**The connective connection:
interstitial cells of Cajal (ICC) and ICC-like cells
establish synapses with immunoreactive cells.
Electron microscope study *in situ*.**

L.M. Popescu^{a, b, *}, Mihaela Gherghiceanu^b, D. Cretoiu^{a, b}, E. Radu^{a, b}

^a Department of Cellular and Molecular Medicine, "Carol Davila" University of Medicine and Pharmacy,
Bucharest, Romania

^b "Victor Babeş" National Institute of Pathology, Bucharest, Romania

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Abstract

We present transmission electron microscope (TEM) evidence that ICC and ICC-like cells frequently establish close contacts (*synapses*) with several types of immunoreactive cells (IRC): lymphocytes, plasma cells, eosinophils, basophils, macrophages and mast cells. Such synapses were found in various organs: *human* mammary gland and myometrium, as well as *rat* stomach, gut, bladder and uterus. Specimens were observed by conventional TEM on ultrathin sections. Based on morphometric analyses and computer-aided 3-D reconstructions from serial sections, we propose an operational definition of ICC-IRC synapses: cell-to-cell *close contacts* where the two cells are separated by only ~15nm, equivalent to twice the plasmalemmal thickness. Two types of such synapses were found: (i) *uniform ('plain') synapses (PS)* – close contact extending for >200 nm, and (ii) *multicontact ('kiss and run') synapses (MS)* – with multiple, focal, close-contact points alternating with regions of wider intermembrane distance. For instance, a typical PS between a rat bladder ICC-like cell and an eosinophil was 2.48 µm long and 11±4nm wide. By contrast, a MS synapse in rat myometrium (between an ICC-like cell and an eosinophil) was 8.64 µm long and had 13 contact points. The synaptic cleft measured 15±8nm at contact points and ~100nm or more in wider areas. These synapses are different from gap junctions usually seen between ICC and between ICC and smooth muscle cells.

We previously proposed that ICC-like cells might represent stromal progenitor cells, participate in juxtacrine/paracrine signaling and play a role in immune surveillance. The nanoscopic distances between the two contiguous membranes suggest a *juxtacrine cell-to-cell signaling (chemical synapse)*, via *juxtacrinins*, a specific case of *phenomenins*. However, the (micro)vesicles found in the synaptic cleft may correspond to an *exosome*-based mechanism.

Keywords: interstitial cells of Cajal • lymphocytes • eosinophils • basophils • plasma cells
mast cells • macrophage • immune synapse • intercellular communication • exosomes

Introduction

Interstitial cells of Cajal (ICC), located in the musculature of the gastrointestinal tract, are well characterized (as much as possible nowadays) from the ultrastructural, cytochemical and functional points of view (recent reviews: [1-8]). Moreover, ICC

* Correspondence to: L.M. POPESCU, M.D., Ph.D.
Department of Cellular and Molecular Medicine,
"Carol Davila" University of Medicine and Pharmacy,
P.O. Box 35-29, Bucharest 35, Romania.
Tel: +40(0)744 535 298; Fax: +40(0)21-312 4885
E-mail: LMP@univermed-cdgm.ro

implication in human pathology is established [3, 9-11]. During the last few years, special interest was focused on ICC-like cells (ICC-LC), outside the musculature of the gastrointestinal tract [12-14]. Our laboratory, based on accepted criteria, reported the identification of ICC-LC in human pancreas [15, 16], uterus [17], Fallopian tube [18] and mammary gland [19, 20]. Thus, occasionally, we found close contacts between ICC-LC and pancreatic stellate cells, macrophages or fibroblasts. ICC contacts with fibroblast-like cells and resident macrophages were also reported [21-23]. Also, Wang *et al.* [24] observed close apposition of

ICC with activated macrophages or migrated lymphocytes in an animal model of inflammation induced by *T. spiralis*.

Such observations prompted us to look for a 'connective connection': synapse-like contacts between ICC/ICC-LC and immunocytes located in the interstitial, non-epithelial, tissue compartments. A concept derived from the classical neuronal synapse, the 'immunological' synapse between T lymphocytes and antigen presenting cells has already been the subject of intensive research during the last 10 years (recent reviews: [25-29]).

Fig. 1 Rat jejunum: TEM, digitally colored image, original magnification 5,700x. An eosinophil (green, with the nucleus not in the plane of section) establishes synapses with ICC prolongations (violet). The red rectangle areas mark *uniform* ('plain') synapses (PS), characterized by flat, long, close-contact plasmalemmal appositions. Blue rectangle shows inside a *non-uniform, multicontact synapse* (MS). Note ICC mitochondria (m) located in the vicinity of synapses. G, Golgi complex; rER, rough endoplasmic reticulum; Gr, granules with a crystalline body, within the less electrondense matrix; N, nucleus; ax, cross-sectioned axons wrapped in Schwann cell cytoplasm. The ICC labyrinthine system is marked by an asterisk.

A

**RAT
STOMACH
(MUSCULARIS
MUCOSAE)**

PLASMA CELL

extensive
rER

m
extensive
rER

N

m

extensive
rER

m

Gr

N

N

Gr

EOSINOPHIL

rER

m

m

m

sER

Caveola

INTERSTITIAL CELL OF CAJAL

**INTERSTITIUM BETWEEN
THE CIRCULAR AND LONGITUDINAL
SMOOTH MUSCLE LAYERS**

Collagen

**Capillary
endothelium**

**FRAGMENTS OF
ICC ?**

RBC

Fig. 2 A–D Rat stomach *muscularis mucosae*: TEM (A, original magnification 1,150x), TEM serial ultrathin sections, (B, C) and 3-D image reconstruction from serial ultrathin sections (D). An ICC (with triangular cell body and 3 processes I–III) appears located near a plasma cell and an eosinophil (A). N, nucleus; sER, smooth endoplasmic reticulum; rER, rough endoplasmic reticulum; m, mitochondria; RBC, red blood cell. Yellow ovals in 2A highlight two contact areas between the eosinophil, the plasma cell, and another cell. A series of 5 ultrathin sections revealed that the ICC process (I) approaches the plasma cell and establishes a contact point with it (compare the dashed-line regions in 2B and 2C - the first and last image of the series, respectively). 3-D image reconstruction (D) from 5 serial sections (the area marked with red dashed line in 2A) shows that ICC processes are rather flattened than cylindrical and that they branch in a 3-D pattern. A short branch establishes a synapse with the plasma cell. In the inset the same synapse is viewed from a different angle (90° rotation). Apparently, different-angle stereoscopic views are necessary to establish if such a synapse belongs to PS or MS type. *Ab initio*, an intermediate type of synapse (IS) cannot be excluded.

We considered, by extension, that the term ‘synapse’ could be applied to direct intercellular contacts that offer a signaling structural platform. Thus, we used it to designate the close plasmalemmal appositions that bring ICC/ICC-LC and immunocytes well within the molecular range.

We present here TEM ultrastructural evidence for a *novel type of synapse* between ICC/ICC-LC and immunoreactive cells in various organs: *human* pancreas, myometrium, Fallopian tube, mammary gland, as well as *rat* stomach, gut, myometrium and bladder. These synapses seem *chemical juxtacrine synapses*, with possible participation of *exosomes*.

Material and methods

Animals

Wistar rats weighing 200–250g were maintained in temperature controlled rooms with a 12h light/dark cycle and had free access to food and water. All animal experiments were carried out in accordance with accepted ethical guidelines. Tissue fixation procedures were performed under deep anesthesia with sodium pentobarbital (100mg/kg of body weight). Animals were perfused through the heart with a fixative mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) followed by a mixture of 2%

3-D RECONSTRUCTION (SERIAL ULTRATHIN SECTIONS) **A**

Fig. 3 A, B Rat stomach *muscularis mucosae*: 3-D image reconstruction from 9 serial ultrathin sections (A), and TEM (B); original magnification 1,500x. **A**: Computer-aided volume rendering shows flattened ICC processes emerging from the cell body (violet), and branching around the plasma cell (red). Other ICC prolongations (blue), probably belonging to another cell, show the same 3-D morphology. The ICC_b-plasma cell synapse is uneven, with 'key-and-lock' areas. Upper inset in A shows contact points where the distance between the two cell membranes is 15nm or less (in violet), seen from the plasma cell cytoplasm. In the lower inset, the ICC process has been rendered transparent in order to depict the same synapse. **B**: The ICC_b (violet dotted outline) seems to establish an *intermediate synapse* (black rectangle) with a plasma cell (red dotted outline). The inset presents a magnification of this synapse, showing that it comprises, besides contact points, also a longer plasmalemmal apposition. Another ICC process (blue dotted outline) and an eosinophil (green dotted outline) are also present in close proximity.

paraformaldehyde and 2.5% glutaraldehyde in the same buffer (all from Sigma Chemical, St. Louis, MO, USA). Total perfusion time was 10 min.

Human tissue samples

Human tissue samples were obtained following approval by the University Ethical Committee. Non-tumoral mammary gland fragments were removed during surgical procedures and areas without macroscopic lesions were further processed. Immediately after excision, samples were placed in sterile cell culture medium and transported as soon as possible to the laboratory.

Electron microscopy

Immersion fixation: Tissue samples were cut into about 1mm³ fragments that were prefixed by immersion for 4h in 4% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, at

4°C. After two baths in 0.1M cacodylate buffer the specimens were post-fixed for 1h in 1% OsO₄ with 1.5% K₄Fe(CN)₆ (to increase membrane contrast) in 0.1M cacodylate buffer, at room temperature. Specimens were then dehydrated in a series of graded ethanols, followed by propylene oxide, and embedded in Epon 812, at 60°C, for 48 h. Semi-thin sections of about one micron thickness were cut and stained with 1% toluidine blue. Specific areas were subsequently chosen for ultrathin sectioning. As usual, ultrathin sections were cut at 60-80 nm, and mounted on Formvar-coated grids, stained with 1% uranyl acetate and Reynolds's lead citrate. For short series (<10 sections), ultrathin sections were cut at 45nm thickness on the ultra-microtome RMC MT-XL (RMC).

TEM examination was performed with a Philips 301 or CM12 Philips transmission electron microscopes, at 60kV. Representative areas were photographed on 6,5/9 cm AZO EM plan films.

Fig. 4 Rat myometrium: TEM; original magnification 7,100x. A *multicontact synapse* (MS) between an ICC-LC and an eosinophil, in the neighbourhood of smooth muscle cells (SMC); m, mitochondria; N, nucleus; db, dense bodies; Note presence of mitochondria (*) in the synaptic vicinity, typical for chemical synapses.

Synapse: - type: multicontact synapse (MS)
 - length: 2.56 μ m
 - cleft width: 26 $\pm$ 17 nm
 - contact points: 4 (11 $\pm$ 5nm wide)

Synapse: - type: multicontact synapse (MS)
 - length: 8.64 μ m
 - cleft width: 74 $\pm$ 26 nm
 - contact points: 13 (15 $\pm$ 8nm wide)

Fig. 5 Rat myometrium: TEM; original magnification 5,700x; image digitally colored. A *multicontact synapse* (MS) between an eosinophil (green) and an ICC-LC prolongation (violet) is indicated by red cassette. An unmyelinated nerve bundle (vermillion) appears in proximity of ICC-LC, but has no direct contact with it. Apparently, ICC-LC 'prefers' the eosinophil !

Computer-aided 3-D image reconstruction and morphometry

3-D spatial relationships between ICC and interstitial immune cells

Synapse:
 - type: plain synapse (PS)
 - length: 0.88 μ m
 - cleft width: 11 $\pm$ 7 nm

Fig. 6 Rat bladder: TEM; original magnification 9,100x; photographic reconstruction. The red cassette delimits a PS-type synapse between an eosinophil and an ICC-LC cell body.

were studied in several ribbons of serial sections from rat stomach *muscularis mucosa*.

Serial electron photomicrographs (negatives) were digitized at 1200 dpi by scanning with a BenQ scanner. Images were processed using AdobePhotoshop© to outline cell contours, then imported in Reconstruct software [30] (Reconstruct 1.0.4.0., 1996-2005 John C. Fiala; <http://synapses.bu.edu>). Image calibration was performed by drawing traces on an image of known size scale. Section thickness was set at 0.045 μ m.

Reconstruct was used to align images with respect to specific cells of interest. Three-dimensional reconstruction and rendering were performed using the cell contours arranged into objects. For cell membranes 3-D representation

tation, a Boissonnat surface reconstruction algorithm was used.

Morphometric analysis was performed on images with known magnifications, using NIH ImageJ, after proper calibration. Statistical analysis was performed also using ImageJ.

Fig. 7 Rat bladder: TEM; original magnification 25,000x; Red outline indicates a very long *plain synapse* (PS) between an eosinophil and the cell body of an ICC-like cell. The distance between the two cells is uniform and about twice the plasmalemma thickness. The aspect of the perinuclear envelope (arrows) shows that the fixation for TEM was correct. N, nucleus; Rbs, ribosomes; rER, rough endoplasmic reticulum; asterisks mark the crystalloid body of the eosinophil specific granules.

Synapse: - type: plain synapse (PS)
- length: 2.48 μ m
- cleft width: 11 $\pm$ 4 nm

For every synapse, the following parameters were recorded:

- total synapse length (μ m),
- average width of synaptic cleft (nm),
- mean intercellular distance at close-contact regions/points (nm).

Results and discussion

We tried to introduce here the idea - based on electron microscopy - that *a new type of synapse exists and can be identified* between ICC/ICC-like cells and immunoreactive cells, apart from the archetypal *neuronal* or the recently-described *immunologi-*

cal synapses. Actually, such ICC/immunocyte synapses set up a structural *connective connection*, since ICC and ICC-LC definitely belong to connective tissue stroma, where immunoreactive cells migrate to perform their functions. We propose the use of a new term, '*stromal synapse*', to describe this specialized connective (conjunctive) connection (Fig. 14).

To identify the ICC (rat intestine and stomach) or ICC-like cells (human myometrium, Fallopian tube and mammary gland stroma, as well as rat myometrium and bladder) we took advantage of the well-known set of criteria established by the classics of electron microscopy of ICC [1, 3, 12, 31, 32].

Fig. 8 Rat myometrium: TEM; original magnification (A) 19,000x. **A:** An ICC-like cell process establishes a **MS**-type synapse with an eosinophil. The inset shows, in the synaptic cleft, four membrane-bound vesicles with an average diameter of 54 ± 7 nm, and homogenous content. The cleft is dilated and delimited by close-contact points of both cells. Extracellular vesicles (exosomes) are similar in size and structure with subplasmalemmal vesicles of the eosinophil (red asterisks). Gr, granules with electron-dense crystalloid (white asterisks); Mito, mitochondrion. **B:** At higher magnification, note the electron-dense structures ('feet') between the two cell membranes (arrows), as well as **focal densities** located underneath the synaptic membrane (red arrowheads). Gr, eosinophil specific granules; rER, rough endoplasmic reticulum.

Synapse: - type: multicontact synapse (MS)
 - length: $4.2 \mu\text{m}$
 - cleft width: 94 ± 32 nm
 - contact points: 11 (12 ± 5 nm wide)

Fig. 9 Rat stomach *muscularis mucosae*: TEM; original magnification 7,100x. A basophil establishes several *synapses* with cell processes belonging to 2 different ICC (ICC_a and ICC_b). Red and green outlines indicate *plain synapses* (PS), while the blue rectangle shows a *multicontact synapse* (MS). Another ICC is also present (ICC_c) in proximity of a blood vessel. N, nucleus; Gr, granules of irregular shape and variable electron-density, which are specific for basophils; m, mitochondria.

Synapse: - type: plain synapse (PS)
- length: 0.87μm
- cleft width: 11±2 nm

Moreover, conditions imposed by the so-called ‘gold standard’ [33] or even ‘platinum standard’ [18] were fulfilled (Figs. 1-3, 4-13). Formerly, we found in human exocrine pancreas synapse-like close contacts between ICC-LC and stellate cells [15].

Synapses appeared located on Cajal-type cell bodies or *more frequently on cell processes*; a simple explanation is that the very long ICC/ICC-LC prolongations have a membrane area significantly larger than the cell body. Intercellular contacts had different length (up to several micrometers), but all comprised longer or shorter segments of close plasmalemmal appositions, with the cell membranes only 10-20 nm apart, in the nanoscopic range.

Quantitative image analysis provided criteria for *stromal synapses* definition and classification. Thus, we propose the following working definition: cell-to-cell *close contacts* where the two cells are separated by only ~15nm, equivalent to about twice the plasmalemmal thickness. Occasionally, we found *synaptic densities* and also *feet* connecting the two contiguous membranes (Fig. 8B). Anyway, very recently evidence for a neuro-immunological synapse [34] and a virological synapse [35] emerged.

Two distinct morphological aspects of intercellular contacts clearly emerged: *uniform* (‘plain’) *synapses* (PS, Fig. 7), where close-contact regions

Synapse:
 - type: plain synapse (PS)
 - length: $3.37\mu\text{m}$
 - cleft width: $21\pm 8\text{ nm}$

Fig. 10 Rat myometrium: TEM; original magnification 9,100x. An *uniform synapse (PS)* between an ICC-LC body and a macrophage plasmalemmal projection. N, nuclei; n, nucleolus; Ly, lysosomes; m, mitochondria; cv, coated vesicle; rER, rough endoplasmic reticulum; Px, peroxisome. Note the macrophage nucleus or a part of it appears in cross-section.

Synapse:
 - type: plain synapse (PS)
 - length: $0.33\mu\text{m}$
 - cleft width: $11\pm 2\text{ nm}$

Synapse:
 - type: multicontact synapse (MS)
 - length: $1.95\mu\text{m}$
 - contact points: 3 ($12\pm 4\text{ nm}$ wide)

Fig. 11 Rat myometrium: TEM; original magnification 25,000x. An ICC-like cell and a plasma cell (with typical abundant rough endoplasmic reticulum - rER) are engaged in two different types of synapses: one *plain synapse (PS)* and one *multicontact synapse (MS)* are visible. A mitochondrion (m) is part of the *multicontact synapse* complex structure.

Fig. 12 Human mammary gland stroma: TEM; original magnification 9,100x. **A:** Lymphocyte establishing a *multicontact synapse* (MS) with an ICC-LC. The blue rectangle delimits the synaptic ‘kiss and run’ region. Contours of the two cell membranes appear traced in **B** (with violet for ICC-LC and orange for lymphocyte) and the distances measured between membranes are shown in **C**. Note (*asterisk*) a peculiar conformation of the endoplasmic reticulum (ER) connecting mitochondria with the cell surface, suggestive for a possible role in synaptic Ca^{2+} homeostasis. m, mitochondria.

Fig. 13 Human mammary gland: TEM; original magnification 19,000x. A mast cell and an ICC-LC process are engaged in a **MS** synapse (**A**). Contours of the two cell membranes appear traced in **B** (with violet for ICC-LC and green for mast cell) and the distances measured between membranes are shown in **C**. Mast cell plasmalemmal protrusions that form contact points are visible in **B** and **C**. Mast cell is typically filled with membrane-bound granules (Gr) that contain dense particles and less dense matrix. A number of mast cell electrondense granules are located in close proximity of the synapse (**asterisks**).

Synapse: - type: multicontact synapse (MS)
 - length: 3.7 μ m
 - cleft width: 101 $\pm$ 48 nm
 - contact points: 6 (28 $\pm$ 9nm wide)

extend for more than 200nm, and *multicontact* ('kiss-and-run') *synapses* (MS, Fig. 12, 13) with multiple, focal, close-contact points, alternating with regions of wider intermembrane distance. Fig. 3b could be an evidence for an *intermediate synapse* (IS), since short PS-like segments are interspaced with contact points resembling MS.

MS, IS and PS might be sequential phases of stromal synapse formation: initially contact points (MS), then cluster of contact points (IS) and finally plain synapse (PS). These would correspond to the stages of immunological synapse formation (T cells / antigen presenting cells) proposed by Brossard *et al.* [36] (see as a sequence Figs. 5, 3B and 7).

Also by analogy to the central region of immunological synapses, where the adjoining membranes are 20-30 nm apart (a distance dictated by the molecular size of the T-cell antigen receptor and HLA molecules), one may speculate that *close-contact areas* of stromal synapses are involved in direct *intercellular signal exchange*. Due to dimensional constraints, *adhesion molecules* would be distributed within the *wider regions* of the stromal synapse, corresponding to the periphery of the immunological synapse, where they maintain the signaling complex structural integrity [27]. Further biochemical and physiological studies are essential to ascertain that the synapses of ICC described here fulfill the criteria formulated by Dustin and Colman [37] for accepting an intercellular connection as chemical synapse: adhesion, stability, and directed secretion.

Eosinophils and plasma cells established three types of stromal synapses, namely:

- *plain synapses* (PS) - see Figs. 1, 6, 7, 11 and 16
- *multicontact synapses* (MS) - shown in Figs. 1, 4, 5, 8, 11 and 15, and possibly
- *intermediate synapses* (IS) - Figs. 2 and 3.

In apparent contradiction to the numerous synapses between eosinophils and ICC or ICC-like cells illustrated here (Figs. 1, 4-8), Wang *et al.* reported that, under their experimental conditions, eosinophils did not make special contacts with ICC [24].

Small vesicles were found in the synaptic cleft of an ICC-LC/eosinophil synapse *in situ* (Fig. 8). Four membrane-bounded vesicles were present, having an electron-transparent contents and a mean diameter of 54 ± 7 nm. Their morphology and size are similar to exosomes (30-100nm), vesicles that, for example, can convey antigenic information from antigen

Fig. 14 Schematic representation of the immunocyte types found to establish '**stromal synapses**' with ICC or ICC-like cells in various human and rat tissues.

presenting cells to lymphocytes [38-41]. Noteworthy, vesicles with dimensions similar to exosomes were found located in the eosinophil cytoplasm, on the intracellular side of the stromal synapse. In our opinion, *in situ* ultrastructural data point towards the idea that an exosome-like mechanism might operate at the level of the chemical stromal synapse. It is attractive to speculate that signaling molecules might be packed and unidirectionally transported through the synaptic cleft *via* extracellular vesicles. Although such a mechanism lacks the speed of electrical synapses, and is different from the quantal transmission of the canonical chemical synapse, it could be a rapid and precise way of intercellular communication.

Basophils: Fig. 9 shows a basophil enclosed by two different ICC processes that establish synapses on a large fraction of the leukocyte membrane. It is tempting to suggest that ICC processes might also act as 'cellular' guides for immune cells that arrive *via* blood stream. Because ICC processes span long distances, it might be that the cells display gradients of membrane-bound or soluble chemoattractants. Thus ICC might contribute to the process of leukocytes homing in connective tissues.

Macrophages: Fig. 10 illustrates a plain synapse (PS) between a macrophage cell process and an ICC-like cell body. ICC/ICC-LC contacts with resident

Fig. 15 An artist's view of *multicontact* ('kiss-and-run') synapse (MS) between ICC/ ICC-LC and an immunocyte (here a lymphocyte). This type of stromal synapse is characterized by irregular cell contact surfaces. The drawing is not to scale.

macrophages were previously reported [15, 18, 21-24, 42, 43]. The macrophage/ICC-LC contacts that we describe here as chemical stromal synapses have an ultrastructure too elaborate to be merely described as 'morphological accidents'. Moreover, the close spatial associations of ICC with macrophages might indicate a juxtacrine signaling function (e.g. cytokine secretion). Noteworthy, Suzuki *et al.* [43] suggested that increased numbers of macrophages and their activation may result in damage to ICC networks.

Lymphocytes: Stromal synapses between ICC-LC and lymphocytes were found in human mammary gland stroma (Fig. 12). However, we have to mention that Wang *et al.* initially reported [24] that lymphocytes formed close contacts with ICC in the intestine of mice infected with *T. spiralis*, but recently [42], in a similar context, they could not find the same ICC-lymphocyte contacts.

Fig. 12 shows that multiple contact points were present between the two type of cells, interspaced with areas of wider intercellular gaps. This ultrastructural aspect resembles immune synapses described between T lymphocytes and dendritic cells [36] or NK cells and their targets [44]. More specifically, the aspect in Fig 12 is similar to the ultrastructure of early synapses, when the two cells are not yet tightly connected [36]. The reported presence of close contacts between ICC/ICC-LC and lymphocytes or plasma cells might indicate an immunomodulatory role of ICC-like cells. Further studies are necessary to elucidate whether these stromal synapses are actually a type of immunological synapse, with ICC/ICC-LC being able to present tissue-derived antigens to lymphocytes.

Mast cells synapses with ICC-LC were found in human mammary gland stroma (Fig. 13) and

Fig. 16 A similar view of an *uniform* ('plain') synapse (PS) between an eosinophil and an ICC/ICC-like cell. The quasi-constant width of the synaptic cleft is typical. Scale is again the subject of artistic license.

myometrium (to be published). A multicontact synapse is visible in Fig. 13, involving a very thin ICC-LC process and the mast cell membrane. Mast cells are able to respond to various stimuli and to trigger innate as well as specific immune responses, apparently playing a role in tissue immune surveillance. They are able to communicate with other cells by direct cell-to-cell contact, secretion of soluble mediators or by releasing immunomodulatory exosomes [45-47]. The close contacts we describe between mast cells and ICC-LC suggest that the latter might also take part in this cellular cross-talk. It is possible that c-kit immunopositive cells (mast cells and myometrial Cajal-like cells [17]) could assist or even function as pacemaker cells of myometrial contractions, the latter being lost in leiomyomata [48].

We previously proposed that ICC-like cells might represent stromal progenitor cells, participate in juxtacrine/paracrine signaling and play a role in immune surveillance [18]. The defining role of the *stromal synapse* will presumably turn out to be intercellular signaling.

Tunneling nanotubules [49, 50] are recently proposed as intercellular communication mechanisms, besides chemical and electrical synapses. However, the nanoscopic distances between the cells engaged in stromal synapses suggest a *juxtacrine cell-to-cell signaling (chemical synapse)*, and the (micro)vesicles found in the synaptic cleft may correspond to an *exosome-based* mechanism. Considering the nowadays molecular euphoria, and the 'phenomenin' trend [51] in cellular and molecular biomedicine, we expect that some '*juxtacrinins*' mediating intercellular communication will be identified and eventually characterized.

In conclusion, we show here that a *third form of synapse* (besides neuronal and immunological synapses) is the '**stromal synapse**', the connective connection between ICC/ICC-LC and immunoreactive cells, located in the interstitial conjunctive tissue.

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