J. Cell. Mol. Med. Vol 12, No 5B, 2008 pp. 1944-1955

Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut

Laura Pieri, Maria Giuliana Vannucchi, Maria Simonetta Faussone-Pellegrini*

Department of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy

Received: July 28, 2008; Accepted: August 5, 2008

Abstract

CD117 (or c-kit) is expressed by the interstitial cells of Cajal (ICC), which are located within the gastrointestinal (GI) muscle coat and directly involved in its motility. CD34 is expressed by several cell types some of which have features and location resembling the ICC; however, a sure identification of these cells is still lacking. In order to establish whether the CD34-positive cells of the human GI tract are to be considered as ICC subpopulation or a novel independent cell type, and to hypothesize their nature and role, we verified CD34 and CD117 receptor expression under light and fluorescence microscope and performed a routine and a CD34-immuno-electron microscopy. CD34-positive cells were seen in the entire human GI tract. In the *muscularis propria*, shared morphologies similar to the c-kit-positive cells, in the *submucosa*, resembled fibroblasts. Their ultrastructure resembled that of the fibrocytes/fibroblasts and of the interstitial Cajal-like cells (ICLC). Double labelling and immunoelectro-microscopy demonstrated that they are unequivocally different to the ICC and, due to the similarities with the ICLC, we identified them as ICLC. The novelty of these results is that two types of interstitial cells are present in the GI muscle coat of humans: the ICC and the ICLC. We hypothesize a mechanical role for the septal ICLC, those at the myenteric plexus level and those bordering the muscle layers; a helping role in neurotransmission is proposed for the ICLC intercalated with the intramuscular ICC, possibly in spreading the slow waves generated by the ICC. Furthermore, the possibility that the ICLC represent the adult mesenchymal stromal cells able to guarantee the ICC renewal deserves to be considered.

Keywords: CD34 immunohistochemistry • c-kit immunohistochemistry • electron microscopy • interstitial cells of Cajal • interstitial Cajal-like cells • mesenchymal stromal cells

Introduction

CD117 is a Kit membrane tyrosine kinase receptor codified by the gene c-kit and expressed on the cell plasma membrane. In the adult, the so-called interstitial cells of Cajal (ICC) and the mast cells are among the cell types that are c-kit (or CD117)-positive. The ICC form networks within the gastrointestinal (GI) muscle coat and there is a general agreement [1–8] that they (*i*) are the cells described and called interstitial cells or interstitial neurons by Cajal [9], (*ii*) are c-kit-positive, (*iii*) have a characteristic ultrastructure and (*iv*) are directly involved in the GI motor activities.

CD34 is a sialylated transmembrane glycoprotein and its expression is a hallmark of haematopoietic stem cells [10–12]. CD34 expression decreases as these cells differentiate and it is no more expressed by the mature blood cells. Interestingly, CD34 is also expressed by cells outside the haematopoietic system. Among them, the endothelial cells [13], cells identified as fibroblasts [14], the so-called ICLC (interstitial Cajal-like cells) described in several organs [15–25], and some cells observed in the GI wall [26, 27]. Most of these cells, in particular the ICLC and the GI cells located in the connective tissue among the muscle bundles and around the myenteric plexus ganglia and nerve bundles, have an elongated and ramified body resembling the ICC; however, a sure identification of these cells is still lacking.

It has been suggested that both GISTs and EGISTs (gastrointestinal and extra-gastrointestinal stromal tumours) originate from the ICC; this hypothesis was built on the observation that almost all these tumours were made by CD117-positive cells [28–33]. Moreover, CD34-positive cells might be present in these tumours, and, therefore, the question rose on whether in the GISTs and EGISTs also these cells are to be considered as ICC. Up-to-now, this

*Correspondence to: M. S. FAUSSONE-PELLEGRINI,
Department of Anatomy, Histology and Forensic Medicine,
University of Florence, Viale Pieraccini 6, 50139 Florence, Italy.
Tel.: (+39)055-4271389
Fax: (+39)055-4271385
E-mail: s_faussone@unifi.it

doi:10.1111/j.1582-4934.2008.00461.x

question remains disputed because only according to reports obtained in the GISTs [see 34 and 35 for review of literature] the ICC are positive for both Kit and CD34 whereas, according to the findings obtained in controls the ICC never express the CD34 [26, 27].

All the reports on the CD34-positive cells are based on immunohistochemical identification and, curiously, nobody has considered the possibility that the cells described in the gut by Cajal might be comprehensive of two cell types: the c-kit- and the CD34-positive cells. The present study was aimed to build a map of the CD34-positive cells within the human GI tract, and, in particular, to morphologically characterize those present in the muscle coat where ICC are undoubtedly present and well recognizable for their location, c-kit-positivity and ultrastructure. At this aim, we made immunohistochemistry for both CD34 and CD117 receptors, verifying their expression under light and fluorescence microscope, and performed a routine and a CD34-immuno-electron microscopy. The results obtained would allow establishing whether the CD34-positive cells can be considered a subpopulation of the ICC or, instead, a novel independent cell type. Furthermore, a definitive morphological characterization of this cell type could be useful to define its nature, to propose a role and to better understand which are the cell types present in the GISTs.

Materials and methods

Samples of gastric (fundus, corpus and antrum) and intestinal (ileum, ascending and descending colon) wall were obtained from patients operated for cancer. Patients were 6 (3 females and 3 males, mean age 68 years) considering this study did not require a high number of cases. Care was taken in taking specimens far from the tumour and in choosing the areas devoid of inflammation. All the patients gave the written consent and a local committee approved the study. Specimens were processed for light, fluorescence and electron microscope immunohistochemistry and for routine electron microscopy.

Light and fluorescence microscope immunohistochemistry

Four-micron-thick sections were cut from formalin-fixed and paraffin-embedded tissue blocks. They were mounted on gelatinized slides, de-waxed in xylene and re-hydrated through a graded series of ethanol. For heat-induced antigen retrieval, the samples were treated for 20 min. at 90–92°C in buffer Tris 10 mM/l and EDTA 1 mM/l pH 9.0.

For light microscope detection, endogenous peroxidase was blocked using 3% H_2O_2 in PBS for 5 min. and then the sections were pre-incubated in 1% bovine serum (BSA) in PBS for 30 min. CD117, c-kit rabbit polyclonal antibody (Dako, MI, Italy), was used at a final dilution of 1: 400; CD34 mouse monoclonal antibody (Dako, MI, Italy) was used at a final dilution of 1: 25. All the antibodies were diluted in BSA 0.1% in PBS and applied for 30 min. at room temperature. After washing, the sections were incubated with a polyclonal biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1: 1000, or with a monoclonal biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1: 300 in 0.1% BSA in PBS, for 90 min. at room temperature. Subsequently, the sections were washed and ABC Reagent (Vectastain ABC/Elite Kit, Vector Laboratories, Burlingame, CA, USA) was added for 20 min. and developed with diaminobenzidine (DAB) (Sigma, St. Louis, MO, USA). Sections not exposed to the primary antibody were included as negative controls for antibody specificity. All the sections were counterstained with haematoxylin for nuclei labelling and observed under the Zeiss Axioscop light microscope.

For fluorescence microscope detection, some sections were pre-incubated in 1% BSA in PBS for 30 min. The c-kit rabbit polyclonal antibody (Dako, MI, Italy) was used at a final dilution of 1: 100 and the CD34 mouse monoclonal antibody (Dako, MI, Italy) was used at a final dilution of 1: 25. All the antibodies were diluted in BSA 0.1% in PBS and applied overnight at 4°C. At the end of the incubation, the sections were washed in PBS and incubated with fluorescein CY™2-conjugated AffiniPure F(ab1)2 fragment goat anti-rabbit IgG (H+L; Jackson Immunoresearch, West Grove, PA, USA) secondary antibody, diluted 1: 50 or with Texas Red conjugated affinity purified IgG (H+L) anti-mouse secondary antibody raised in horse (Vector Laboratories, Burlingame, CA, USA) diluted 1: 100. All the secondary antibodies were diluted in BSA 0.1% in PBS and applied for 2 hrs, in the dark, at room temperature. For CD117/CD34 double labelling, the sections were incubated as above mentioned putting together the primary antibodies. Some sections never exposed to the primary antibodies were included as negative controls. All the sections were then mounted in an aqueous medium (Gel Mount, Biomeda Corp., Foster City, CA, USA) and observed under an epifluorescence Zeiss Axioskop microscope and photographed.

Transmission electron microscopy

For conventional electron microscopy, full-thickness strips of the muscle coat of the stomach and intestine were immersed in a fixative solution of 2% cacodylate-buffered glutaraldehyde (pH 7.4) and kept in this solution for 6 hrs. Then, they were rinsed in a cacodylate-buffered solution supplemented with sucrose, post-fixed with 1% phosphate-buffered OsO_4 (pH 7.4) for 2 hrs, dehydrated with graded alcohol, clarified in propylene oxide and embedded in Epon using flat moulds.

For pre-embedding immuno-electron microscopy, full-thickness thin sections of the muscle coat were fixed for 20 min. at room temperature with the PLP fixative that was prepared by mixing immediately before use equal volumes of paraformaldehyde 8% in distilled water and 0.2M PBS containing 0.055 g/l $NaIO_4$ and 0.04M lysine, added with 0.5% glutaraldehyde. After washing in 0.1M PBS, the specimens were pre-incubated in 0.1 M PBS (pH 7.4), added with 1% BSA, for 15 min. at room temperature and, then, incubated in the presence of the CD34 primary antibody. After washing, the samples were incubated in the presence of horseradish peroxidase (HRP) biotinylated anti-rabbit secondary antibody (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA), 1 drop/5 ml 0.1M PBS, for 30 min. at room temperature and biotinylated anti-mouse secondary antibody. The specimens were then washed and treated with avidin–biotin complex system (Vectastain ABC kit; Vector Laboratories) for 10 min. and reaction products were developed by diaminobenzidine (Sigma Aldrich, St. Louis, MO, USA) 0.015 g in 10 ml of 0.5M Tris buffer (pH 7.4) and two drops of H_2O_2 3%. Samples were observed under a light microscope to verify the presence of the immunoreaction and the selected areas were cut and post-fixed in 1% phosphate-buffered OsO_4 (pH 7.4) for 2 hrs at room temperature, dehydrated and embedded as described above for conventional electron microscopy.

The semi-thin sections of both types of samples were obtained with a LKB NOVA ultra-microtome, either unstained or stained with a solution of

toluidine blue in 0.1M borate buffer, and then observed under a light microscope. Ultra-thin sections of the selected areas were obtained with the same ultra-microtome using a diamond knife and for conventional electron microscopy stained with an alcoholic solution of uranyl acetate, followed by a solution of concentrated bismuth subnitrate; for the immunostained specimens, some sections were observed without staining them and some others were stained with a solution of lead citrate. All the ultra-thin sections were examined under a JEOL 1010 electron microscope and photographed.

Results

Immunocytochemistry, light and fluorescence microscopy

Everywhere in the GI wall, the endothelial cells are CD34-positive and c-kit-negative (Fig. 1A and C), and mast cells are CD34-negative and c-kit-positive (Fig. 1B and D). Moreover, other types of CD34-positive cells are present in the submucosa (Fig. 1E and F) and either CD34- or c-kit-positive cells are located in the muscularis propria.

Muscularis propria. At all GI levels examined, the CD34-positive cells are located around and within the muscle bundles (Fig. 2A and B), around myenteric ganglia (Fig. 2C) and around and within nerve strands (Fig. 2D). These cells are polymorphic, but have in common an elongated shape, a small body and thin processes (Fig. 2E and F). The body contains the nucleus and a variable amount of cytoplasm and its shape might be oval or triangular. Processes are thin and long and their number is highly variable, as well as that of their ramifications (Fig. 2E and F). Small knobs, oval or triangular in shape, are present along the ramifications. The CD34-positive cells make an almost continuous sheath around myenteric ganglia (Fig. 2C and G) and a discontinuous layer at the submucosal border of the circular muscle layer (Fig. 2A) and at the mesothelial border of the longitudinal muscle layer. Although often close to each other, these cells do not clearly form networks, either within the muscle coat (Fig. 2A and B) or at the myenteric plexus level (Fig. 2G). On the contrary, the c-kit-positive ICC, although sharing similar morphologies with the CD34-positive cells being elongated and provided of processes, form networks either intramuscularly or at the myenteric plexus level (Fig. 2H). Moreover, in the small intestine the CD34-positive cells (Fig. 2A) are only occasionally encountered at the level of the deep muscular plexus (DMP), where c-kit-positive cells identifiable as ICC-DMP are numerous and form a network. By double labelling, CD34- and c-kit-positivity do not co-localized (Fig. 3A–C), although the CD34- and the c-kit-positive cells are often so close to each other to suggest that a single cell has a c-kit-positive body and CD34-positive processes or a CD34-positive body and c-kit-positive processes. Moreover, it can be undoubtedly appreciated when the bodies and the processes of the CD34-positive and the c-kit-positive cells are strictly intermingled (Fig. 3B) as well as when the CD34-positive cells run in rows intercalated with the c-kit-positive cells (Fig. 3C).

In the *mucosa* and *submucosa,* mast cells only are c-kit-positive. None of the other connective tissue cells is CD34-positive in the mucosa, while most of them are CD34-positive in the submucosa (Fig. 1E and F). Moreover, some of the CD34-positive cells characteristically form a thin and almost continuous layer that encircles large vessels (Fig. 1C and F) and submucous plexus ganglia (Fig. 1E).

Ultrastructure of the CD34 positive cells located in the muscularis propria

At all level examined, there are cells identifiable as ICC for their ultrastructural characteristics [36–39]. Moreover, other cells frequently run close to the ICC (Figs. 4 and 5), thus sharing a similar distribution. Many of these cells are located at the myenteric plexus level (Fig. 4A and B), covering ganglia (Fig. 4B) and entering nerve strands where form an apparently discontinuous three-dimensional network (Fig. 4A). Some of these cells form a discontinuous layer at both the submucosal and mesothelial borders of the circular (Fig. 5A and B) and longitudinal muscle layers. In the stomach (antrum), cells with these features surround groups made by nerve bundles and ICC (Fig. 5C and D) and some of their processes apparently contribute to form the aforementioned submucosal sheath (Fig. 5D). Some other cells (Fig. 4C) are located in the intramuscular connective septa, where they form a discontinuous sheath around muscle bundles in continuity with the submucosal one. Finally, some other cells are sparsely distributed among the smooth muscle cells (Fig. 4D). All these cells, at variance with the ICC, have a very small oval or triangular body containing the nucleus (Figs. 4A–D, 5C, D) and extremely long and thin processes (Fig. 5). The cytoplasm is scarce and contains cisternae of the rough endoplasmic reticulum (Figs. 4A, D, 5C), especially numerous in the nucleated portion, a small Golgi apparatus and mitochondria sparsely distributed (Figs. 4 and 5). Triangularly shaped enlargements (knobs) occur all along the processes, where both mitochondria and rough endoplasmic reticulum cisternae are contained (Figs. 4A and 5D). Occasionally, caveolae and small bundles of intermediate filaments are present. The basal lamina is always absent. Appositions between the processes of these cells (Fig. 4A and D, inset Fig. 4A) and between these processes and the ICC body can be seen; on the contrary, none of these cells establishes specialized contacts with smooth muscle cells and is in contact with nerve endings. As already described by one of us [38], the intramuscular ICC are often intercalated with these cells.

Immunoelectro-labelling demonstrates all these cells (Fig. 6A–F) and the endothelial cells (Fig. 6C) are CD34-positive. Conversely, none of the ICC is CD34-positive. The labelling is exclusively present on the plasma membrane and distributed all along of the entire cell contour, caveolae included. The plasma membrane appears as a dark and thick layer from which regularly distanced spherules protrude within the glycocalyx (Fig. 6F).

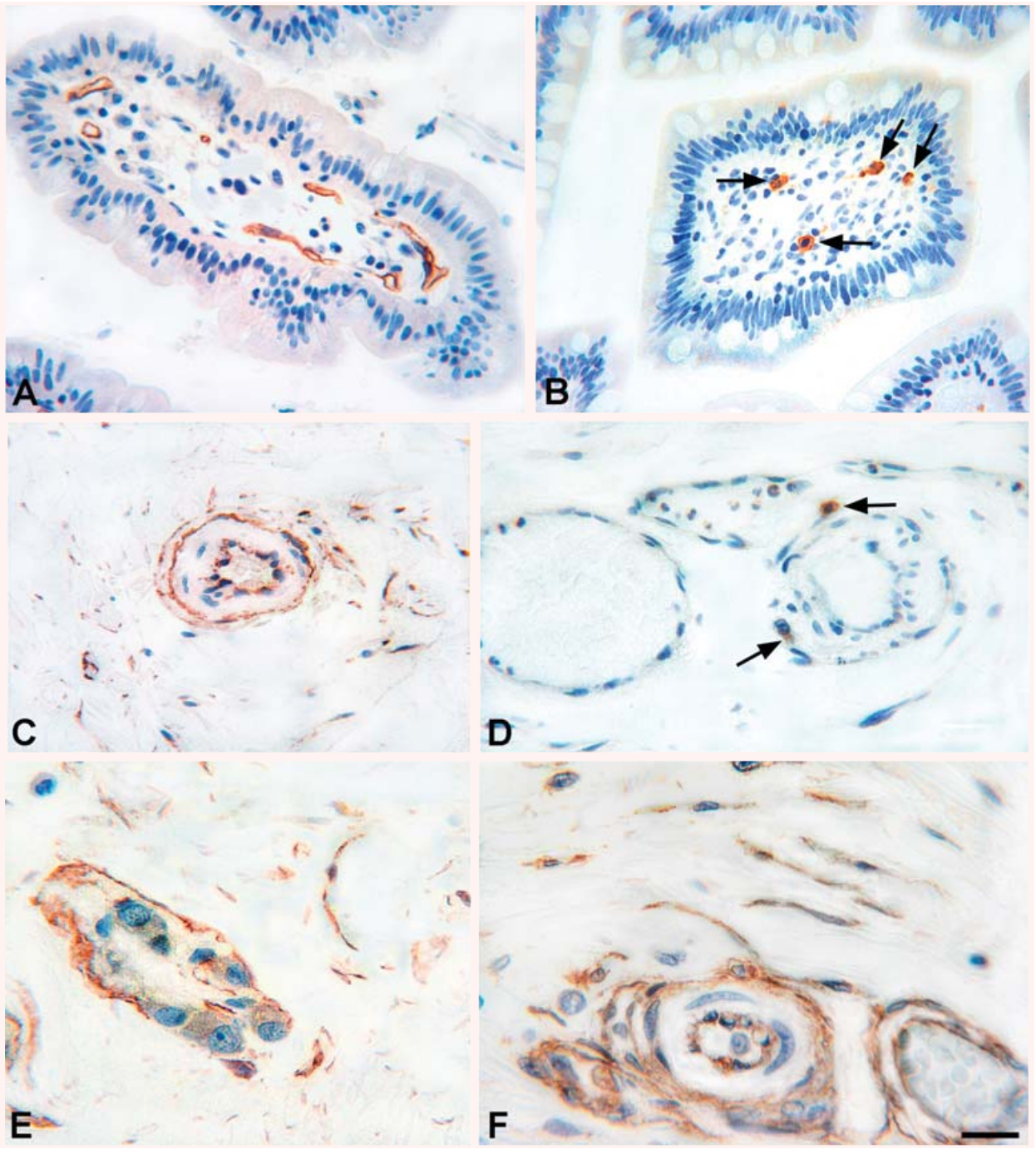

Fig. 1 **A**, **C**, **E** and **F**: CD34-immunoreactivity; **B** and **D**: c-kit-immunoreactivity. (**A**) Intestinal villous, endothelial cells only are CD34-positive. (**B**) Intestinal villous, mast cells only are c-kit- positive (*arrows*). (**C**–**F**) Submucosa. Most of the CD34-positive cells are sparsely distributed (**C**, stomach, **E**, large intestine, **F**, small intestine) and some others form a thin and continuous layer that encircles large vessels (**C**, stomach, **F**, small intestine) and submucous plexus ganglia (**E**, large intestine). The CD34-positive cells resemble fibrocytes/fibroblasts having an oval body and two processes. Mast cells only are c-kit positive (**D**, small intestine). Bar: **A**–**D** = 35 μm; **E**, **F** = 20 μm.

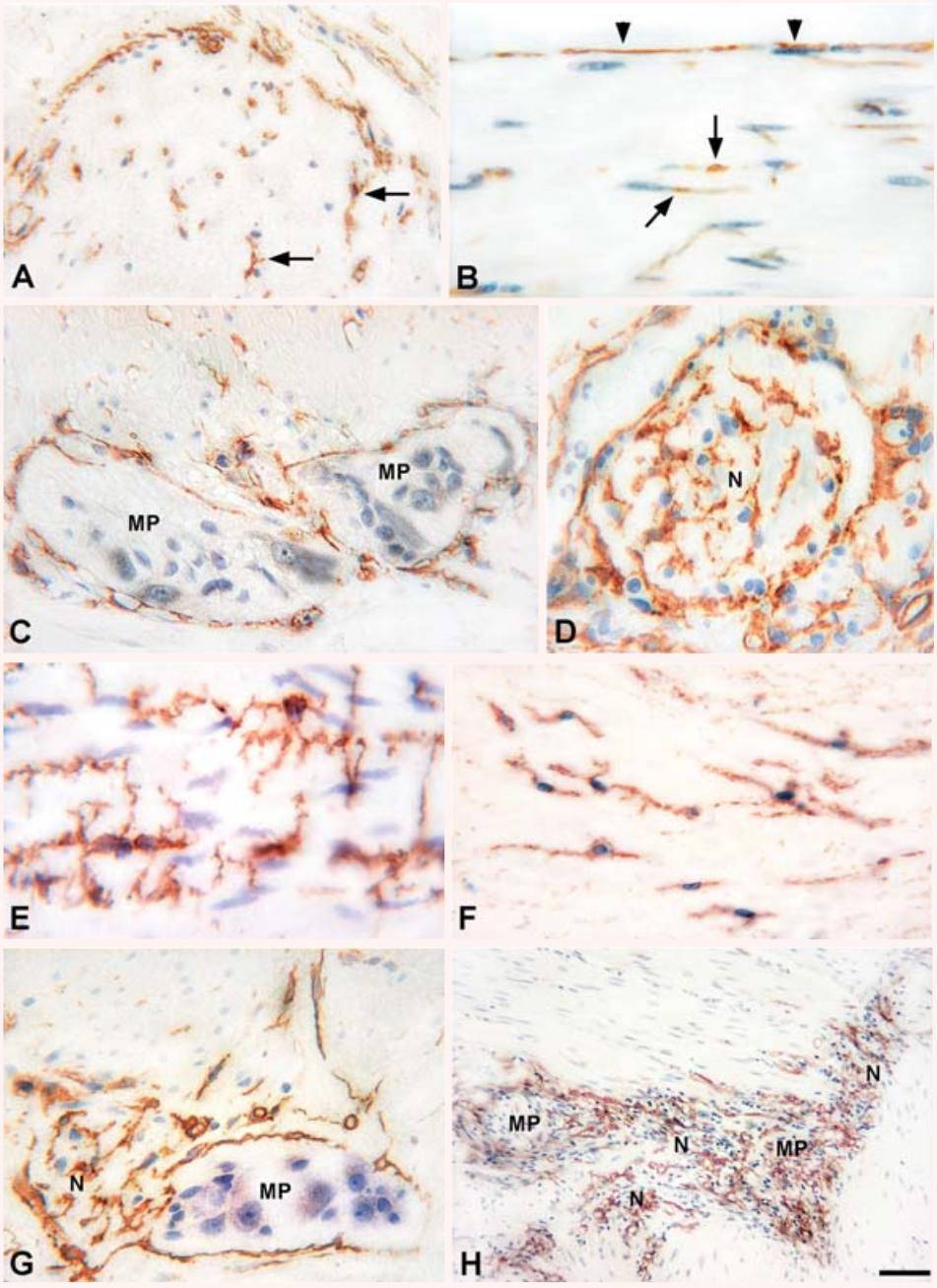

Fig. 2 A–**G**: CD34-immunoreactivity; **H**: c-kit-immunoreactivity **A**: small intestine. The CD34-positive cells form a discontinuous layer at the submucosal border of the circular muscle layer and some of them are intramuscularly located (*arrows*). **B**: stomach. CD34-positive cells (*arrowheads*) located in the connective septa form a discontinuous layer close to muscle bundles; some other cells (*arrows*) are within the muscle bundles. **C**, **D** and **G**: CD34-positive cells make an almost continuous sheath around myenteric ganglia (**C**, small intestine and **G**, stomach) and nerve strands (**D**, large intestine). At the level of nerve strands and in between the ganglia these cells form a discontinuous three-dimensional network (**D** and **G**). **E** and **F**: everywhere in the GI tract, the CD34-positive cells have an elongated shape, a small body and a variable number of thin and long processes. The processes have knobs along their length. **H**: small intestine; a high number of c-kit-positive cells at the myenteric plexus level where form networks. **MP**: ganglia at the myenteric plexus; **N**: nerve strands at the myenteric plexus. Bar: **A** = 35 μm; **B**–**G** = 20 μm; **H** = 80 μm.

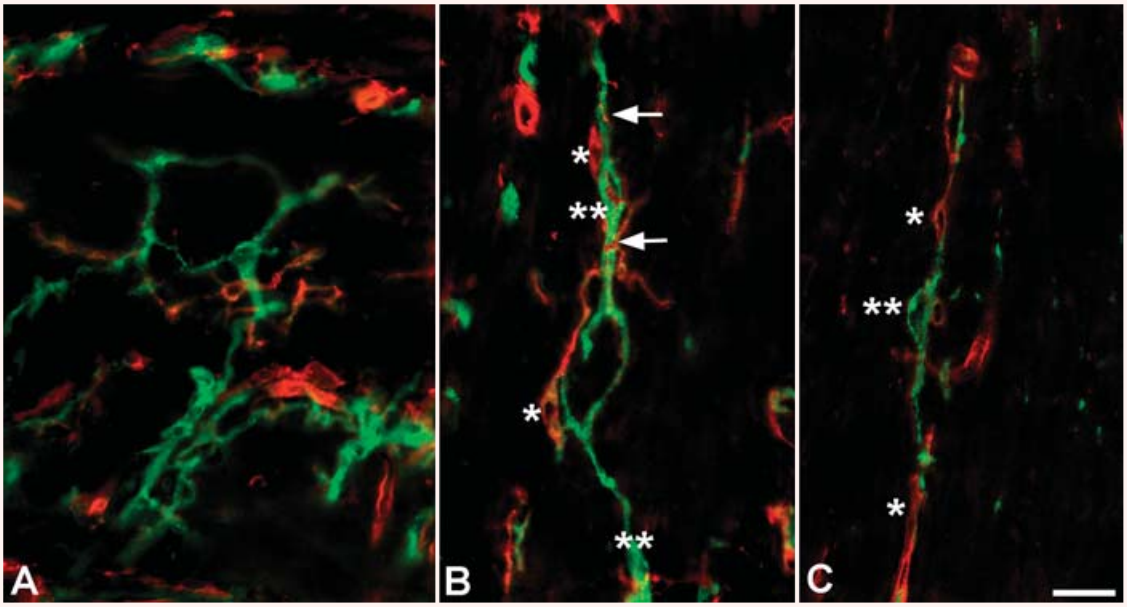

Fig. 3 Double CD34/c-kit labelling. **A–C**: stomach. CD34-positivity red and c-kit-positivity green. (**A**) None of the CD34-positive cells is c-kit-positive. (**B**) The CD34-positive and the c-kit-positive cells are often very close to each other. *Arrows* indicate the processes of a CD34-positive cell passing over the body of a c-kit-positive cell. **C**: a row formed by a c-kit-positive cell intercalated between two CD34-positive cells. *: CD34-positive cells; **: c-kit-positive cells. Bar: **A–C**: 25 μm.

Discussion

In the present study, the distribution of the CD34-positive cells in the wall of the entire human GI tract has been identified by immunohistochemistry. Some differences, however, were seen between the cells located in the *muscularis propria* and those located in the *submucosa*. Indeed, in the *muscularis propria*, the CD34-positive cells shared some morphological characteristics similar to those of another cell type present at the same location, the c-kit-positive cell or ICC, while in the *submucosa*, where the ICC are absent, the CD34-positive cells resembled fibrocytes/fibroblasts. Moreover, routine electron microscope examination allowed providing an unequivocal identification and an ultrastructural characterization of the CD34-positive cells.

Under the electron microscope, we presently observed cells not yet identified and having specific characteristics. Their ultrastructure is simple and mainly similar to that of both the fibrocytes (quiescent cells) and the fibroblasts (activated cells) and different from that of the typical human ICC [36–39]. These cells, therefore, might correspond to the cells recently described in the GI muscle coat as 'fibroblast-like cells'. Importantly, under transmission electron microscope, only the cells with these features were CD34-positive and the ultrastructural distribution of the CD34-immunoreactivity is in agreement with the fact that CD34 is a sialylated transmembrane glycoprotein. Indeed, labelling was specifically located within the thickness of the plasma membrane and on spherules protruding outside at the level of the glycocalyx. Recently, Popescu group [15–24] described in several organs of humans a cell type, named ICLC (interstitial Cajal-like cells), having ultrastructural features practically identical to those presently observed for the CD34-positive cells located within the muscle coat of the human GI tract. Briefly, we can reasonably conclude that the CD34-positive fibroblast-like cells we identified in the GI muscle coat of man might be identified as ICLC. In the Table 1, the ultrastructural differences and similarities among the human ICC, the ICLC, the fibroblasts and the fibrocytes are summarized. Interestingly, due to the fact that the ICLC have been described in a great variety of organs, these cells can be considered as a ubiquitous cell type. Popescu group has also studied these cells by immunohistochemistry and found a larger numbers of cells stained for CD34 than for c-kit, suggesting not all of the ICLC co-express the two molecules. Moreover, in the ICLC isolated from the fallopian tube, these authors could see that in the double-labelled cells the c-kit antibody mainly stained the cell body, while CD34 antibody preferentially stained the cell processes [16].

The question on whether the CD34-positive cells are or not to be considered as ICC is solved by the present data. In agreement with Vanderwinden *et al.* [26, 27], the c-kit- and the CD34-immunoreactivities were never seen to co-localize at any level of the human GI tract, although some cells seemed to possess the two immunoreactivities due to the vicinity of the cell bodies and

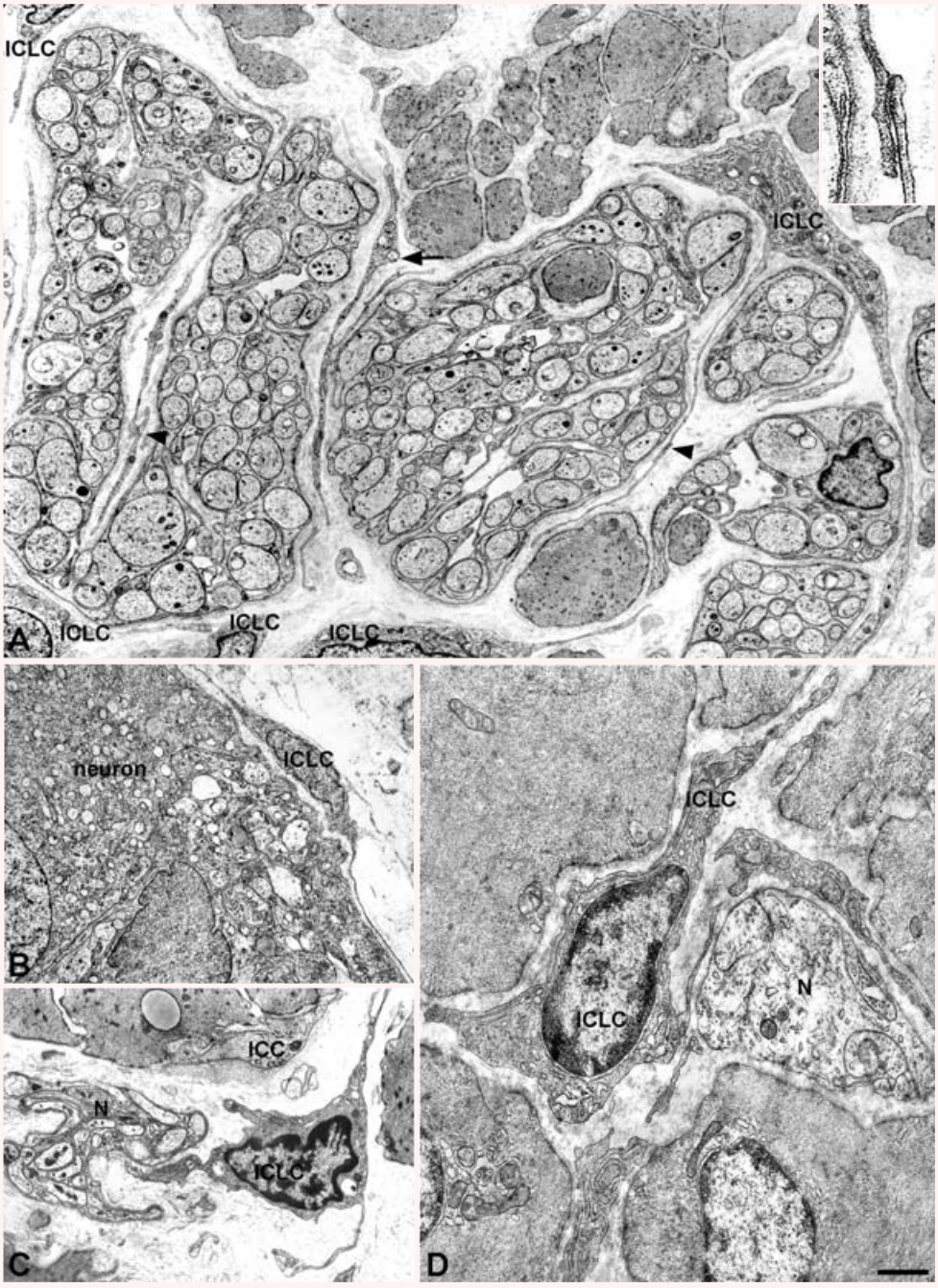

Fig. 4 Transmission electron microscope. **A** and **B**: large and small intestine, respectively. **C** and **D**: stomach. **A**: cells (**ICLC**) with a small oval or triangular body, long and thin processes and fibroblastic features cover nerve strands (**A**) and myenteric ganglia (**B**). Along the processes are present knobs containing mitochondria (*arrow*). *Arrowheads* indicate contact areas between the cell processes, one of which is enlarged in the inset. Neuron: myenteric neuron. **C**: one fibroblast-like cell (ICLC) located in a connective septum, in the vicinity with a nerve bundle (**N**) and an ICC (**ICC**). The ICC contacts two smooth muscle cells. **D**: two interconnected fibroblast-like cells (**ICLC**) located among the smooth muscle cells and in the vicinity of a nerve bundle (**N**). Bar: **A–C** = 1.3 μm; **D** = 0.6 μm; **inset** = 0.3 μm.

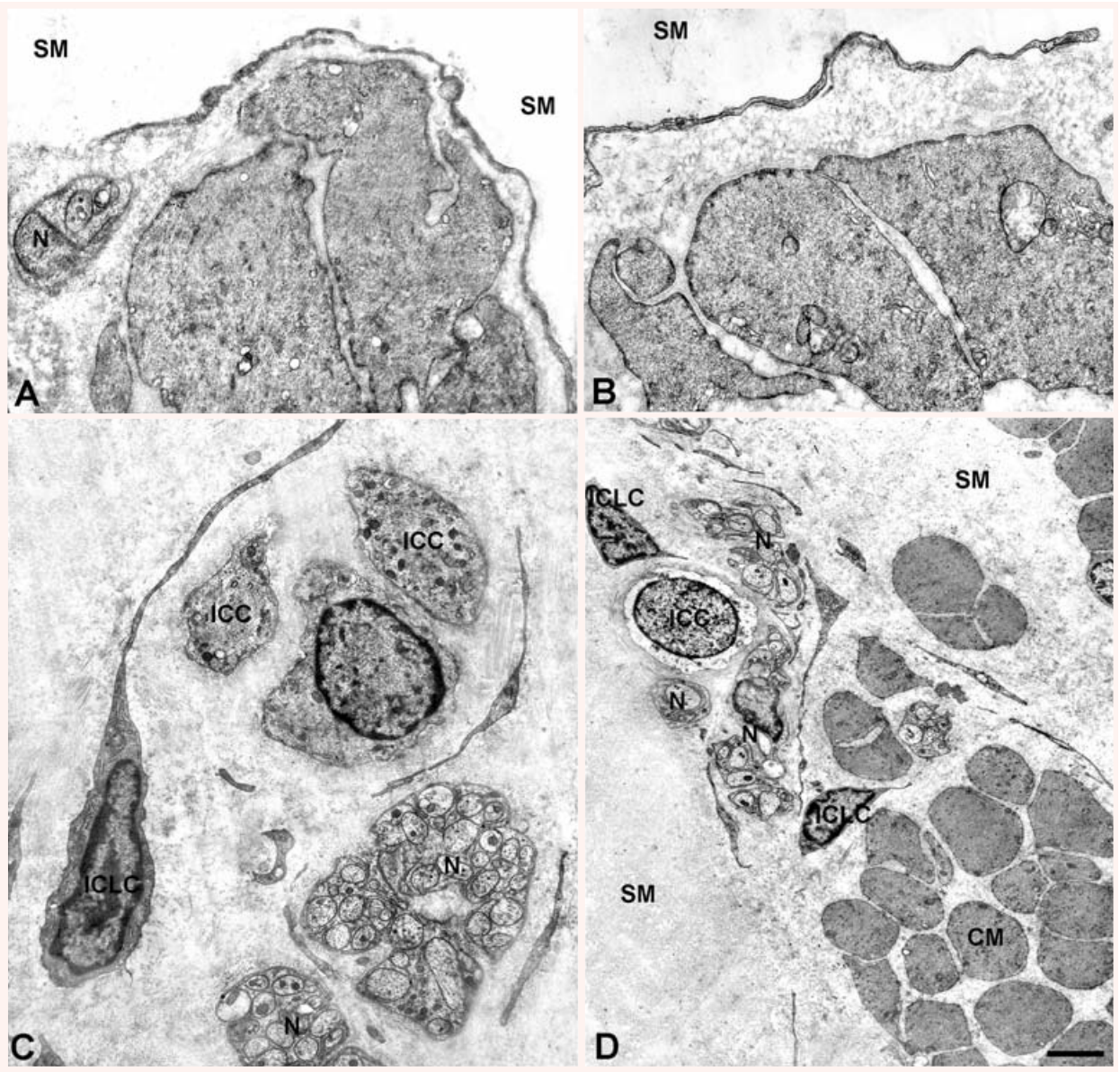

Fig. 5 Trasmission electron microscope. **A**, **C** and **D**: stomach. **B:** small intestine**. A** and **B**: thin processes of the ICLC cover the submucosal border of the circular muscle layer. **C** and **D**: cells with fibroblastic features (ICLC) surround nerve bundles and groups of ICC (ICC) located at the submucosal border of the circular muscle layer of the antrum. In **D**, some of the ICLC processes run towards the circular muscle (CM) to cover its submucosal surface. SM: submucosa; **N**: nerve bundles. Bar: **A** and **B** = 0.9 μm; **C** = 1.3 μm; **D** = 2 μm.

the intermingling cell processes. The absence of a co-localization is in contrast with other reports according to which ICC – or at least a large subset of them – are CD34-positive in the human gut. These studies, however, are mainly conducted in GISTs and EGISTs [see 33–36 for review of literature] and the hypothesis of a co-localization is based on the fact that the c-kit- and the CD34-positive cells share a similar localization rather than on a double labelling. Moreover, it has also to be considered that most of the reports are in favour for a co-expression in these tumours and not in controls. In this regard, it has never been considered that the CD34-positive cells present in the tumours might be the endothelial cells. Finally, the presence in the GISTs of CD34- and c-kit-positive cells might also be explained not by a CD34/c-kit co-localization, rather by the presence of both cell types which, as presently

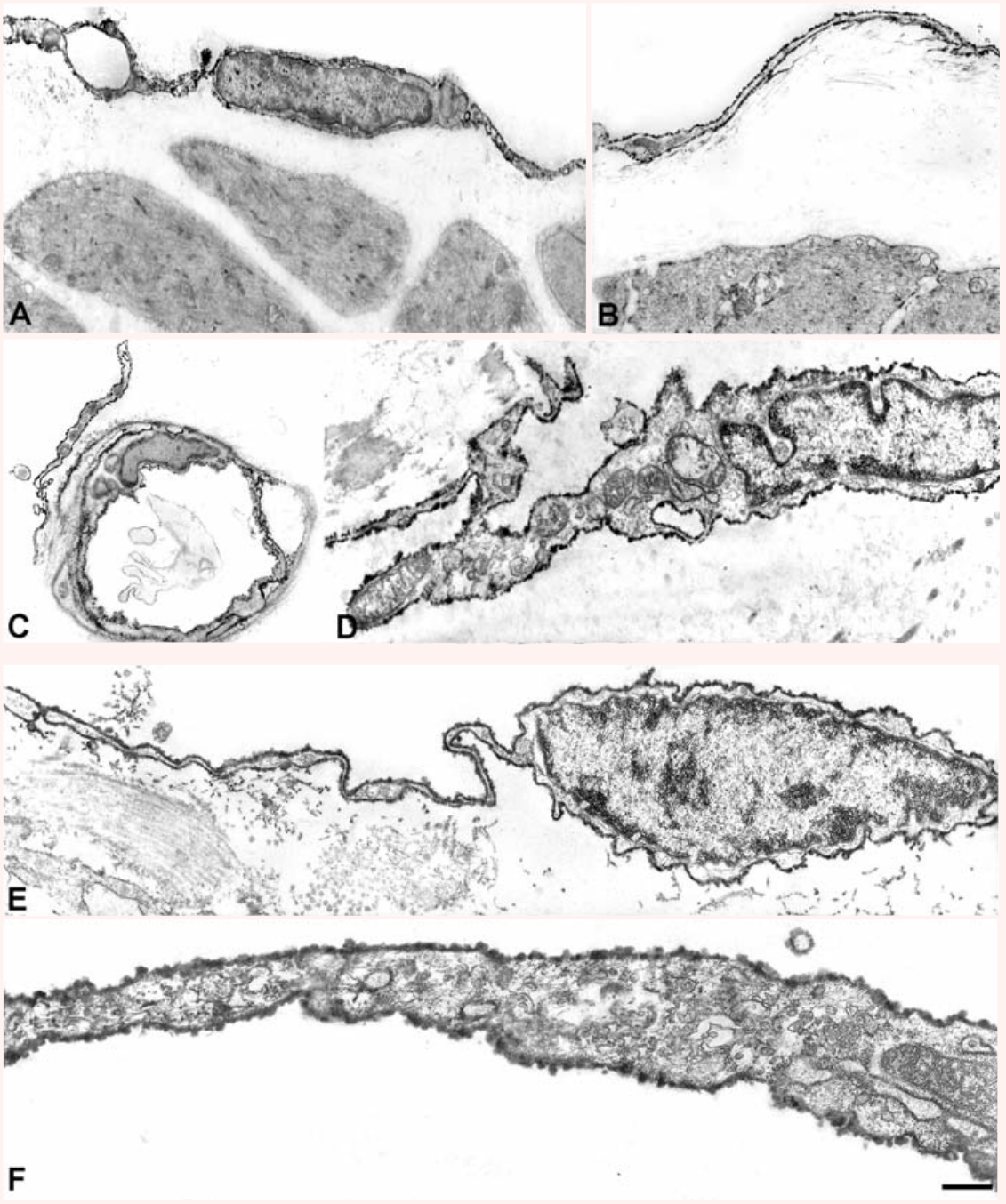

Fig. 6 CD34-immunoelectro-labelling. **A**, **E, F**: small intestine. **B**–**D**: stomach. CD34-immunoelectro-labelling is present on cells with ICLC features (**A**–**F**) and on the endothelium (**C**). The labelling appears as an electron-dense material distributed all along the plasma membrane, from which spherules protrude outside. Bar: **A** and **B** = 1 μm; **C** = 2 μm; **D, E** = 0.5 μm; **F** = 0.4 μm.

Table 1 Ultrastructural characteristics of the ICC, ICLC, fibrocytes and fibroblasts of the human GI tract

ICC	ICLC	Fibrocytes	Fibroblasts
(see ref. 39)	(present results)	(quiescent fibroblast)	(activated fibrocyte)
Spindle-shaped and large-sized cells	Oval-shaped and small-sized cells	Oval-shaped and small-sized cells	Oval-shaped and large-sized cells
Oval body, containing the nucleus and a large quantity of cytoplasm	Oval or triangular body, containing the nucleus and a small quantity of cytoplasm	Oval body, containing the nucleus and a small quantity of cytoplasm	Oval body, containing the nucleus and a large quantity of cytoplasm
Two or more processes, thick at the starting point from the body, of variable length and possessing several ramifications	Two or more processes, very thin and extremely long, ramified and with knobs along their length	Two thin processes, length (?) ramifications (?)	Two short and thick processes, ramifications (?)
Clear chromatin and one nucleolus	Dense or clear chromatin, nucleolus (?)	Dense chromatin, nucleolus (?)	Clear chromatin and a large nucleolus
Golgi apparatus normal-sized	Small Golgi apparatus	Small Golgi apparatus	Large Golgi apparatus
Extended smooth endoplasmic reticulum and few cisternae of rough endoplasmic reticulum	Smooth endoplasmic reticulum absent and several cisternae of rough endoplasmic reticulum	Smooth endoplasmic reticulum absent and few cisternae of rough endoplasmic reticulum	Smooth endoplasmic reticulum absent and extended rough endoplasmic reticulum
Many mitochondria everywhere distributed	Several mitochondria in the perinuclear cytoplasm and within the knobs	Few mitochondria	Several mitochondria
Thin and intermediate filaments (many)	Thin and intermediate filaments (many)	Filaments (?)	Filaments (?)
Many caveolae	Few caveolae	No caveolae	No caveolae
Discontinuous basal lamina	No basal lamina	No basal lamina	No basal lamina
Close contacts to each other and with smooth muscle cells and nerve endings	Close contacts to each other and with smooth muscle cells	No contacts to each other and with smooth muscle cells and nerve endings	No contacts to each other and with smooth muscle cells and nerve endings

shown, are normally resident and intermingled in the GI muscle coat.

Cajal described in the gut the cells, which are now known as ICC and similar cells in other organs [9]. Cajal called all of them interstitial cells (neurons) and used methods, such as methylene blue vital staining and silver impregnations, which are highly capricious. As an example, these methods do not label, at least in the gut, all the ICC populations and work differently in the various animal species. As already stressed [40], we must accept that the cells corresponding to the GI interstitial cells of Cajal are the cells that in the gut are c-kit-positive, have a specific ultrastructure and a well ascertained role in GI motility. The conventional name of these cells is ICC. Nobody has up to now considered the possibility that the interstitial cells Cajal described in the gut might be a mixture of c-kit- and CD34-positive cells. *The novelty of the present study is that in the muscle coat of the human GI tract, together with the 'typical' ICC, there is another type of cell we consider to correspond to the ICLC described by Popescu group and, even though also this cell might belong to the 'interstitial cells' described by Cajal, it has not to be confused with the ICC.*

We have presently provided a detailed description of the ultrastructural features of this new cell type but it still remains to understand its role. The ICLC located in the rat mesentery have been proposed to form a three-dimensional network at the same time resistant and deformable following stretches consequent to intestine movements, mainly avoiding blood vessels closure [25]. Yamazaki and Eyden [41] suggested for the network of CD34-positive interstitial cells located in human fallopian tube a putative immune surveillance role. Both roles might be proposed also for the ICLC we found in the GI muscle coat, in particular the presence at the myenteric plexus level of a three-dimensional network resistant to and deformable following intestine movements is easily conceivable and in this case these cells might correspond to the 'covering cells' [42]. Conversely, it is hard to identify the role of the ICLC accompanying nerve bundles and ICC at the submucosal border of the circular muscle layer of the gastric antrum. These ICLC seem to continue with those covering the entire submucosal border of the circular muscle layer and with the septal ones. At present, the unique explanation might be that they play a mechanical, supporting role. Finally, since some of the intramuscular ICC and

ICLC seem to be part of a unique network in which ICC only are innervated, we would suggest these intramuscular ICLC play a role in neurotransmission, possibly contributing to spread the slow waves generated by the ICC.

Taking into account the formers, another more intriguing role might be hypothesized for the gastrointestinal ICLC. It has been reported, both in humans and laboratory mammals, that the immature ICC are fibroblast-like during the pre- and post-natal life [43, 44]; similarly, after BAC treatment, the newly differentiating ICC have fibroblast-like features [45]. Furthermore, although ICC undergo to apoptosis, their number does not change significantly with aging [46]; nevertheless, dividing ICC have never been observed either in the foetal stages [47] or in the adults whereas mitotic figures were found in both the smooth muscle and fibroblast-like cells (Faussone-Pellegrini, personal observations). Keeping in mind all these data, especially considering that the ICLC share fibroblast-like features with the immature ICC and are often located in proximity or even intercalated with the ICC, *we hypothesize that the ICLC might be identified with the mesenchymal stromal cells derived from the mesenchymal cells and which are present in the adults and reasonably comprehensive of a pool of ICC precursors*. In agreement with this hypothesis, it has been recently reported [48] that murine immature gastric cells expressing *in vitro* CD34 at high level and c-kit at very low level might differentiate into ICC by stopping to express CD34 and increasing c-kit labelling, and it has also been proposed [49] that the starting cell is the mesenchymal stem cell and the intermediate cell the fibroblast-like cell.

In conclusion, cells we identified as ICLC are present within the human gut. In the muscle coat, these cells are very often close to the ICC and also intercalated with them; however, their ultrastructure is different from that of the human ICC. These cells are sometimes in contact with the smooth muscle cells but never with nerve endings. Hypothetically, we have considered these ICLC to be the adult mesenchymal stromal cells. Several roles can be proposed: a mechanical supporting role, to be involved in neurotransmission, to be important for ICC renewal and GISTs genesis possibly representing a pool of ICC precursors.

References

1. **Faussone-Pellegrini MS, Cortesini C, Romagnoli P.** Sull'ultrastruttura della tunica muscolare della porzione cardiale dell'esofago e dello stomaco umano con particolare riferimento alle cosiddette cellule interstiziali del Cajal. *Arch It Anat Embriol.* 1977; 82: 157–77.
2. **Thuneberg L.** Interstitial cells of Cajal: intestinal pacemaker cells? *Adv Anat Embryol Cell Biol.* 1982; 71: 1–130.
3. **Thomsen L, Robinson TL, Lee JCF, Farraway L, Hughes MJG, Andrews DW, Huizinga JD.** Interstitial cells of Cajal generate a rhythmic pacemaker current. *Nat Med.* 1998; 4: 848–51.
4. **Koh SD, Sanders KM, Ward SM.** Spontaneous electrical rhythmicity in cultured interstitial cells of Cajal from the murine small intestine. *J Physiol.* 1998; 513: 203–13.
5. **Ward SM, Morris G, Reese L, Wang XY, Sanders KM.** Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterology.* 1998; 115: 314–29.
6. **Wang XY, Vannucchi MG, Nieuwmeyer F, Ye J, Faussone-Pellegrini MS, Huizinga JD.** Changes in interstitial cells of Cajal at the deep muscular plexus are associated with loss of distention-induced burst-type muscle activity in mice infected by Trichinella spiralis. *Am J Pathol.* 2005; 167: 437–53.
7. **Faussone-Pellegrini MS**. Interstitial cells of Cajal: once negligible players, now blazing protagonists. *Ital J Anat Embryol.* 2005; 110: 11–31.
8. **Yin J, Chen JD**. Roles of interstitial cells of Cajal in regulating gastrointestinal motility: *In vitro vs in vivo* studies. *J Cell Mol Med.* 2008; 12: 1118–29.
9. **Cajal SR**. Histologie du système nerveux de l'homme et des vertébrés, vol. 2, Grand sympathique. Paris, Maloine; 1911. pp. 891–942.
10. **Civin CI, Strauss LC, Brovall C, Fackler, MJ, Schwartz JF, Shaper JH.** Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol.* 1984; 133: 157–65.
11. **Berenson RJ, Bensinger WI, Hill RS, Andrews RG, Garcialopez J, Kalamasz, DF, Still BJ, Spitzer G, Buckner CD, Bernstein ID, Thomas ED.** Engraftment after infusion of CD34 marrow cells in patients with breast cancer or neuroblastoma. *Blood.* 1991; 77: 1717–22.
12. **Siena S, Bregni M, Brando B, Belli N, Ravagnani F, Gandola L, Stern AC, Lansdorp PM, Bonadonna G, Gianni AM.** Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. *Blood.* 1991; 77: 400–09.
13. **Fina L, Molgaard HV, Robertson D, Bradley NJ, Monaghan P, Delia D, Sutherland DR, Baker MJ, Greaves MF.** Expression of the CD34 gene in vascular endothelial cells. *Blood.* 1990; 75: 2417–26.
14. **Brown J, Greaves MF, Molgaard HV.** The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts. *Int Immunol.* 1991; 3: 175–84.
15. **Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu N, Ceausu M, Gherghiceanu M, Braga RI, Vasilescu F, Zagrean L, Ardeleanu C.** Novel type of interstitial cell (Cajal-like) in human fallopian tube. *J Cell Mol Med.* 2005; 9: 479–523.
16. **Popescu LM, Hinescu ME, Ionescu N, Ciontea SM, Cretoiu D, Ardelean C.** Interstitial cells of Cajal in pancreas. *J Cell Mol Med.* 2005; 9: 169–90.
17. **Hinescu ME, Popescu LM.** Interstitial Cajal-like cells (ICLC) in human atrial myocardium. *J Cell Mol Med.* 2005; 9: 972–5.
18. **Gherghiceanu M, Popescu LM.** Interstitial Cajal-like cells (ICLC) in human resting mammary gland stroma. Transmission electron microscope (TEM) identification. *J Cell Mol Med.* 2005; 9: 893–910.
19. **Ciontea SM, Radu E, Regalia T, Ceafalan L, Cretoiu D, Gherghiceanu M, Braga RI,**

Malincenco M, Zagrean L, Hinescu ME, Popescu LM. C-kit immunopositive interstitial cells (Cajal-type) in human myometrium. *J Cell Mol Med*. 2005; 9: 407–20.

20. **Popescu LM, Gherghiceanu M, Hinescu ME, Cretoiu D, Ceafalan L, Regalia T, Popescu AC, Ardeleanu C, Mandache E.** Insights into the interstitium of ventricular myocardium: interstitial Cajal-like cells (ICLC). *J Cell Mol Med*. 2006; 10: 429–58.
21. **Hinescu ME, Gherghiceanu M, Mandache E, Ciontea SM, Popescu LM.** Interstitial Cajal-like cells (ICLC) in atrial myocardium: ultrastructural and immunohistochemical characterization. *J Cell Mol Med*. 2006; 10: 243–57.
22. **Popescu LM, Ciontea SM, Cretoiu D.** Interstitial Cajal-like cells in human uterus and fallopian tube. *Ann N Y Acad Sci*. 2007; 1101: 139–65.
23. **Hinescu ME, Ardeleanu C, Gherghiceanu M, Popescu LM.** Interstitial Cajal-like cells in human gallbladder. *J Mol Histol*. 2007; 38: 275–84.
24. **Suciu L, Popescu LM, Gherghiceanu M.** Human placenta: *de visu* demonstration of interstitial Cajal-like cells. *J Cell Mol Med*. 2007; 11: 590–97.
25. **Hinescu ME, Popescu LM, Gherghiceanu M, Faussone-Pellegrini MS.** Interstitial Cajal-like cells in rat mesentery: an ultrastructural and immunohistochemical approach. *J Cell Mol Med*. 2008; 12: 260–70.
26. **Vanderwinden JM, Rumessen JJ, De Laet MH, Vanderhaeghen JJ, Schiffmann SN.** CD34+ cells in human intestine are fibroblasts adjacent to, but distinct from, interstitial cells of Cajal. *Lab Invest*. 1999; 79: 59–65.
27. **Vanderwinden JM, Rumessen JJ, De Laet MH, Vanderhaeghen JJ, Schiffmann SN.** CD34 immunoreactivity and interstitial cells of Cajal in the human and mouse gastrointestinal tract. *Cell Tissue Res*. 2000; 302: 145–53.
28. **Sircar K, Hewlett BR, Huizinga JD, Chorneyko K, Berezin I, Riddell RH.** Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. *Am J Surg Pathol*. 1999; 23: 377–89.
29. **Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM.** Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am J Pathol*. 1998; 152: 1259–69.
30. **Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad TG, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y.** Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998; 279: 577–80.
31. **Sakurai S, Fukasawa T, Chong JM, Tanaka A, Fukayama M.** Embryonic form of smooth muscle myosin heavy chain (SMemb/MHC-B) in gastrointestinal stromal tumor and interstitial cells of Cajal. *Am J Pathol*. 1999; 154: 23–8.
32. **Robinson TL, Sircar K, Hewlett BR, Chorneyko K, Riddell RH, Huizinga JD.** Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. *Am J Pathol*. 2000; 156: 1157–63.
33. **Bussolati G.** Of GISTs and EGISTs, ICCs and ICs. *Virchow Arch*. 2005; 447: 907–8.
34. **Streutker CJ, Huizinga JD, Driman DK, Riddell RH.** Interstitial cells of Cajal in health and disease. Part I: normal ICC structure and function with associated motility disorders. *Histopathology*. 2007; 50: 176–89.
35. **Streutker CJ, Huizinga JD, Driman DK, Riddell RH.** Interstitial cells of Cajal in health and disease. Part II: ICC and gastrointestinal tumours. *Histopathology*. 2007; 50: 190–202.
36. **Faussone-Pellegrini MS, Cortesini C.** Some ultrastructural features of the muscular coat of human small intestine. *Acta Anat*. 1983; 115: 47–68.
37. **Faussone-Pellegrini MS, Pantalone D, Cortesini C.** An ultrastructural study of the interstitial cells of Cajal of the human stomach. *J Submicrosc Cytol Pathol*. 1989; 21: 439–60.
38. **Faussone-Pellegrini MS, Pantalone D, Cortesini C.** Smooth muscle cells, interstitial cells of Cajal and myenteric plexus interrelationships in the human colon. *Acta Anat*. 1990; 139: 31–44.
39. **Faussone-Pellegrini MS, Thuneberg L.** Guide to the identification of interstitial cells of Cajal. *Microsc Res Tech*. 1999; 47: 248–66.
40. **Huizinga JD, Faussone-Pellegrini MS.** About the presence of interstitial cells of Cajal outside the musculature of the gastrointestinal tract. *J Cell Mol Med*. 2005; 9: 468–73.
41. **Yamazaki K, Eyden BP.** Ultrastructural and immunohistochemical studies of stromal cells in lamina propria of human fallopian tube ampullar mucosa: the recognition of 'CD34 positive reticular network' and its putative function for immune surveillance. *J Submicrosc Cytol Pathol*. 1995; 28: 325–37.
42. **Gabella G.** Innervation of the gastrointestinal tract. *Int Rev Cytol*. 1979; 59: 129–93.
43. **Faussone-Pellegrini MS.** Cytodifferentiation of the interstitial cells of Cajal related to the myenteric plexus of mouse intestinal muscle coat. An E.M. study from fetal to adult life. *Ana Embryol*. 1985; 171: 163–16.
44. **Faussone-Pellegrini MS.** Cytodifferentiation of the interstitial cells of Cajal of mouse colonic circular muscle layer. An E. M. study from fetal to adult life. *Acta Anat*. 1987; 128: 98–109.
45. **Faussone-Pellegrini MS, Vannucchi MG, Ledder O, Tian-Ying Huang T-Y, Hanani M.** Plasticity of interstitial cells of Cajal: a study of mouse colon. *Cell Tissue Res*. 2006; 325: 211–7.
46. **Gibbons SJ, De Giorgio R, Faussone-Pellegrini MS, Garrity-Park MM, Miller SM, Schmalz PF, Young-Fadok TM, Larson DW, Dozois EJ, Camilleri M, Stanghellini V, Szurszewski JH, Farrugia G.** Apoptotic cell death of human interstitial cells of Cajal. *Neurogastroenterol Motil*. 2008. in press.
47. **Faussone-Pellegrini MS, Vannucchi MG, Alaggio R, Strojna A, Midrio P.** Morphology of the interstitial cells of Cajal of the human ileum from foetal to neonatal life. *J Cell Mol Med*. 2007; 11: 482–94.
48. **Lorincz A, Redelman D, Horváth VJ, Bardsley MR, Chen H, Ordög T.** Progenitors of interstitial cells of Cajal in the postnatal murine stomach. *Gastroenterology*. 2008; 134: 1083–93.
49. **Huizinga JD, White EJ.** Progenitor cells of interstitial cells of Cajal: on the road to tissue repair. *Gastroenterology*. 2008; 134: 1252–3.