cell types to be distinguished.

Colon

The pattern of distribution of ICC in the colon was the same for the right, transverse, and left colon. In the longitudinal muscle layer ICC were identified in the muscle bulk in parallel orientation with the muscle fibers and also in association with penetrating blood vessels. In the intermuscular plane, ICC formed a network encasing the myenteric nerve plexus (Fig. 2). In the circular muscle layer, ICC were again found in the bulk of the muscle in parallel orientation with the muscle fibers (Fig. 3) and in association with blood vessels. ICC were also seen lining the intramuscular...
septa. In the muscle layers, ICC were observed to be connected through dendritic processes to other ICC, most often in a linear arrangement. ICC were identified at the inner layer of smooth muscle fibers at the submucosal border of the circular muscle (Fig. 4). ICC were not identified in the submucosa, muscularis mucosa, or mucosa, although c-kit-positive cells identified morphologically as mast cells were present (Fig. 5). Mast cells were also present in all layers of the muscularis propria.

Rectum

In the longitudinal and circular muscle layers of the rectum, ICC were seen in the muscle bulk and in association with penetrating blood vessels. The ICC were mainly in parallel orientation with the muscle fibers. ICC formed networks in the muscle layers and dendritic processes ramified between the muscle fibers more frequently than was observed in the colon. In the intermuscular plane, ICC were seen in association with elements of the myenteric plexus. At the inner margin of the circular muscle, occasional ICC were observed and were also seen in association with neural elements of the submucosal plexus. As in the colon, in the lamina propria and mucosa of the rectum, c-kit-positive cells were present but on a morphological basis were classified as mast cells. Mast cells were again also present in all layers of the muscularis propria.

Transmural and Regional Distribution of ICC

Colon. The density of ICC in the muscularis propria of the colon and rectum, respectively, was graded on the basis of the number of ICC cell bodies seen in 10 high-power fields (×400). In the colon, χ² distribution analysis revealed significant differences in the density of ICC between the myenteric plexus and circular muscle layer and between the myenteric plexus and the longitudinal muscle layer in the right colon (Fig. 6A), transverse colon (Fig. 6B), and left colon (Fig. 6C). There were no significant differences in the density of

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Fig. 3. ICC (dark stain; arrowheads) in circular muscle layer of left colon. The whole of the field is not visualized but the density grade in the myenteric plexus would be moderate. Original magnification, ×400. Scale bar, 20 µm.

Fig. 4. An ICC at inner border of circular muscle layer (arrowhead). A mast cell (MC) is also seen. Original magnification, ×400. Scale bar, 20 µm.

Fig. 5. Mast cells (dark stain) in lamina propria of rectum. Original magnification, ×400. Scale bar, 20 µm.
ICC between the longitudinal muscle layer and circular muscle layer in any of the colonic regions.

Rectum. In contrast to the colon, the distribution of ICC was homogenous throughout the layers of the muscularis propria in the rectum (Fig. 6D). There were no significant differences in ICC density between the circular muscle layer and longitudinal muscle layer, the circular muscle layer and myenteric plexus, or between the longitudinal muscle layer and myenteric plexus.

Longitudinal muscle layer. Segmental variations in the density of ICC in the layers of the muscularis propria were apparent. In the longitudinal muscle layer (Fig. 7A), the only significant differences were between the rectum and right colon (P = 0.005) and between the rectum and left colon (P = 0.02), with the rectum having the higher density of ICC.

Intermuscular plane. At the level of the myenteric plexus (Fig. 7B), the transverse colon had a significantly different ICC density compared with the right colon (P = 0.038), the left colon (P = 0.006), and the rectum (P = 0.008), with a higher proportion of sections with the higher density gradings.

Circular muscle layer. In the circular muscle layer (Fig. 7C), the rectum had a significantly greater density of ICC than the left colon (P = 0.004); no other significant regional differences were apparent in the circular muscle layer.

DISCUSSION

In this study, immunohistochemistry using a rabbit anti-c-kit antibody identified two different cell types. On a morphological basis, these were determined to be ICC or mast cells. In the lamina propria, muscularis mucosa, and mucosa, the c-kit-positive cells present were deemed to be mast cells, which is in agreement with a previous report as to the nature of c-kit-positive cells in the human colon (34). Mast cells were also present in the muscularis propria. The morphological characteristics of ICC that we identified in human colonic ICC are similar to those recently described in a study of the human colon using anti-c-kit immunohistochemistry (34).

In animal studies it has been suggested that the expression of c-kit by ICC may wane with increased age (32). In a small group of subjects, with an age range of 1 day to 30 years, the distribution of ICC in the colon was reported as similar across the age range (34). In this study, c-kit expression by ICC was present in tissue taken from patients over 90 years of age. In our personal experience of ICC in colonic tissue from chil-
In each segment of the colon, the greatest density of ICC was observed in the intermuscular plane in which ICC encased the myenteric plexus. The density of ICC in the intermuscular plane was significantly greater in comparison to the circular and longitudinal muscle layers in the right, transverse, and left colon. Our finding is in disagreement with a recent report (34) of an even pattern of distribution of ICC in the muscularis propria of the colon. Descriptions of the distribution of ICC in the human colon on the basis of electron microscopic evidence have reported the presence of ICC around the myenteric plexus (11) and within the bulk of the circular muscle layer in the right, transverse, and sigmoid colon (25). We demonstrate that ICC are also present within the bulk of the longitudinal muscle layer.

We observed ICC throughout the colon, and also in the rectum, at the inner layer of the circular muscle layer in association with the submucosal plexus. This is in agreement with Rumessen et al. (25) who identified ICC throughout the colon, at the inner layer of the circular muscle, and contradicts the observations of Faussone-Pellegrini et al. (10) who identified ICC at the inner layer of the circular muscle but in the right colon only. We identified ICC at the inner margin of the circular muscle layer in association with the submucosal plexus in all regions of the colon and the rectum; however, these ICC were extremely sparse and certainly did not appear to form a continuous lining as has been previously described in other animal species (2).

Segmental variations in the density of ICC at various levels of the muscularis propria were apparent. At the myenteric plexus, the transverse colon had a higher proportion of moderate and/or many density gradings than the rectum or right or left colon. In the circular muscle layer, the rectum exhibited a significantly higher density of ICC than the left colon, and the right and transverse colon tended to have a higher density than the left colon, but this did not achieve significance. In the longitudinal muscle layer, the rectum displayed a significantly higher density of ICC compared with the right and left colonic regions. Such regional variations in the human colon have not been previously documented. In a small study (34) of five human colonic specimens, there were no segmental differences re-

Fig. 7. Regional distribution of ICC in longitudinal muscle layer (A), myenteric plexus (B), and circular muscle layer (C). See Fig. 6 legend for explanation of density % and grading scale. In longitudinal muscle layer, the only significant differences were between rectum and right colon (P = 0.005) and between rectum and left colon (P = 0.02). At the level of myenteric plexus, transverse colon had a significantly different ICC density compared with right colon (P = 0.038), left colon (P = 0.006), and rectum (P = 0.008). In circular muscle layer, rectum had a significantly greater density of ICC than left colon (P = 0.004); no other significant regional differences were apparent in circular muscle layer.
ported. In animal studies (4), regional variations of density of ICC in the colon have been described. In the cat, dog, rabbit, and opossum, ICC were sparse in the cecum, reached maximal density in the middle part of the colon, and became progressively less dense toward the rectum (4); this pattern is similar to that which we observed in humans. However, in the animals (4) this regional variation was observed at the submucosal plexus, whereas we observed the regional variation at the myenteric plexus. In these animals (4), few ICC were observed in association with the myenteric plexus, in which the density of ICC was proportional to the density of the plexus itself, and ICC were less conspicuous in the cecum and rectum compared with the rest of the colon.

Proposed functions for ICC include acting as pacemakers (21, 31, 32, 35, 36), intermediaries in the neural modulation of muscle activity (5), spatial coordinators of muscle activity (7), and as stretch receptors (8, 9). These roles are not mutually exclusive. The evidence that ICC act as pacemakers of gut muscular activity is derived from animal studies. In the dog, colonic ICC at the inner margin of the circular muscle layer in association with the submucosal plexus generate slow waves (1, 18, 20, 28) and thus act as pacemakers; a second pacemaker region at the myenteric plexus is also present (29). Our study would suggest that the ICC at the inner layer of the circular muscle layer would not form a sufficiently dense network to act as the main pacemaker region. A more plausible candidate for the main pacing region would be the intermuscular plane, in which the density of ICC is greatest. The electrical activity of the longitudinal muscle of the colon is similar in nature across the species (3). In the human longitudinal muscle layer, electrical activity is characterized by oscillatory activity in a narrow frequency range [24–36 counts/min (cpm)] with superimposed spiking activity with or without periods of quiescence. The electrical activity in the circular muscle layer shows interspecies variation: in the dog slow waves with constant amplitude and frequency are observed (3) and in the human colon a range of electrical frequencies is apparent (3, 14). In the human circular muscle layer, the slow wave frequency is very variable and lies within the range of 4–60 cpm (14). The frequency and amplitude are both variable and change with stimulation, and periods of electrical quiescence are apparent (14). The variable slow wave frequency suggests that multiple pacemakers exist. Pacing of the circular muscle in the human may not primarily reside in the submucosal ICC as suggested by the canine model (6, 30), and another pacing region in the intermuscular region may be present and may play the more dominant role, as suggested by our observations.

It has been suggested from animal studies of the cat, dog, ferret, guinea pig, opossum, rabbit, and rat that the density of ICC in the stomach and colon roughly parallels the prominence of slow waves (4). In the human colon, regional variations in slow wave activity have been reported, and in vivo studies have demonstrated that the overall dominant frequency of contractions is highest in the midcolonic region (27). This correlates well with our observation that the ICC density in the myenteric plexus is greatest in the transverse colon.

ICC may act as intermediaries in the enteric neural control of gut muscle activity (5). The association of ICC in the colon and rectum with neural elements of the myenteric plexus, and to a lesser extent with the submucosal plexus, would support this hypothesis.

In the human colon and rectum, contractile activity is characterized by propagated and nonpropagated contractions. The nonpropagated motor activity may reflect pacing of the smooth muscle by sparsely distributed ICC in the bulk of the muscle layers. Propagated motor activity in the form of colonic motor complexes, rectal motor complexes, or high amplitude propagated contractions would require communication between different regions of the colon and/or rectum through neural or ICC networks. Similarly, transmural differences in ICC density may explain the initiation of segmental contractions in the colon and the lack of segmenting activity in the rectum. Data from animal studies suggest that ICC networks mediate communication between different areas of the gut, whether between different muscle layers (17) or along the long axis of the gut (13, 19). The arrangement of ICC in linear chains or branching networks would be consistent with this role. ICC in the intermuscular plane may coordinate the activity of the circular and longitudinal muscle layers and may allow neural networks to modify muscular activity through alteration of ICC activity.

In summary, in the muscularis propria of the colon, ICC density was found to be greatest in the intermuscular plane compared with the muscle layers, but in the rectum the distribution of ICC was more even (Fig. 8).
Segmental density variations of ICC were identified; in the intermuscular plane the greatest density of ICC was observed in the transverse colon, which is the region of proposed pacemaking activity. Abnormal distribution of ICC has been reported in the affected tissue in gut dysmotility disorders, such as infantile pyloric hypertrophic stenosis (16, 33) and Hirschsprung’s disease (34, 37), and abnormal structure of ICC has been identified in patients with ulcerative colitis (22). Labeling ICC using an anti-c-kit antibody will help in the understanding of normal human colonic physiology and pathophysiology of diseases in which abnormal gut motor activity is an underlying feature.

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