

Interstitial Cajal-like cells in rat mesentery: an ultrastructural and immunohistochemical approach

M. E. Hinescu^{a,b}, L. M. Popescu^{a,b,*}, M. Gherghiceanu^a, M.-S. Faussonne-Pellegrini^{c,*}

^a 'Victor Babes' National Institute of Pathology, Bucharest, Romania

^b Department of Cellular and Molecular Medicine, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania

^c Department of Anatomy, Histology and Forensic Medicine, Section of Histology, University of Florence, Florence, Italy

Received: September 12, 2007; Accepted: November 15, 2007

Abstract

Interstitial Cajal-like Cells (ICLC) were recently recognized in a plethora of non-digestive organs. Here, we describe a cell type of rat mesentery sharing ultrastructural and immunohistochemical features with ICLC. Mesenteric ICLC were demonstrated by transmission electron microscopy (TEM) and further tested by light microscope immunohistochemistry. The cell described here fulfils the TEM diagnostic criteria accepted for ICLC: location in the connective interstitium; close vicinity to nerves, capillaries and other interstitial cells; characteristic long, moniliform cell processes; specialized cell-to-cell junctions; caveolae; mitochondria at 5–10% of cytoplasmic volume; rough endoplasmic reticulum at about 1–2%; intermediate and thin filaments, microtubules; undetectable thick filaments. The processes of this mesenteric ICLC were particularly long, with a mean length of 24.91 μm (10.27–50.83 μm), and a convolution index of 2.32 (1.37–3.63) was calculated in order to measure their potential length. Mean distances versus main target cells of ICLC–nerve bundles, vessels, adipocytes and macrophages–were 110.69, 115.80, 205.07 and 34.65 nm, respectively. We also tested the expression of CD117/c-kit, CD34, vimentin, α -smooth muscle actin, nestin, NK-1, tryptase and chymase and the antigenic profile of the mesenteric ICLC was comparable if not identical with that recently observed in ICLC from other extra-digestive tissues. Due to the peculiar aspect of the mesenteric ICLC processes it can be hypothesized that these cells form a three-dimensional network within the mesentery that is at the same time resistant and deformable following stretches consequent to intestine movements, mainly avoiding blood vessels closure or controlling blood vessels rheology. It remains, however, to be established if and how such cells are connected with the archetypal enteric ICC.

Keywords: interstitial cells of Cajal • CD117/c-kit • CD34 • vimentin • rat mesentery • immunohistochemistry

Introduction

The presence of interstitial Cajal-like cells (ICLC) outside the digestive tract was compellingly documented mainly by electron microscopy [1, 2] but also

by specific chemical markers, such as CD117. However, questions remained if a clear-cut between ICLC and fibroblast-like cells and between ICLC and

*Correspondence to: L.M. POPESCU, MD, PhD, Department of Cellular and Molecular Medicine, 'Carol Davila' University of Medicine and Pharmacy, P.O. Box 35-29, Bucharest 35, Romania.
E-mail: lpopescu@jcm.org

S. FAUSSONE-PELLEGRINI MD, PhD, Department of Anatomy, Histology and Forensic Medicine, Viale Pieraccini 650139 Florence, Italy.
Tel.: +39 0554271389
Email: s_faussone@unifi.it

enteric interstitial cells of Cajal (ICC) can still be drawn on both ultrastructural and immunohistochemical bases. Extra-gastrointestinal stromal tumours (EGISTs) are now a recognized concept in pathology [3]. Isolated case reports [4, 5] or more systematic analysis [6–8] demonstrated omental and mesenteric EGISTs representing about 1.5% of the mesenchymal stromal tumours throughout the gastrointestinal tract [9]. EGISTs originating primary from omentum and mesentery exhibit phenotypical similarities with other gastrointestinal stromal tumours (GISTs) [6]. Omental and mesenteric GISTs were typically positive for CD117 and less consistently for CD34 [6–8]. However, the cell of origin of EGISTs is not known [9]. Since it is largely accepted that most of GISTs are originating from the ICC, it appears logical to presume that the origin of EGISTs might be the ICLC. Such a hypothesis can be supported by the fact that kit-positive cells were identified in the omentum [10] and ICLC cells were recently described in the wall of mesenteric vessels [11, 12], or portal veins [13]. The aim of the present study was to examine by the means of immunohistochemistry and electron microscopy, according to the accepted guidelines for their identification [14–17], if ICLC exist in normal rat mesentery.

Material and methods

Animals

Normal adult male Wistar rats, body weight 200–250 g, were used. They were housed in temperature- and light controlled rooms, and had free access to standard food and tap water. The study was conducted in accordance with the moral, ethical, regulatory and scientific principles governing research as set out in 'Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC)'. This study was approved by the Bioethics Committees of the 'Victor Babes' Institute of Pathology Bucharest.

Electron microscopy

Under sodium pentobarbital anaesthesia (60 mg/kg), rats were perfused with PBS at 50 ml/min for 2 min. at room

temperature and then with a freshly prepared solution of 2.5% glutaraldehyde in 15 mM cacodylate buffer, pH 7.2, for 5 min. The mesentery was quickly excised and immersed 90 min., at 4°C, in the same fixative. Tissue samples (fragments of about 1 mm³) were then processed for ultrastructural investigation as previously described [18–20].

Electron microscopy examination was performed with a Philips CM 12 transmission electron microscope at 60 kV. The images were recorded with Morada 11 megapixel CCD camera and analysed with ITEM SIS software (Olympus). Data obtained by digital morphometric analysis are expressed as mean ± SD.

Immunohistochemistry

Immunocytochemistry was performed on 3 µm thick sections from 10% formalin-fixed paraffin-embedded specimens, according to the Avidin-Biotin-Complex method as we previously described [18–20]. These sections were tested with the following antibodies: CD117, polyclonal, 1:100 (DAKO); CD34, 1:100, clone QBEND10 (Biogenex); smooth muscle (αactin (SMA), monoclonal, 1:1500, clone 1A4 (Sigma Chemical); S-100, polyclonal, 1:500 (DAKO); NK1, monoclonal, 1:50, clone NK1, (DAKO); vimentin, monoclonal, 1:100, clone V9 (DAKO); nestin, monoclonal, 1:100, clone 5326 (Santa Cruz); chymase, monoclonal, 1:100, clone CC1 (NeoMarker) and tryptase, monoclonal, 1:100, clone AA1 (NeoMarker). All specimens were counterstained with Mayer's haematoxylin, examined and photographed on a Nikon Eclipse 600 microscope.

Results

Electron microscopy

Cells with an interstitial location and resembling ICLC were found in the vicinity of and intermingled with capillaries (Figs. 1–5), nerve bundles (Figs. 1, 2, 4), adipocytes (Figs. 2, 4A) and other interstitial cells, mainly macrophages (Figs. 1, 2, 4A) and fibroblasts (Fig. 3). These cells, that we named ICLC, showed a stellate morphology with a nucleus located in the central part of the body and surrounded by a tiny cytoplasm from which minimum two processes emerged (Figs. 1A, 4). Processes branched in a dichotomous pattern and shared a very unusual aspect. All of them were extremely long and had a tortuous course to create a complex labyrinthic system all around collagen bundles, all other types of cells, vessels and nerves (Figs. 1, 2, 4). Moreover,

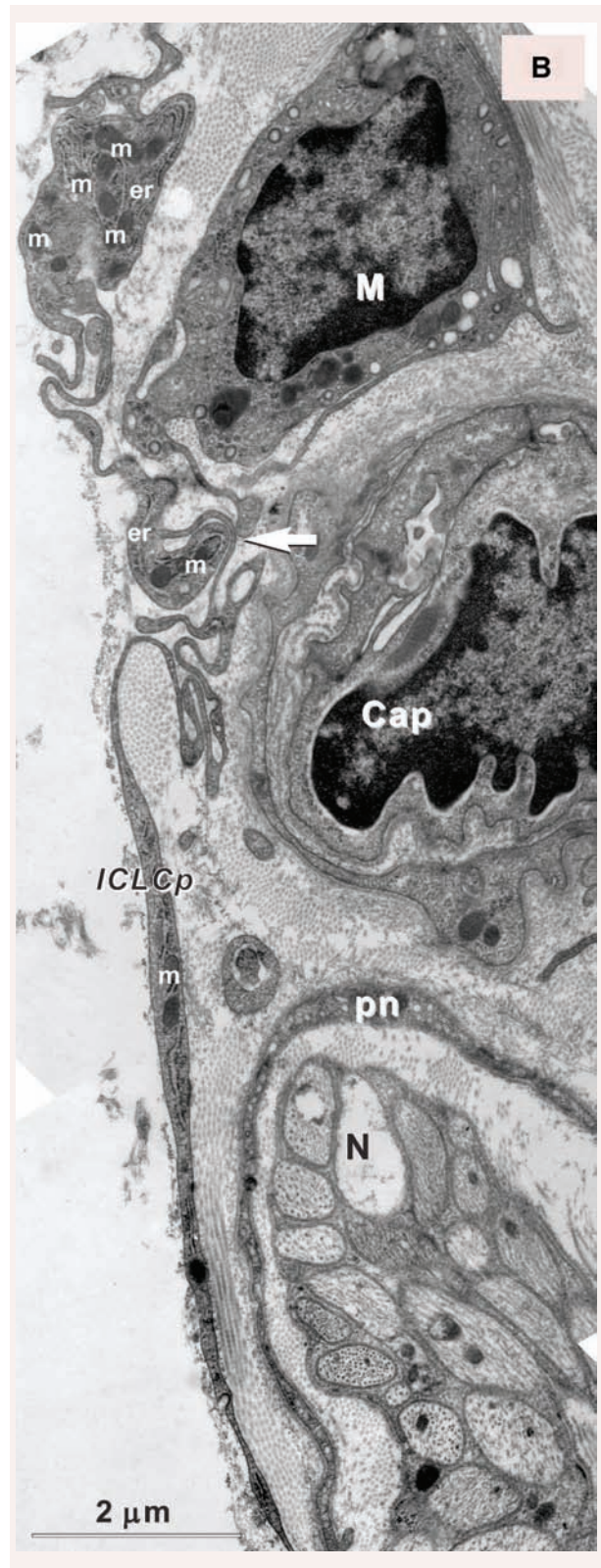
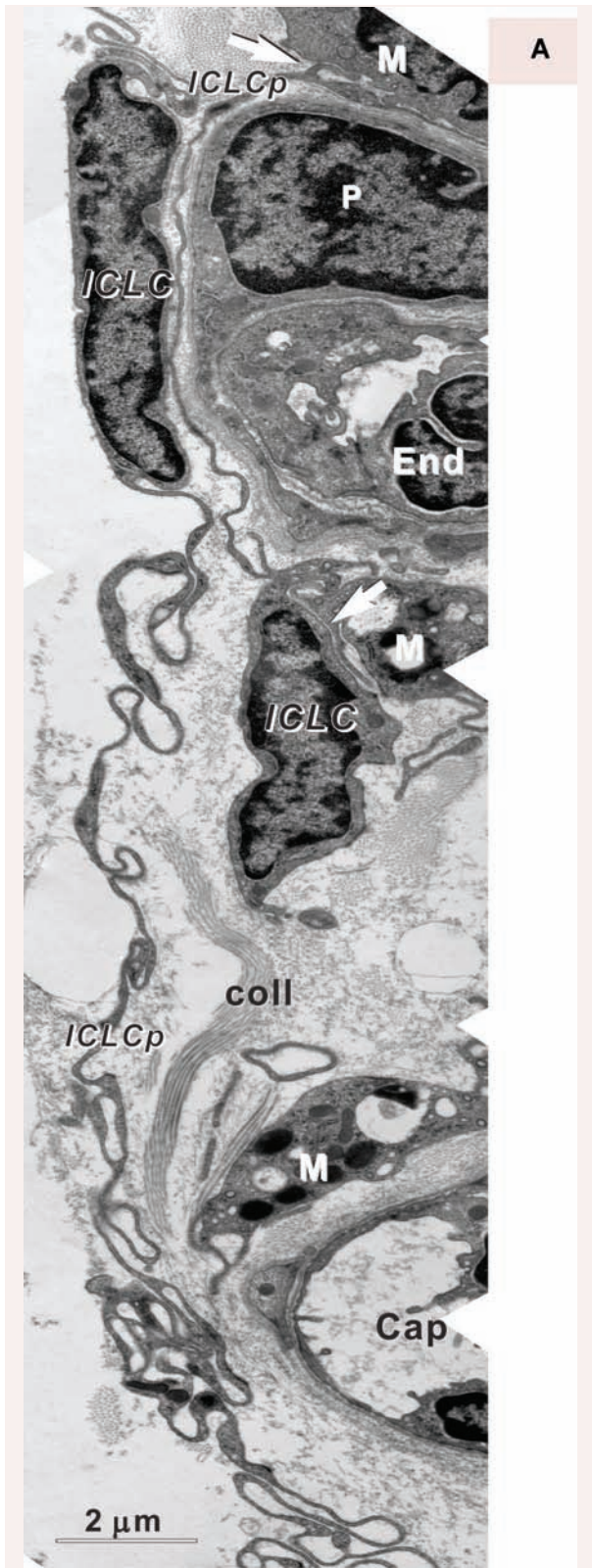




Fig. 1 Transmission electron microscopy (TEM) image of rat mesentery. **(A)** Two interstitial Cajal-like cells (ICLC) with long and slender cellular processes (ICLCp) in the region of blood vessels. Note close contacts (arrows) between ICLC and macrophages (M). **(B)** TEM image showing a labyrinthine segment of the ICLCp and its close contact with a macrophage (arrow) in the proximity with capillary (Cap) and nerve bundle (N). The ICLC moniliform process has cytoplasmic dilatations containing mitochondria (m) and endoplasmic reticulum (er). End, endothelial cell; P, pericyte; Cap, capillary; coll, collagen fibres; pn, perineural cell.

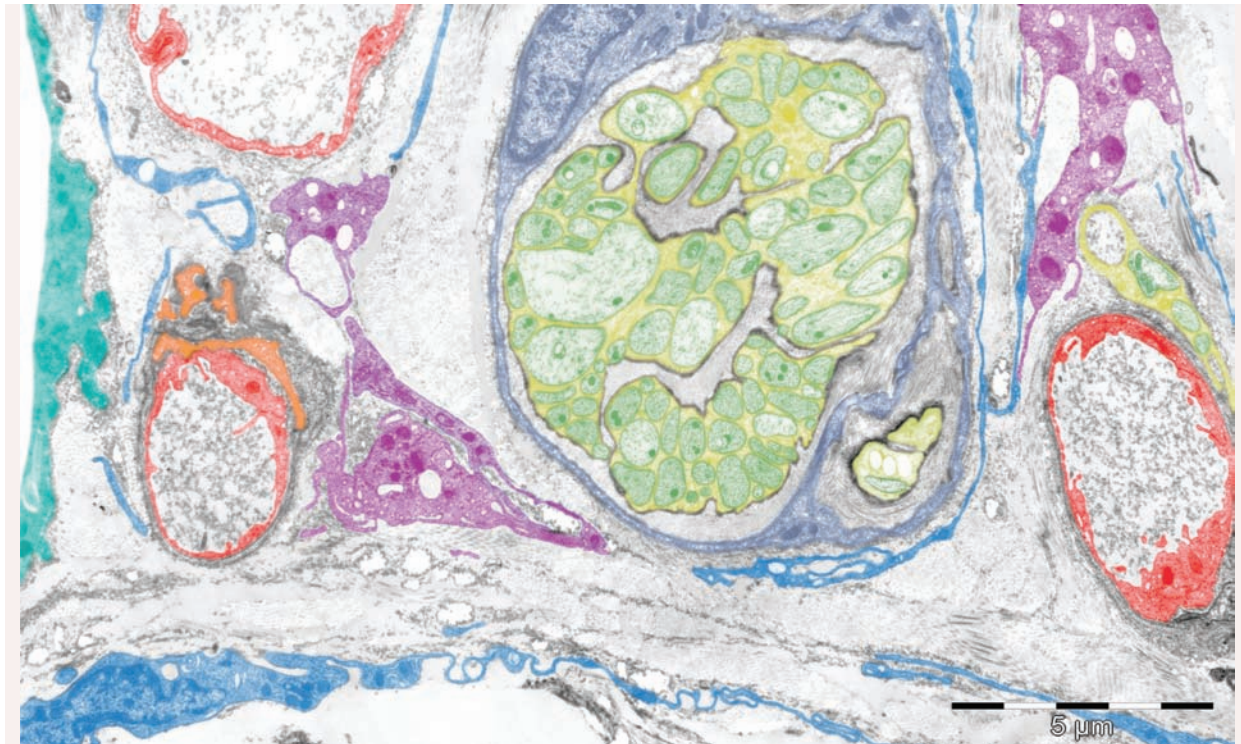


Fig. 2 Digitally coloured transmission electron microscopy micrograph. A colour code was imagined: red = endothelium; orange = pericyte; green-yellow = nerve bundles; purple = macrophage; blue = ICLC; brilliant green = adipocyte.

these processes had moniliform features being uneven thin and bearing mitochondria into dilatations (Figs. 1, 4A, 5). Their mean length was $24.91 \mu\text{m}$ ($10.27\text{--}50.83 \mu\text{m}$) and the mean thickness was $104.78 \pm 75.08 \text{ nm}$ ($33.78\text{--}301.59 \text{ nm}$). In order to measure the potential length of the ICLC processes a convolution index was expressed as their actual length divided by the end-to-end distance in a straight line (an adaptation after [ref 21]). The mean convolution index was calculated with a value of 2.32 (minimum 1.37; maximum 3.63). The nucleated portion of the ICLC contained cisternae of the rough endoplasmic reticulum (ER), a small Golgi apparatus and several mitochondria (Figs. 1B, 5, 7). ER cisternae were also present within the processes and the

richness in them varied among the cells. Occasionally, a primitive cilium was contained in a cytoplasmic pocket at the level of the nucleated portion (Fig. 7). Caveolae (Fig. 6) and attachment plaques connecting ICLC processes with the extracellular matrix (Figs. 1B, 5) were found along the ICLC processes. Basal lamina was never observed. Cell-to-cell small contacts were usually observed between the ICLC (Figs. 1, 6), either between their body and the process of another ICLC or between processes (Fig. 6). These junctions resembled the so-called desmosome-like junctions. The quantitative estimations of the cytoplasmic volume occupied by mitochondria (5–10%) and ER (1–2%) are very similar with results for ICLC in other locations. The mean

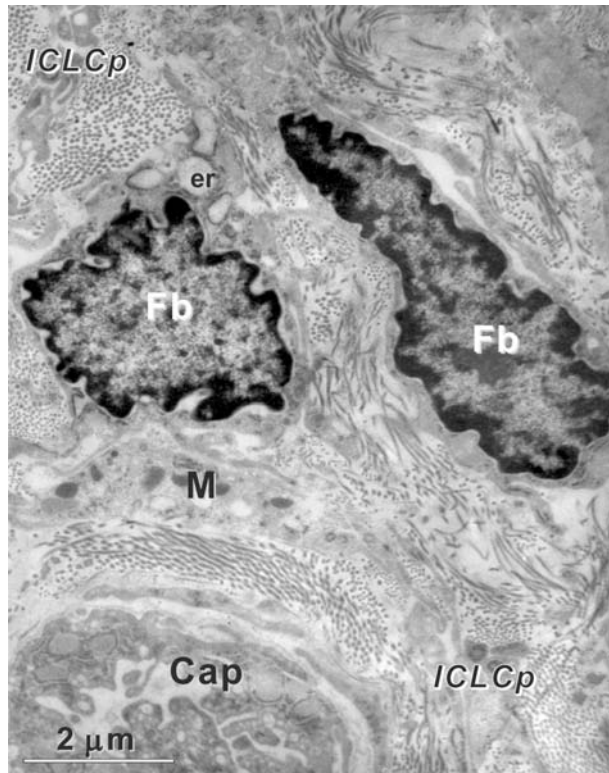


Fig. 3 TEM image of rat mesentery shows two fibroblasts (Fb), ICLC process (ICLCp), a macrophage (M) and a capillary (Cap). The fibroblasts have convoluted nuclei, several cisternae of endoplasmic reticulum (er) and short cytoplasmic protrusions.

distances *versus* main target cells for ICLC–nerve bundles, vessels, adipocytes and macrophages—were 110.69, 115.80, 205.07 and 34.65 nm, respectively.

Immunohistochemistry

ICLC were positively labelled for many of the markers we tested: CD117 (Fig. 8A and B), CD34 (Fig. 8C and D), vimentin (Fig. 8E), actin (Fig. 8F) and nestin (Fig. 8G). In particular, CD117, CD34, as well as vimentin and actin, labelled cells with an almost identical location and morphology: an ovoid cell body provided with a nucleus and a few cytoplasm and long, thin processes. Nestin labelled similar cells, but processes were lesser identifiable. Mast cells were also CD117 positive, but mast cell identifying markers, such as chymase or tryptase (Fig. 8H) used for differential diagnosis, labelled these cells only and

never labelled cells with the features of the ICLC. Immunostainings for NK-1 and S-100 were negative.

Discussion

Unnoticed until now, a specific cell type was characterized by transmission electron microscope (TEM) and immunohistochemistry in rat mesentery. The morphological features of this cell type bear a resemblance to those displayed by ICLC from extra-digestive organs and some of them are common features of the better-characterized ICCs [22]. Up-to-now, the presence of ICLC outside the digestive tract was documented mainly by electron microscopy [1, 3]. Therefore, to evaluate the possibility that the mesenteric cells presently observed might be ICLC, we first looked for the fulfilment of TEM diagnostic criteria accepted for ICLC [14, 16, 23]: (1) location in interstitium; (2) close vicinity with nerve bundles, capillaries, other interstitial cells; (3) characteristic cell processes; (4) specialized cell-to-cell junctions; (5) inconspicuous basal lamina; (6) caveolae; (7) mitochondria at 5-10% of cytoplasmic volume; (8) ER at about 1-2%; (9) presence of intermediate and thin filaments and of microtubules, (10) undetectable thick filaments. The richness in rough ER observed in some cells (Fig. 6) may raise questions about what products such cells can synthesize and also questions if a clear-cut between ICLC and fibroblast-like cells can be drawn. However, we could observe very typical fibroblasts clearly different from ICLC, since having cell processes short and less thin at the emergence point, different nuclear shape and a more extended rough ER. Since electron microscopy revealed a very clear relationship to vessels and intrinsic nerves as both ICLC and ICC generally have, the positivity of the mesenteric interstitial cells to CD117/c-kit, CD34, and some other markers such as actin and nestin usually present on ICC/ICLC cells, supported the idea that these cells could be called ICLC also in terms of criteria at a light microscopy level. It has to be noticed, however, that the very peculiar distribution of the interstitial cells in mesentery (intermingled with adipocytes) makes difficult sometimes to interpret what cell type is positive to c-kit, CD34, actin or vimentin. In any case, mast cell identifying markers, such as chymase or tryptase, did not label these cells. Moreover, since our study was conducted on normal rat mesenteric tissue specimens, the differen-

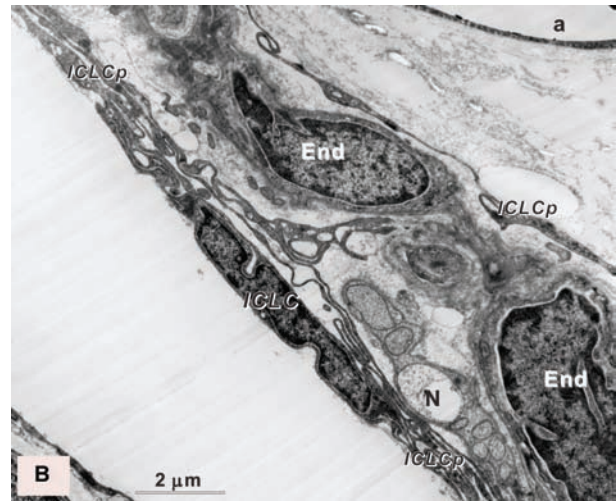
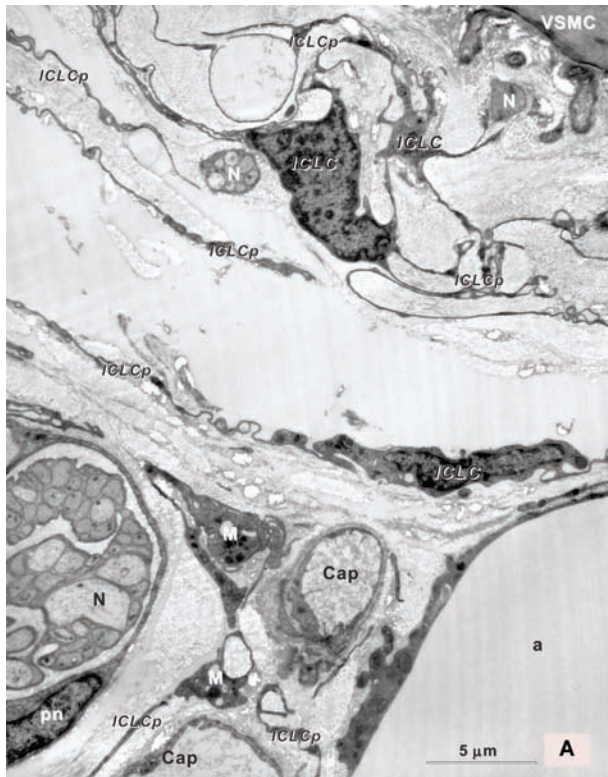


Fig. 4 TEM images of rat mesentery illustrate the long ICLC processes (ICLCP) and their sinuous trajectory around adipocytes (a), macrophages (M), nerve fibres (N), capillaries (Cap) and arteries. (A) On the upper side, note the interstitial network made by ICLC processes. (B) Two ICLCP encloses a blood capillary and nerve fibres (N). End, endothelial cells; VSMC, vascular smooth muscle cell.

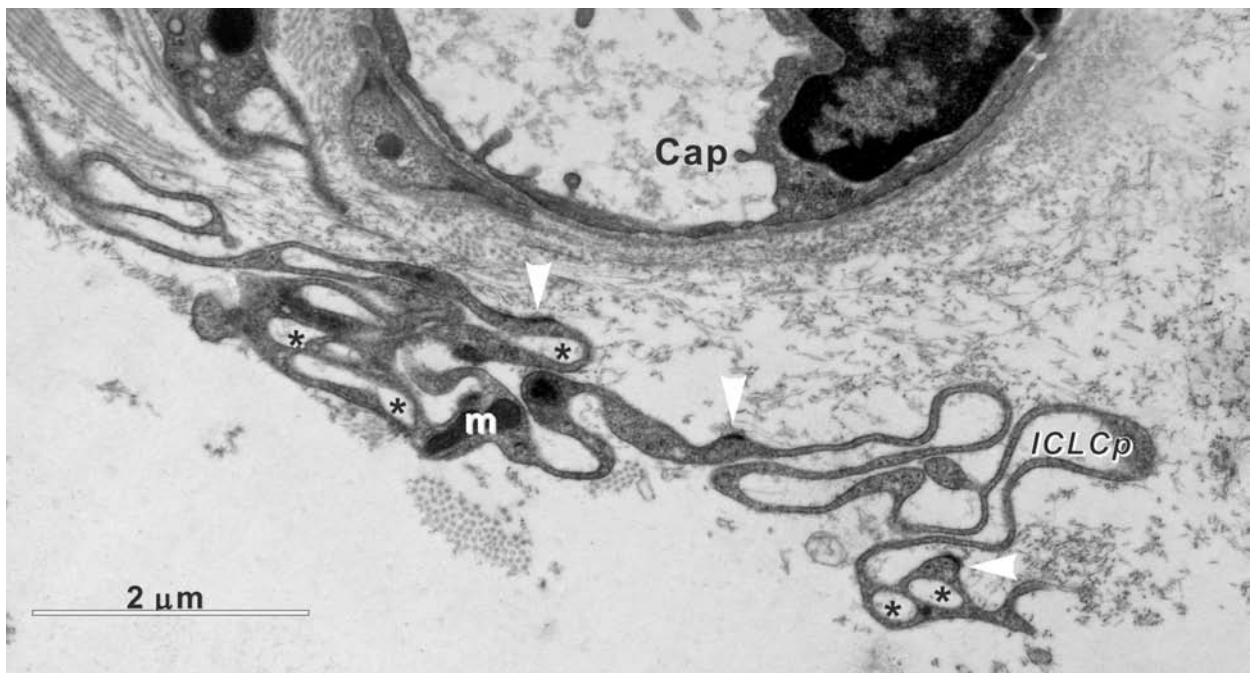


Fig. 5 Detail of a 28 μm long ICLC process (ICLCP) in the rat mesentery. Attachment plaques (arrowheads) can be seen on the cellular membrane. Small buttonholes (asterisks) made by the ICLCP convolutions often appear on TEM images. The ICLCP has a convoluted contour and forms a labyrinthine system which increases its length (convolution index = 3.29). Cap, capillary; m, mitochondria.

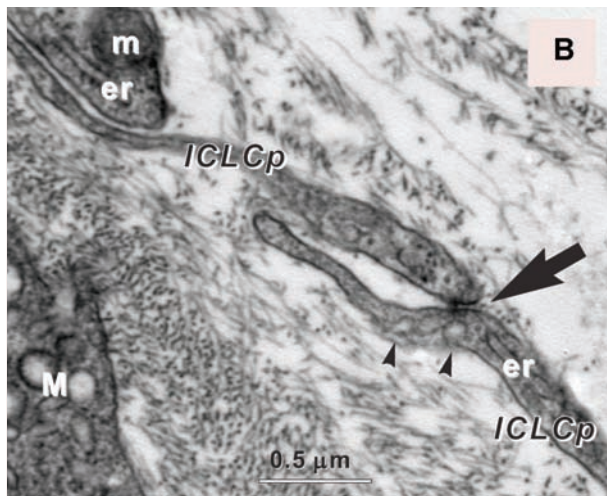
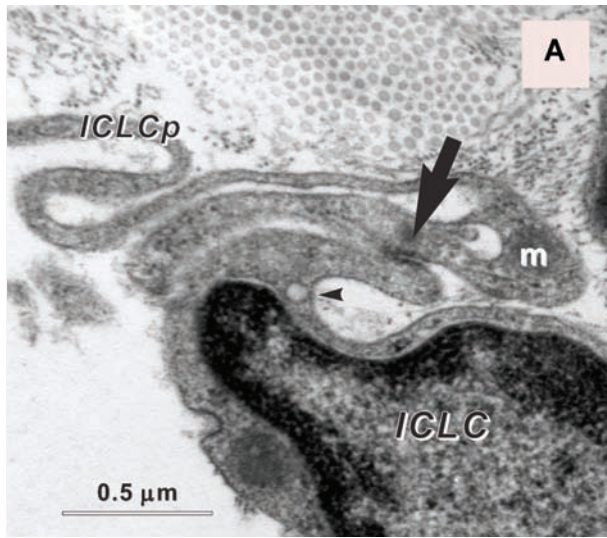


Fig. 6 (A and B) TEM images of rat mesentery. Details of specialized cell-to-cell contacts (arrows) between ICLC body and processes (A) and between ICLC processes (B) M, macrophage; m, mitochondria; er, endoplasmic reticulum; arrowheads, caveolae.

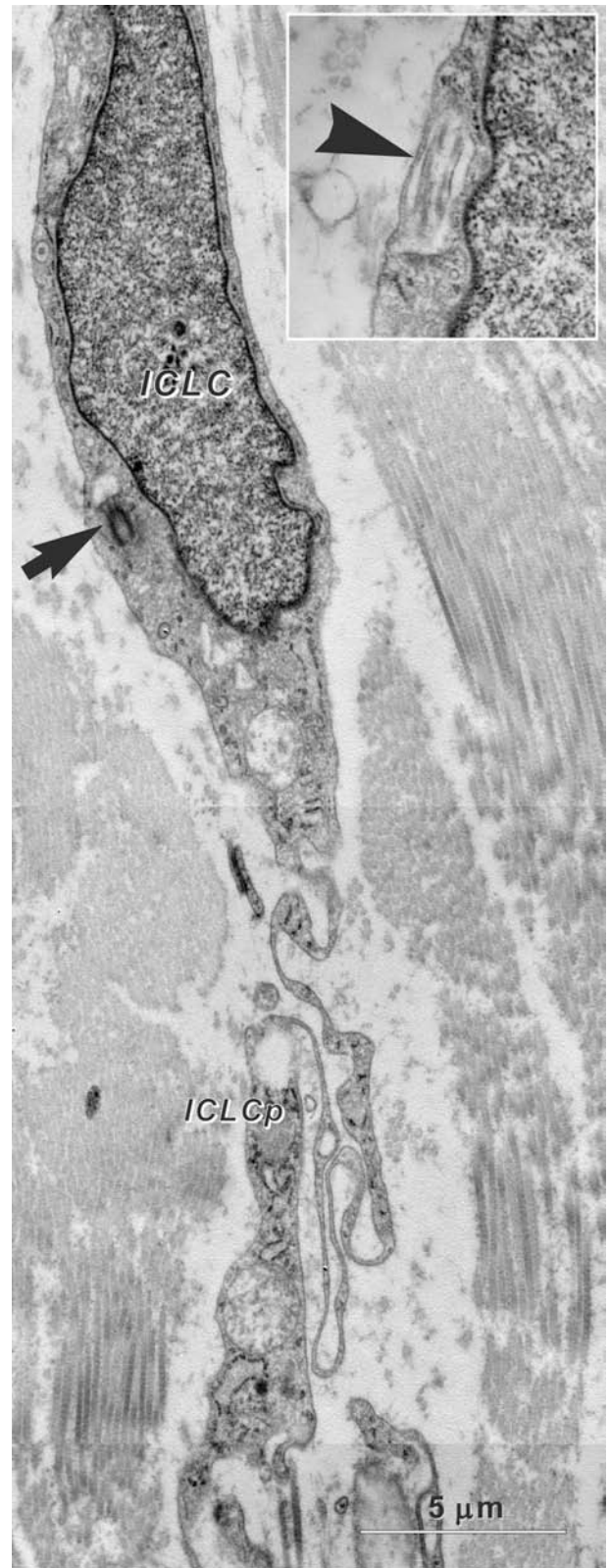


Fig. 7 TEM image of rat mesentery. The ICLCs in the loose connective tissue of the mesentery occasionally have a cilium which appears to lie in a cytoplasmic pocket (arrow). The inset illustrates the cilium (arrowhead) in the adjacent section. ICLCp, ICLC process. →

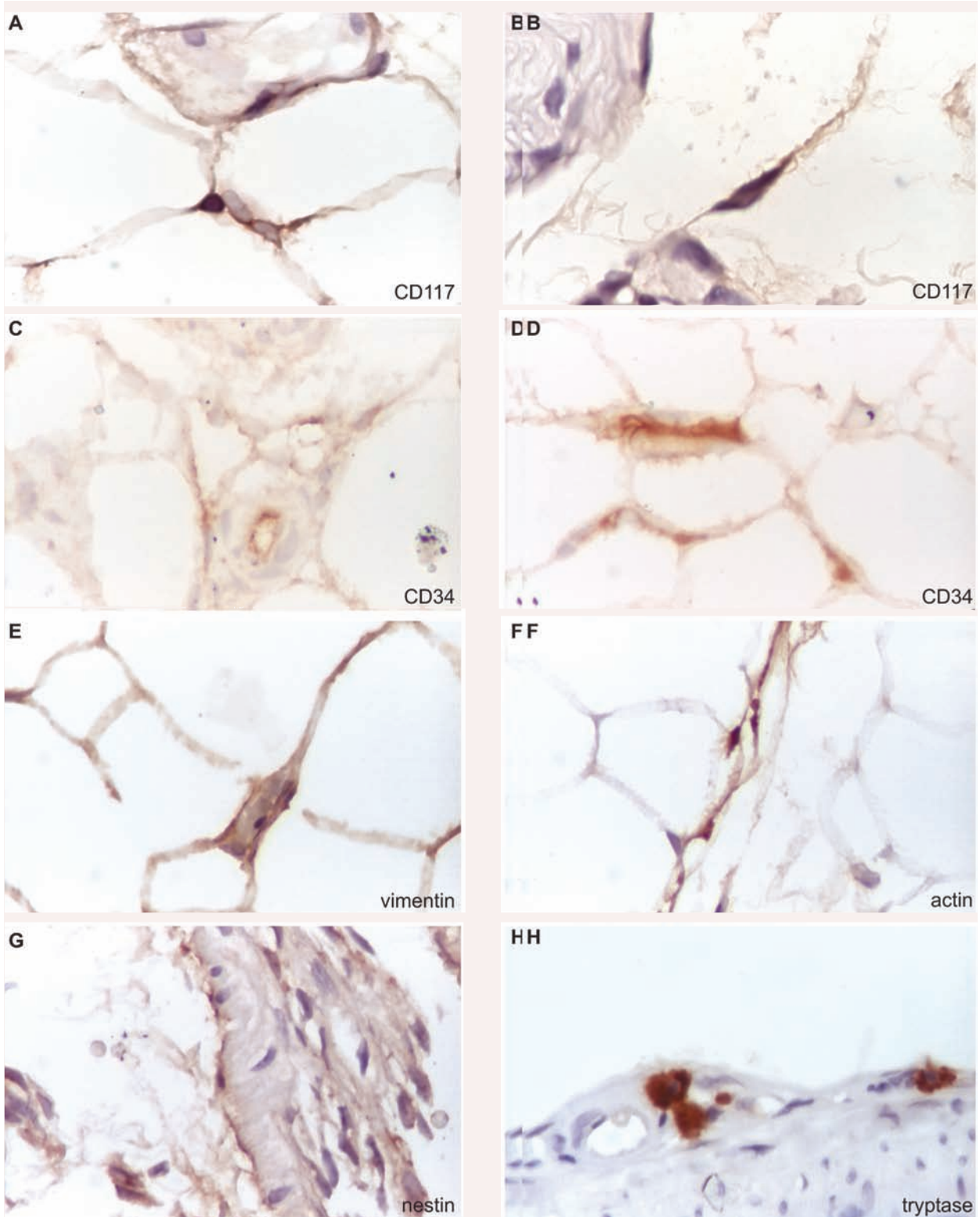




Fig. 8 Cells of the rat mesentery immunostained for CD117/c-kit (**A, B**), CD34 (**C, D**), vimentin (**E**), actin (**F**), nestin (**G**), tryptase (**H**). All the sections were counterstained with Mayer's haematoxylin. Original magnification: A, B, D, 60x; C, E–H, 40x.

tial diagnosis with myofibroblasts, a cell type present only in pathology [24], was not considered. Briefly, in the rat mesentery both the shape and location of the labelled cells perfectly matched those of the ultra-structurally identified ICLC. Moreover, the antigenic profile of these ICLC was comparable with that recently observed in ICLC from other extradigestive tissues like pancreas [18], fallopian tube [23], myometrium [19, 25], atrial [20, 26] and ventricular myocardium [27], gallbladder [28, 29], mammary gland [30, 31], placenta [32], blood [11–13] or lymphatic vessels [33], excretory ducts [34–36]. We still consider that electron microscopy remains the essential to identify ICC or ICLC. For instance, electron microscopy showed that ICLC in mesentery share a common ultrastructural feature with some ICC from rabbit duodenum [37], the presence of a single cilium. Unfortunately, as previous studies underlined, a direct comparison electron microscope *versus* immunohistochemistry, that would be useful for an undoubted identification and complete characterization of both ICC and ICLC, is often difficult to accomplish and can rarely be carried out [1]. A new and interesting finding of the present study is that the rat mesenteric ICLC have very peculiar processes. Indeed these processes are extremely long and have a very tortuous course. It is conceivable that, following a stretch, these processes can become straight. Moreover, since each ICLC has several processes giving to the cell a stellate aspect, and by means of desmosome-like junctions each ICLC is attached to the neighbouring ones, these cells form a continuous network throughout the entire mesentery. Due to the fact that the processes are anchored to the stroma by means of attachment plaques, it is reasonable to hypothesize that the meshes of the ICLC network might change shape and size following intestinal loops repletion/depletion without giving raise to discontinuities. In summary, it can be hypothesized that the mesenteric ICLC form a three-dimensional network within the mesentery that is at the same time resistant and deformable following stretches consequent to intestine movements, likely avoiding blood vessels closure. Such network may provide new explanations for experimental observations conclud-

ed before mesenteric ICLC were characterized (*e.g.* decreased expression of ICC after experimental mesenteric transection [38] or ischaemia and reperfusion after occlusion of mesenteric vessels [39]). It remains also to be established if the plasticity of such a network [40, 41] involves other signalling molecules than kit. Morphology alone is inadequate to predict the role of the ICLC in the mesentery physiology and pathology. However, the GIST *versus* EGISTs approach seems now susceptible to re-interpretations, in areas going from signalling [42] to drug-responses [43], or from conceptual models [44–46] to surgery [47]. Similarly, it appears more reasonable to reinterpret the hypothesis that 'omental and mesenteric GISTs are derived from stomach and small intestine, respectively, representing tumours that for some reasons have detached from their gastrointestinal origin during their development [8]. An excess of speculations and myths continues to be present in the literature on both ICLC and ICC [48]. Complex interactions between ICC located in the digestive tract organs and their counterparts located in mesentery and/or blood vessels may be speculated and even the network of ICC/ICLC could be more complex than presently known. Three important questions remain: (1) what and how determinants are functioning in the development and physiological function of ICLC, (2) whether and to what extent mesenteric ICLC may play a causal or a facilitating role in gastrointestinal abnormalities, (3) whether ICLC interactions with archetypal enteric ICC are inherent (or not).

Acknowledgements

The authors thank Prof. Kenton Sanders, University of Nevada, Dr. Markku Miettinen, Armed Forces Institute of Pathology, Washington, DC, Dr. Brian Eyden, Christie Hospital, Manchester, for comments on some illustrative material. We also thank Prof. Carmen Ardeleanu for constant help concerning immunohistochemistry and Dr. D. Cretoiu and Dr. C. Manole for assistance with the illustrations and photoediting. Many thanks are also expressed to Prof. Sanda Clejan, Tulane University, for critical reading of

the manuscript. This study was supported by National Agency for Scientific Research, Project CEEEX 112/2006.

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