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Characterization of Interstitial Cells of Cajal in Bowel of Cattle (*Bos taurus*)

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ABSTRACT

Interstitial cells of Cajal (ICC) have been described in the gastrointestinal tract of different mammals including humans, horses, pigs, rats, dogs, mice and guinea-pigs. In the present study, ICC were identified in the jejunum of *Bos taurus* using polyclonal anti-c-Kit antibodies in immunohistochemical assays. Vimentin and desmin intermediate filaments were also determined using monoclonal antibodies. ICC were found in the tunica muscularis either in a palisade distribution pattern between the outer longitudinal and the inner circular layers (ICC-MP) or freely distributed in clusters in the longitudinal layer (ICC-LM). Morphometric studies determined that ICC have a fusiform shape presenting cytoplasmic prolongations. ICC were positive to c-Kit and vimentin antigens but negative to desmin. We have observed and described for the first time the presence of ICC in a ruminant. As observed in the aforementioned mammals, bovine ICC were associated with the myenteric plexus. Nevertheless, the presence of widespread ICC in the longitudinal muscular layer of the jejunum differs from previously described studies of other mammals.

Keywords: bovine, c-Kit, enteric nervous system, immunohistochemistry, intermediate filaments, interstitial cells of Cajal, morphometry

Abbreviations: ICC, interstitial cells of Cajal; ICC-LM, ICC distributed in small groups in the intestinal muscular longitudinal layer; ICC-MP, ICC adopting a palisade distribution between the intestinal muscular outer longitudinal and the inner circular layers

INTRODUCTION

Interstitial cells of Cajal (ICC) (Cajal, 1899), a variety of the myofibroblast cell, have been found in the gastrointestinal tube of humans (Faussone-Pellegrini *et al.*, 1977), dogs (Langton *et al.*, 1989; Torihashi *et al.*, 1994), horses (Hudson *et al.*, 1999), rats (Faussone-Pellegrini *et al.*, 1996; Ekblad *et al.*, 1998), mice (Faussone-Pellegrini, 1985; Torihashi *et al.*, 1995; Ordog *et al.*, 1999; Daniel and Wang, 1999), guinea-pigs (Zhou and Komuro, 1992; Komuro and Zhou, 1996; Burns *et al.*, 1997; Wang *et al.*, 1999) and

birds (Lecoin *et al.*, 1996). Their origin has been debated for decades, and they are now considered as mesodermal cells. ICC present a precursor in common with the smooth-muscle cells (Ward and Sanders, 2001; Wu *et al.*, 2000). The ICC are considered the pacemakers of the gastrointestinal tube, acting as mediators of the enteric nervous system, and the initiators of the gastrointestinal slow-wave activity (Thuneberg, 1982). They have also been associated with gastrointestinal motility disorders (Vanderwinden, 1999) and gastrointestinal stromal tumors (Vanderwinden and Rumessen, 1999; Hagger *et al.*, 1998).

According to Hanani and Freund (2000), ICC are located in association with the myenteric plexus (ICC-MP); at the border between the circular muscle and the submucosa (ICC-SMP); between the internal thin layer and the thick outer layer of the circular muscle (ICC-DMP); in the longitudinal muscle (ICC-LM) and in the circular muscle (ICC-CM). The latter two localizations are referred to as intramuscular ICC (ICC-IM).

The gastrointestinal motility pattern in ruminants is different from that in other mammals (Leek, 2001). Considering that there are no bibliographic references concerning ICC in ruminants and that bovines may suffer from motility disorders that could interfere with livestock productivity we decided to determine the presence of these cells in bovine intestines.

MATERIALS AND METHODS

Five portions of the middle jejunum were obtained from six 2-year-old female Hereford cattle slaughtered at a commercial abattoir. Organs were fixed in 10% buffered formaldehyde, embedded in paraffin and processed according to the technique described by Hagger and colleagues (1998) and Robinson and colleagues (2000).

Immunohistochemistry was performed using the Envision method (Dako Corp., Carpinteria, CA, USA). A commercial rabbit affinity-purified polyclonal anti-c-Kit antibody (CD117) (Dako, Japan A4502) was used as a primary antibody for detecting ICC. Anti-vimentin (prediluted V9, Dako) and anti-desmin (prediluted D33, Dako) monoclonal antibodies were used for detecting intermediate filaments. Ten transversal sections of each sample were cut at 5 μm and mounted on silane-coated slides. The slides were then deparaffinized with xylene, incubated with 0.3% methanolic H_2O_2 for 30 min at room temperature in order to inhibit endogenous peroxidase activity, hydrated through a series of ethanol solutions of decreasing concentration, and finally rinsed with deionized water and PBS, pH 7.6. No antigen retrieval was carried out because of results obtained in preliminary studies. To prevent non-specific adsorption of immunoglobulins, the sections were incubated for 20 min in 0.1% BSA. The sections were then incubated either with the c-Kit antibody diluted 1/50 in PBS or with the other two antisera for 2 h at room temperature. An incubation of the sections with the polymer K1490 (Dako) for 20 min at room temperature was then performed. Each incubation period was followed by gentle washing in PBS. The use of 3,3′-diaminobenzidine (Dako) revealed the bound complex. The sections were counterstained with Meyer's haematoxylin, dehydrated and mounted with DPX.

To distinguish mast cells, transversal sections of the middle jejunum were stained with the toluidine blue metachromatic technique (Bancroft and Stevens, 1990).

To perform morphometric studies, 10 images of each slide were captured with an analogue RGB video-camera (DXC-151A CCD, Sony, Tokyo, Japan) mounted on a microscope (BX50, Olympus, Tokyo, Japan), at various magnifications, and digitized with a frame grabber (Flashpoint 128, Integral Technologies, Inc., Indianapolis, IN, USA) connected to a computer. Images were processed using the Image-Pro Plus v4.50 software (Media Cybernetics, Silver Spring, MA, USA) with an RGB depth of 24 bits, and saved in TIFF format. The images had a resolution of 640×480 pixels with a spatial calibration yield of either 0.13 ($\times 1000$) or 0.31 ($\times 400$) μm/pixel. ICC were quantitatively characterized based on their area (μm^2), major and minor axis (μm) (to obtain the aspect ratio of the object), perimeter (μm) and roundness. Table I describes the morphometric parameters evaluated.

The number of ICC present were counted either per 100 lineal micrometre (palisade distribution) or per 1×10^4 μm^2 (free distribution).

TABLE I
Morphometric parameters used to evaluate ICC characteristics

Parameter	Description
Area	The area of each object
Aspect	The ratio between the major axis and the minor axis of the ellipse equivalent to the object (i.e. an ellipse with the same area and first and second degree moments), as determined by major/minor axis
Major axis	The length of the major axis of the ellipse equivalent to the object (i.e. an ellipse with the same area and first and second degree moments)
Minor axis	The length of the minor axis of the ellipse equivalent to the object (i.e. an ellipse with the same area and first and second degree moments)
Perimeter	The length of the outline of each object
Roundness	The roundness of each object, as determined by the formula: $(\text{perimeter}^2)/(4\pi \times \text{area})$. Circular objects have roundness = 1; other shapes have roundness >1

RESULTS

The immunohistochemical analysis revealed the presence of two different anti-c-Kit-positive cell localizations. Cells were observed either in the tunica muscularis or in the lamina propria and tunica submucosa (Figure 1a,b).

Cells found in the lamina propria and tunica submucosa were morphologically recognized as mast cells. This was confirmed using metachromatic techniques. On the other hand, cells found in the tunica muscularis showed either a palisade distribution pattern between the outer longitudinal and the inner circular layers of the jejunum

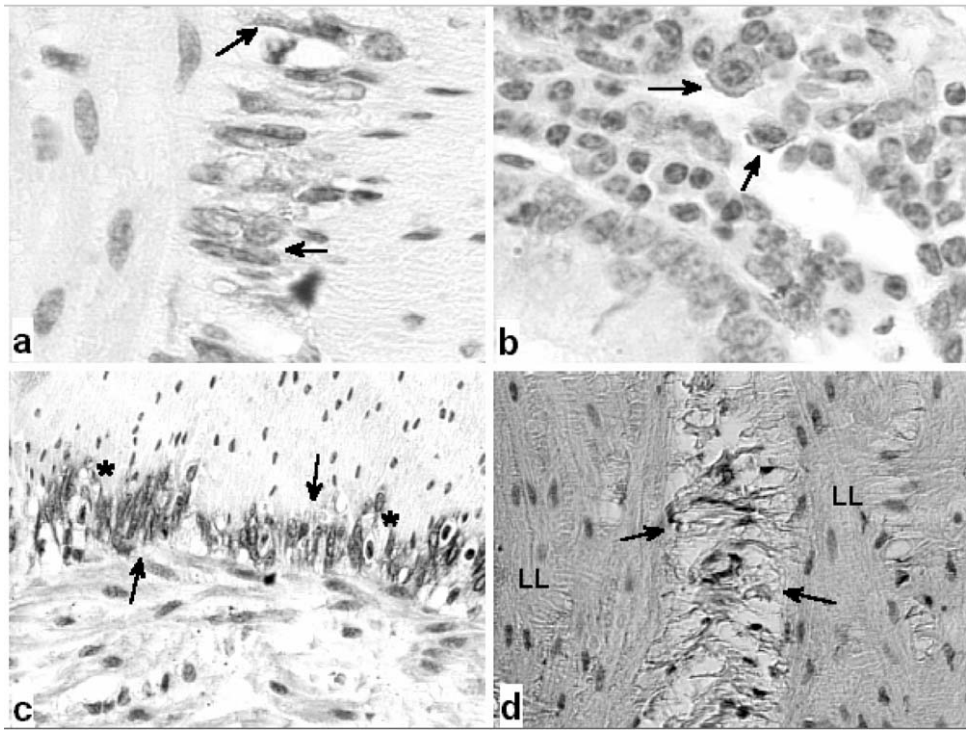

Figure 1. Cells showing anti-c-Kit-positive response in bovine jejunum. (a) ICC (arrows) are present in the tunica muscularis (original magnification $\times 1000$). (b) anti-c-Kit-positive cells, morphologically resembling mast cells (arrows), are distributed along the mucosa (original magnification $\times 1000$). (c) ICC (arrows) are distributed either in a palisade pattern (original magnification $\times 400$) or (d) distributed in clusters (arrows) in the longitudinal layer (LL) (original magnification $\times 400$). Asterisks mark ganglionar cells

(ICC-MP) (Figure 1c) or were distributed in small groups in the longitudinal layer (ICC-LM) (Figure 1d). The observed palisade pattern was disrupted when ICC-MP surrounded the ganglionar cells present in the layer (Figure 1c). We measured 100 lineal μm and found a total of 25.05 ± 6.25 ICC-MP. The ICC-LM present in the tunica muscularis (9.52 ± 3.86 per 1×10^4 μm^2) were observed throughout the outer longitudinal layer without a defined distribution pattern (Figure 1d).

Using immunohistochemistry of paraffin-embedded tissues, we found that ICC-MP and ICC-LM presented a fusiform cell body with a large oval nucleus. The ICC-MP had short dendritic processes (Figure 2a) while ICC-LM had large ones (Figure 2b). Morphometric characteristics determined for cells found in both muscular anatomical locations are shown in Table II.

c-Kit-positive cells present in the tunica muscularis were also vimentin-positive but desmin-negative (Figure 3a,b).

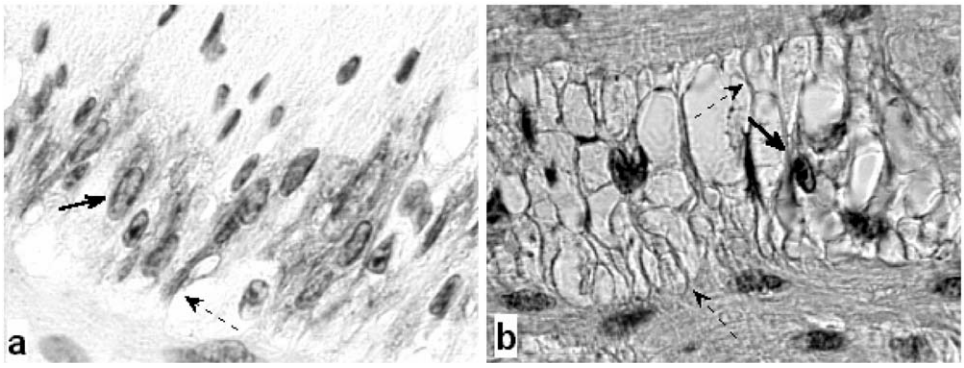

Figure 2. Morphological aspects of immunohistochemically stained ICC. ICC-MP and ICC-LM show a fusiform cell body with a large oval nucleus (arrows). (a) ICC-MP have short dendritic processes (dashed arrow). (b) ICC-LM have large dendritic processes (dashed arrows) (original magnification $\times 1000$)

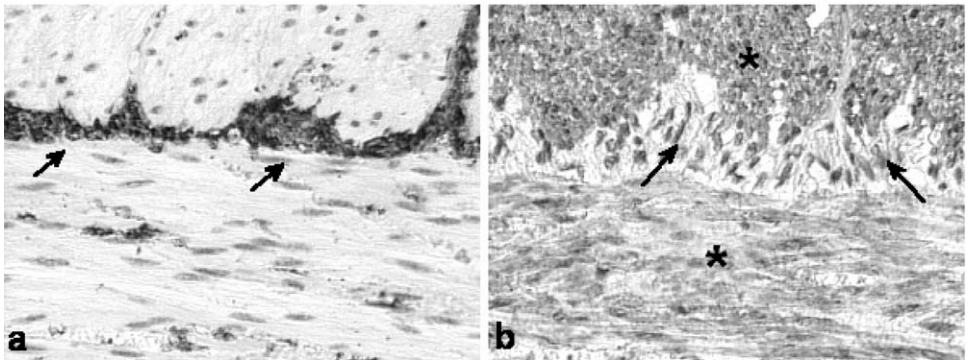

Figure 3. Intermediate filaments of ICC. ICC are positive to anti-vimentin antibodies (arrows) (a) and negative to anti-desmin antibodies (arrows) (b). Asterisks indicate an anti-desmin positive reaction in circular and longitudinal muscle layers (original magnification $\times 400$)

DISCUSSION

According to the distribution pattern and morphology of the cells present in the tunica muscularis and their positive reaction to anti-c-Kit and anti-vimentin antibodies, we speculate that these cells may be ICC. This finding constitutes the first evidence of the existence of ICC in ruminants, a group of mammals with a digestive mechanics different from that of the other species analysed (Forbes and Barrio, 1992; Leek, 2001).

TABLE II
Morphometric characteristics of ICC

	Area (μm^2)	Axis (major) (μm)	Axis (minor) (μm)	Aspect	Perimeter (μm)	Roundness
ICC-MP						
Cytoplasm	55.93 ± 17.84	16.67 ± 3.50	4.45 ± 1.05	3.94 ± 1.25	39.36 ± 7.63	2.32 ± 0.64
Nucleus	20.52 ± 8.82	8.42 ± 1.88	3.04 ± 0.84	2.87 ± 0.72	19.16 ± 4.07	1.51 ± 0.26
ICC-LM						
Cytoplasm	31.29 ± 7.19	12.77 ± 1.19	3.14 ± 0.43	4.07 ± 2.77	27.91 ± 3.98	1.99 ± 0.11
Nucleus	4.45 ± 0.30	4.75 ± 0.38	1.22 ± 0.16	3.89 ± 2.40	10.04 ± 0.97	1.83 ± 0.47

Values represent means $\pm$ standard deviation (n = 6 two-year-old female Herefords)

In the present study, we determined the presence of ICC-LM in the small intestine of bovines. These were the only type of ICC-IM found in this species. In humans, only ICC-CM and ICC-DMP have been observed (Vanderwinden and Rumessen, 1999) while in dogs, rats and guinea-pigs ICC-DMP were the only cells found (Komuro, 1999). In horses, ICC were found in both longitudinal and circular muscle (Hudson *et al.* 1999, 2002). ICC-MP were found in association with ganglionar cells and smooth muscle as it occurs in humans, dogs, horses, rats, mice, guinea-pigs and birds (Hudson *et al.*, 1999, 2002; Sanders *et al.*, 1999; Epperson *et al.*, 2000). This association in cattle could indicate a functional relationship between these three types of cells, as has been postulated for other species (Sanders *et al.*, 1999).

Although the ICC of the small intestine of bovines are fusiform cells and have processes similar to those observed in the other species (Komuro, 1999; Hanani and Freund, 2000; Sanders *et al.*, 2002), we cannot compare the morphometric data with those of other species since no similar studies have been carried so far, except for the guinea-pig. In guinea-pigs two types of morphologies for the ICC-MP were found in the ileum (Belzer *et al.*, 2002); the major axis of both type of cells is similar to what we observed in the jejunum of bovines. Nevertheless, the minor axis in bovines is half the size found in guinea-pigs. This difference could be due to species variation or the localization of the cells.

Our immunohistochemical studies have shown that bovine ICC express vimentin and lack desmin, similarly to other species (Powell *et al.*, 1999).

We have shown that bovines present ICC in their small intestine, as occurs in different mammals and birds. However, the ICC found in the jejunum of bovines present a distribution pattern different from that in other species.

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