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IDENTIFICATION OF INTERSTITIAL CELLS OF CAJAL IN THE HUMAN RECTUM

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1. ABSTRACT

It is postulated that the electric waves of the gut are generated by interstitial cells of Cajal (ICC). We hypothesized the presence of ICC in the rectum as initiating the rectal electric activity. The current study investigated this hypothesis. Two rectal specimens were taken from healthy areas of excised rectum of 22 rectal cancer patients (age 44.6±9.2 SD years, 12 men, 10 women). The study specimens were subjected to c-kit immunohistochemistry. Controls for antisera specificity consisted of tissue incubation with normal rabbit serum substituted for the primary antiserum. C-kit positive branched ICC-like cells were detected in the rectal musculature of the studied specimens. They were distinguishable from the c-kit-negative non-branched smooth muscle cells and from the c-kit positive but non-branched mast cells. Immunoreactivity was absent in the negative controls. We have identified in the rectum for the first time cells with morphologic and immunologic phenotypes similar to the ICC of the gut. The role of these cells in normal physiologic and pathologic conditions of the rectum needs further studies.

2. INTRODUCTION

The motor activity of the rectum has attracted the attention of many investigators. The principle function of the rectum is to contract in order to expel the fecal matter. The rectal motility, aided by various physiologic reflexes, serves specifically this function (1-4).

The rectum of healthy volunteers exhibits electric activity in the form of slow waves (SWs) and fast activity spikes or action potentials (APs) (5-7). The APs are coupled with rectal pressure elevation (5-7). The electromechanical activity of the rectum increases with rectal distension (5). The electric waves appear to be responsible for rectal motility (5-7). The source of these waves is not exactly known; previous studies have shown that they are transmitted through the smooth muscles of the rectum and are partially controlled by the intrinsic and extrinsic rectal innervation (8,9).

It is postulated that the gut electric waves are generated from the interstitial cells of Cajal (ICC) (10-15). These cells are located at the level of the myenteric plexus in the deep muscular plexus and within the circular muscle layer itself (10-15). They are considered to procreate the spontaneous pacemaker activity in the smooth muscle layers of the gut (16-18) and to be involved in neurotransmission as well (8,9,15). Moreover, they are claimed to pace the gastrointestinal phasic activity and are regarded as the pacemaker cells in the gastrointestinal muscles. They mediate or transduce inputs from enteric motor nerves to the smooth muscle syncytium (8,9,15). Interstitial cells of Cajal were recorded in the gastrointestinal tract (10-18); however a study of the presence of these cells in the rectum could not be traced in the literature. As the rectum exhibits electric waves, we hypothesized that it contains such cells; this hypothesis was investigated in the current study.

3. MATERIAL and METHODS

We obtained one 1x1 cm rectal specimen from each of 22 rectal cancer patients (mean age 44.6±9.2 SD years, range 36-58, 12 men, 10 women). The specimens were taken from the normal areas of the surgically excised rectum. The histologic sections were examined by a pathologist to exclude any malignant areas present in the prepared specimens. An informed consent was obtained from the patients to do investigations on their excised rectum.

For immunocytochemical processing, tissue sections were rehydrated in KPBS at room temperature for 20 min., blocked with 10% normal goat serum for 20 min., and incubated overnight at 4°C with the primary antiserum, a rabbit polyclonal IgG antibody to the human c-kit protein (Oncogene Research Products, Cambridge, MA), diluted 1:100 in KPBS, 0.05% goat serum and 0.1% Triton X-100. The next day, slides were rinsed with KPBS three times (each 10 min.) and then incubated with a Cy3-conjugated

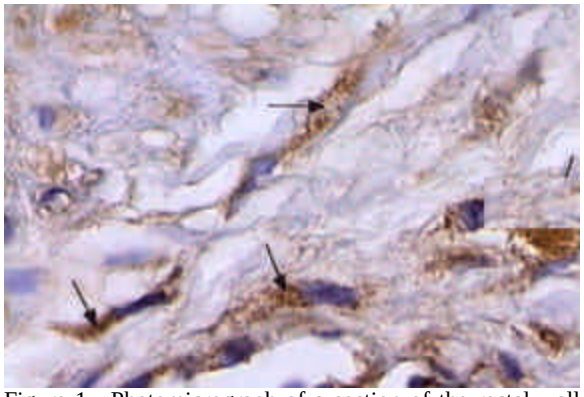

Figure 1. Photomicrograph of a section of the rectal wall showing c-kit-positive cells (arrow). The cells appear elongated with dendritic processes (c-kit immunostain counterstained with hematoxylin X400)

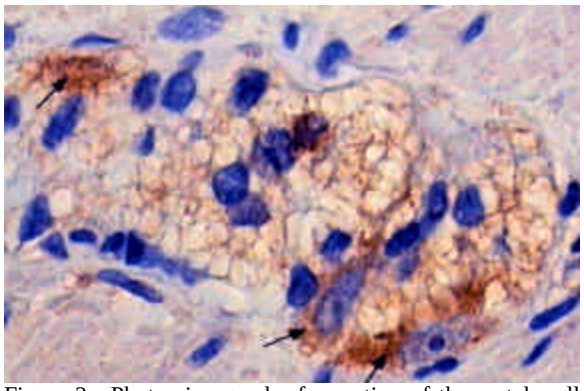

Figure 2. Photomicrograph of a section of the rectal wall showing c-kit-positive cells. Cells have either two (bipolar) or more (multipolar) dendritic processes (arrows). (c-kit immunostain counterstained with hematoxylin X400).

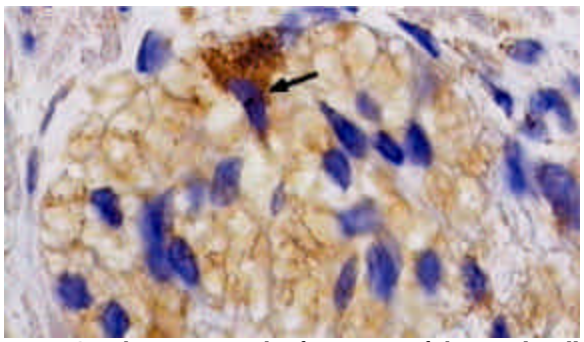

Figure 3. Photomicrograph of a section of the rectal wall showing c-kit-positive cells (arrow) which are included in the enteric nerve plexus. (c-kit immunostain counterstained with hematoxylin X400).

goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA) at room temperature for 2 hours at a dilution of 1:800 in KPBS 0.05% goat serum, and 0.1% Triton X-100. Because mast cells also contain the c-kit receptor and thus stain positive with c-kit antibodies, dual staining with fluoroscein-avidin DCS (Vector Laboratories, Burlingame, CA) diluted 1:200 for 2 hours was used to specifically identify mast cell staining. Slides were again washed three times with KPBS and overslipped. They were then imaged using an Olympus Fluoview 500 scanning confocal microscope.
Controls for the specificity of the antisera consisted of incubation of the tissue with normal rabbit serum substituted for the primary antiserum.

The results were analysed statistically using the Student's t test. Values were given as the mean ± SD and differences assumed significance at $p<0.05$.

4. RESULTS and DISCUSSION

Morphologically, cells that were different from the smooth muscle cells (SMC) were detected in the examined sections. These cells were c-kit positive and varied in morphology and size (Figure 1). Two morphological types could be identified: multi and bipolar. The multipolar type had many processes laterally while the bipolar had the processes at each end of the cell (Figure 2). The cells were arranged separately or in groups which were surrounded by connective tissue cells. They were located at the junctional area between the inner circular and outer longitudinal smooth muscle layers and/or insinuated between the smooth muscle fibers of the circular muscle layer. They were found close to, or included in, the enteric nerve plexus (Figure 3). The cells had different sizes varying from 48 to 142 µm (mean 84.2±38). They were fusiform, the cytoplasm had fine granules showing strong immunoreaction, and the nucleus was large and oval (figs.1, 2). As all branched cells were c-kit positive, they were identified as ICC.

Also mast cells appeared in the section as c-kit positive (Figure 4); however they differed morphologically from the ICC. They showed as rounded cells with rounded nuclei. Their body did not bear processes. This is in contrast to the fusiform cell body of the ICC with dendritic processes and large, oval nuclei (figs.1,2).These findings regarding the ICC also contrasted with those of the surrounding SMC. The latter had a typical spindle shape with more regular dimensions compared to the morphologic heterogeneity of the ICC (figs.1, 2, 5). They were generally larger than the ICCs and had a size varying from 122-175 µm (mean 148.2±14.4). The SMC had no dendritic processes like those of the ICC and had no immunoreactivity to the c-kit antibodies.

Immunoreactivity was absent in the negative controls in which the primary antibody had been omitted.

Recent investigations suggest a central role for the ICC in the control of intestinal motility in that they act as pacemakers (10-18). The current study is the first to demonstrate the presence in the rectal wall of c-kit positive, ICC-like cells that may act as pacemakers responsible for generation of the electric waves. The cellular morphology of these c-kit positive cells is characteristic of the cells which were previously identified as the ICC or pacemaker cells in the gastrointestinal tract. These cells were easily

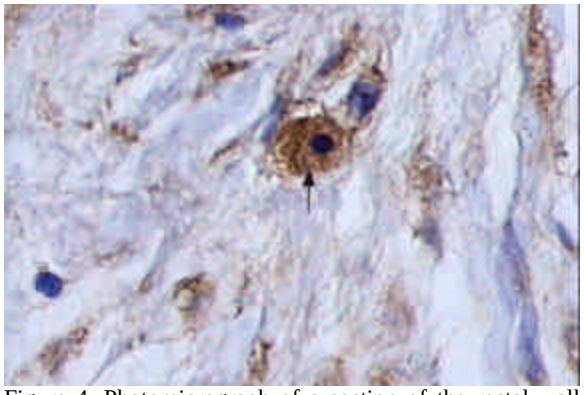

Figure 4. Photomicrograph of a section of the rectal wall showing c-kit-positive mast cells with rounded body which do not bear dendritic processes (c-kit immunostain counterstained with hematoxylin X400).

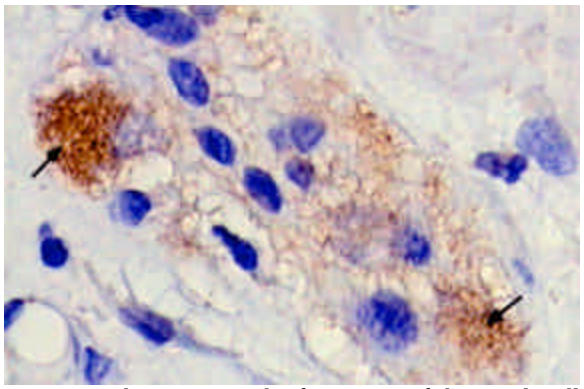

Figure 5. Photomicrograph of a section of the rectal wall showing c-kit-positive cells and the c-kit negative smooth muscle fibers (c-kit immunostain counterstained with hematoxylin X400).

distinguishable as above described from the mast cells and SMC. The mast cells were c-kit positive but their body was not speculated, while the SMC were c-kit negative. The cells were found in the muscle layers of the rectal wall close to, or included in, the enteric nerve plexuses.

It is reported that ICCs function as pacemakers, signaling to the bulk smooth muscle to contract, and also as intermediaries in the transmission of nerve signals to smooth muscle cells (10-18).Spontaneous electric activity has been recorded from the rectum of humans(6,7). It was generally assumed that this was an inherent property of the smooth muscle cells themselves and that no specialized pacemaker cells were necessary to initiate this activity.

However, the current study suggests that the spontaneous SWs recorded from the rectum may have originated in the ICCs and been conducted to the smooth muscle cells. The resting rectal electric activity seems to be controlled by the constant firing of pacemaker cells which are most likely the ICCs, the activity of which is conducted to the smooth muscle. It is believed that the release of small amounts of excitatory transmitter acts either directly on the rectal smooth muscle fibers or indirectly through stimulation of the pacemakers' firing with a resulting rectal smooth muscle contraction. Furthermore, as the ICC apparently mediate the response to nerves in the gastrointestinal tract (10-18), such a pattern may also exist in the rectum. Thus, it is suggested that the ICC mediate the effects of neurally released nitric oxide, provided that the ICC are preferentially innervated.

The current study may thus indirectly support the role of the ICC as mediators and/or initiators of rectal motility. Therefore, we believe that recognition of abnormalities of the ICC system in the rectum may offer an insight into the causes of disorders of rectal motility. Deficiency of ICC is suggested to play a role in inefficient rectal motility. Further studies would be necessary to determine the functional role of the c-kit-positive ICC in rectal motility and motility disorders.

In conclusion, we have identified cells in the rectum with morphologic and immunologic phenotypes similar to the ICC of the gut. The role of these cells in normal physiologic and pathologic conditions of the rectum needs further studies.

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