SYMPOSIUM REPORT

Structure and organization of interstitial cells of Cajal in the gastrointestinal tract

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The morphological features of interstitial cells of Cajal (ICC) in the gastrointestinal (GI) tract are described based on observations of laboratory animals including mice, rats and guinea-pigs, using immunohistochemical staining for Kit and electron microscopy. ICC show a specific distribution, arrangement and cell shape depending on their location within various regions and tissue layers of the GI tract. Hence they are classified into several subtypes. The stomach shows distinct regional variations in the distribution of subtypes of ICC from the cardia to pylorus, whereas the small intestine and colon both seem to retain nearly the same distribution pattern of subtypes of ICC throughout each organ. All subtypes of ICC share common ultrastructural features, such as the presence of numerous mitochondria, abundant intermediate filaments, and formation of gap junctions with the same type of cells and with smooth muscle cells. In addition, depending on their species and anatomical location, some subtypes of ICC show some features typical of smooth muscle cells including a basal lamina, caveolae, subsurface cisterns and dense bodies. ICC are somewhat heterogeneous morphologically. A question is raised on a special relationship between their ultrastructural features and dependency on Kit/stem cell factor system. As the neuromediator function of ICC, reciprocal distribution of ICC and gap junctions in the muscle coat is demonstrated by the comparison of Kit immunoreactive cells and gap junction protein connexin 43 in both small intestine and colon.

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In 1982, Thuneberg surprised the researchers of the GI tract by proposing the hypothesis that interstitial cells of Cajal (ICC) might act as pacemaker cells and as an impulse conduction system in the gut musculature in a way analogous to the pacemaker cells in the heart (Thuneberg, 1982). Since then, an accumulation of evidence from morphological and physiological studies has made it clear that this hypothesis is compatible with experimental observations and that ICC are a distinct mesenchymal cell type (Lecoin *et al.* 1996; Young *et al.* 1996) with functions of either pacemaker or neuromediator cells in the tunica muscularis of the GI tract (Thuneberg, 1989; Sanders, 1996; Huizinga *et al.* 1997; Komuro *et al.* 1999; Ward & Sanders, 2001). As further evidence for their neuromediator role, synaptic specializations beween ICC and nerves were recently demonstrated by immunohistochemical staining for synapse-associated proteins (Beckett *et al.* 2005).

ICC have been found throughout the digestive tract from the oesophagus (Faussone-Pellegrini & Cortesini, 1985; Torihashi *et al.* 1999) to the inner sphincter region of the anus in the human (Hagger *et al.* 1998), but they show different distribution patterns and morphological features depending on their anatomical locations, and according to which they are classified into several subtypes (Hanani *et al.* 2005). The structural arrangement of ICC appears to reflect their physiological tasks and thus provides a clue for critical understanding of intestinal motility. This article deals with the morphological features of each subtypes of ICC revealed by the immunohistochemical and ultrastructural observations of the stomach, small intestine and colon of laboratory animals such as mouse, rat and guinea-pig.

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Organization of ICC networks

The location of the different subtypes of ICC is shown schematically in Fig. 1. The cell shape and arrangement

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of each subtype of ICC is mainly determined by their relationships to local nerve plexuses, the orientation of the smooth muscle layer in which they are contained, and the frequency of connections between ICC themselves.

ICC of the myenteric plexus (ICC-MP). ICC-MP are multipolar cells with three to five primary cytoplasmic processes that project secondary, tertiary and further branching processes that interconnect with their counterparts. They form a cellular network around the myenteric plexus in the space between the circular and longitudinal muscle layers (Fig. 2*a* and *b*). The size of ganglia and pattern of the myenteric plexus vary greatly between different parts of the GI tract (Gabella, 1979). In addition to features reflecting the local myenteric plexus, ICC-MP are fewer in number and their cellular networks are relatively looser in the gastric corpus and colon than in the small intestine (Fig. 2*b*).

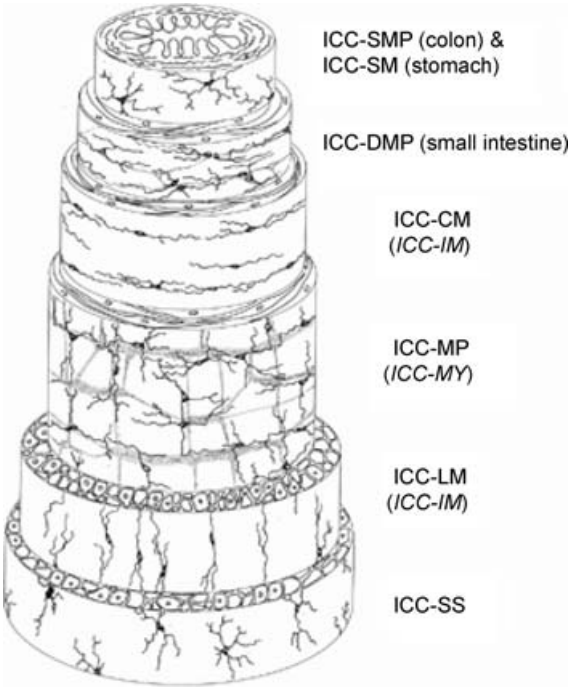

Figure 1. Schematic representation of the types of ICC located in different tissue layers of the GI tract
ICC-SMP and ICC-SM are seen at the interface between the submucosa and circular muscle layer in the colon and gastric pylorus, respectively. ICC-DMP are associated with the deep muscular plexus located between the inner and outer circular muscle sublayers in the small intestine. ICC-CM and ICC-LM are found within the circular and longitudinal muscle layers, respectively. ICC-MP are associated with the myenteric plexus between the circular and longitudinal muscle layers. ICC-SS are found in the subserosal connective tissue space. (Reproduced with permission from Hanani *et al.* 2005.). As to terminology in this article, ICC located in the myenteric (Auerbach's) plexus are designated as ICC-MP instead of ICC-MY or ICC-AP, to keep consistency with the abbreviation for ICC-DMP (deep muscular plexus) and ICC-SMP (submuscular plexus) and because of uncommon usage of the term Auerbach's plexus in the recent literature. ICC-CM and ICC-LM are separately designated, because some morphological and functional differences between them have not been ruled out.

ICC of the circular muscle (ICC-CM). ICC-CM of the circular muscle layers are mainly bipolar cells orientated along the long axis of surrounding smooth muscle cells. However, the distribution and cell density of ICC-CM differs considerably from organ to organ. ICC-CM of the small intestine often show secondary cytoplasmic branches and are sparsely distributed in association with rather thicker nerve bundles (Fig. 2*c*). They appear not to form their own developed cellular network. In contrast, ICC-CM of the stomach and colon show a simple elongated spindle shape, but are densely distributed along nerve bundles (Fig. 2*d*). They are also found in the connective tissue septa, where they have been specially designated as ICC-SEP in the literature (Horiguchi *et al.* 2001; Mazet & Raynier, 2004).

ICC of the longitudinal muscle (ICC-LM). ICC-LM are similar to ICC-CM in cell shape but are usually less numerous than the latter in nearly the whole GI tract, i.e. in the stomach, small intestine and colon (Komuro, 2004). ICC-CM and ICC-LM are often collectively termed ICC-IM.

ICC of the deep muscular plexus (ICC-DMP). ICC-DMP are closely associated with the nerve bundles of the deep muscular plexus of the small intestine that extends two-dimensionally in a plane between the inner thin and outer thick sublayers of the circular muscle (Rumessen *et al.* 1982; Zhou & Komuro, 1992). ICC-DMP are also multipolar cells, but the majority of their processes show a distinct unidirectional orientation along the circumference, due to their close association with both nerve bundles and circular muscle fibres (Komuro, 2004).

ICC of submucosa and sumucosal plexus (ICC-SM and ICC-SMP). ICC-SM and ICC-SMP are found at the interface between the submucosal connective tissue and the innermost circular muscle layer of the pyloric region of the stomach (Horiguchi *et al.* 2001; Seki & Komuro, 2002; Mitsui & Komuro, 2003) and of the colon, respectively (Berezin *et al.* 1988; Ishikawa & Komuro, 1996). Their cell axes are parallel with those of adjacent circular muscle cells, but they contain multipolar cells with a few secondary processes and seem to form a loose network with each other (Kunisawa & Komuro, 2004), unlike the adjacent ICC-CM.

ICC of the subserosa (ICC-SS). Stellate interstitial cells in the subserosal layer were observed in the mouse small intestine with supravital methylene blue staining (Thuneberg, 1982) and in the mouse colon by Kit immunohistochemistry (Vanderwinden *et al.* 2000).

Distribution of ICC

Each subtype of ICC is almost uniformly found in its own tissue layer throughout the entire length of the small intestine and colon, though some differences have been reported in the mouse colon (Ward *et al.* 2002) and human colon (Horisawa *et al.* 1998). In this respect, the stomach is unique in that ICC have a different distribution in proximal and distal regions of the same organ (Burns *et al.* 1997*;* Seki & Komuro, 2002). In the mouse stomach, for example, ICC-CM and ICC-LM are densely distributed throughout the thick circular and longitudinal muscle layers of the cardia, fundus and most of the squamous epithelial portion of the corpus. However, ICC-MP are completely lacking from the myenteric region in these areas. ICC-MP emerge in the area adjacent to the glandular corpus and become well-developed in the pyloric antrum, while both ICC-CM and ICC-LM decrease in number in this area. Along the circumferential axis of the antrum, ICC-MP are distributed more densely in the greater

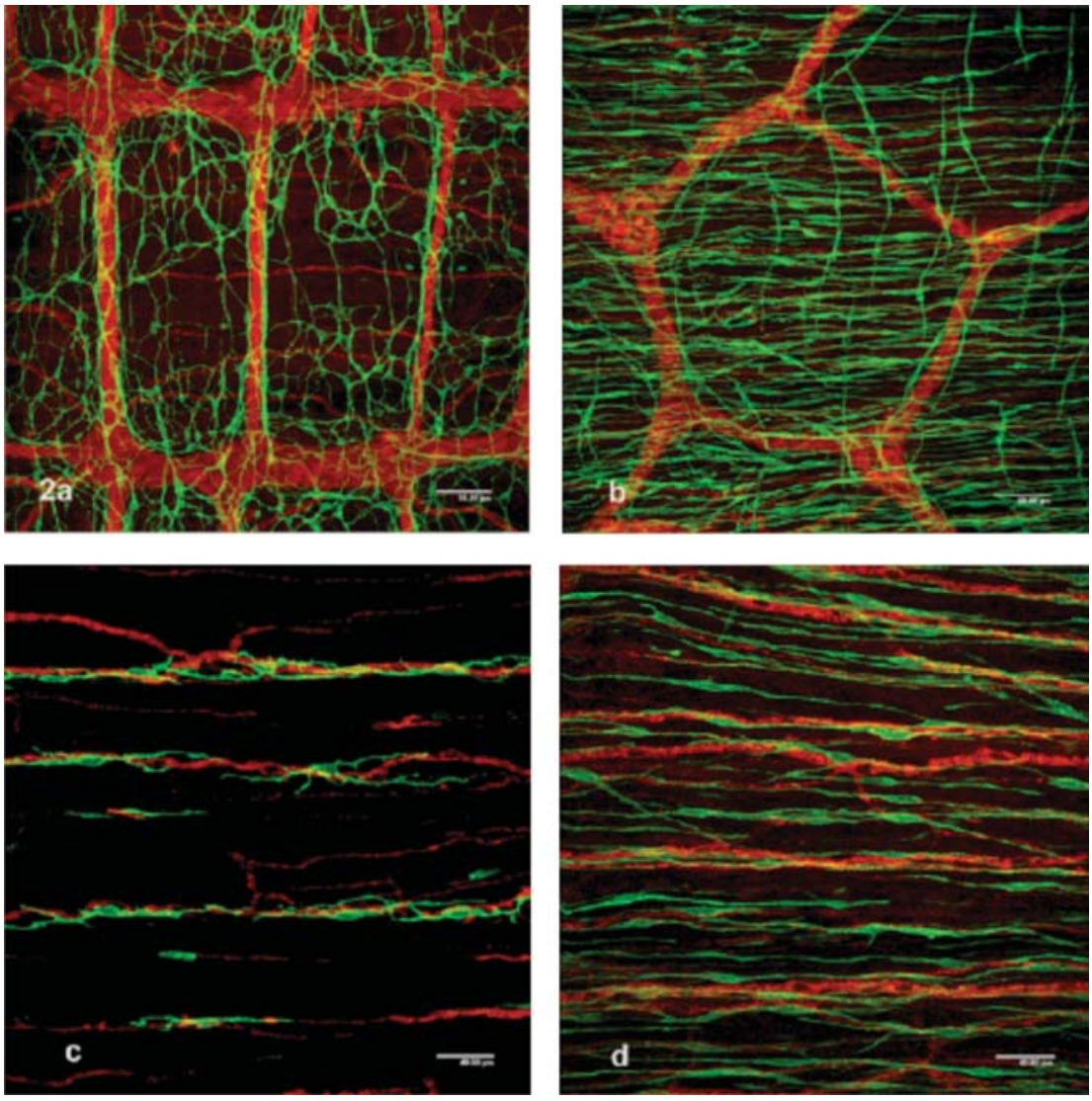

Figure 2. Immunohistochemical staining for Kit and PGP 9.5 to demonstrate cellular networks of ICC (green fluorescence of Alexa 488) and nerves (red fluorescence of TRITC), respectively
a, the network of ICC-MP over the myenteric plexus of the guinea-pig small intestine in which ganglia are orientated parallel to the circular muscle fibres and are connected with thick nerve bundles orientated almost perpendicularly to the ganglia. Scale bar, 80 μm. b, around the myenteric plexus of the guinea-pig colon, ICC-MP are not clearly observed because of their loose arrangement and dense distribution of ICC-CM orientated in a horizontal direction. A few ICC-LM are also seen perpendicularly. Scale bar, 80 μm. c, sparsely distributed ICC–CM in association with rather thick nerve bundles within the circular muscle layer of the guinea-pig small intestine. Scale bar, 40 μm. d, ICC-CM within the circular muscle layer of the guinea-pig gastric corpus. Scale bar, 40 μm.

Table 1. Three types of ICC classified by their ultrastructural features

ICC type	Basal lamina	Caveolae	Gap junctions	Intermediate filaments	Mitochondria	Nerve contacts
Type 1 ICC (least like smooth muscle cells)	–	+/–	++	++	++	++
Type 2 ICC (intermediate type)	+/–	++	++	++	++	++
Type 3 ICC (most like smooth muscle cells)	++	++	++	++	++	++

Abundance, (++) present or numerous; (+/–), fuzzy or few; (–) absent. All types of ICC are positive for Kit immunoreactivity.

curvature than in the lesser curvature in the mouse (Hirst *et al.* 2002) and the guinea-pig (Kunisawa & Komuro, 2004; Mazet & Raynier, 2004).

Another characteristic feature of the pylorus is the presence of ICC-SM at the submucosal border of the circular muscle layer in a confined area directly adjacent to the sphincter (Horiguchi *et al.* 2001; Mitsui & Komuro, 2003).

Coupling of ICC and smooth muscle cells

Ultrastructural observations revealed that certain types of ICC are intercalated between nerves and smooth muscle cells (Thuneberg, 1982; Zhou & Komuro, 1992; Ishikawa *et al.* 1997; Seki & Komuro, 1998) and indicated that they may act as a route for neurotransmission. Such ICC make close contacts with nerve varicosities containing many synaptic vesicles on the one-hand and form gap junctions with neighbouring muscle cells on the other (Fig. 3). More concrete morphological evidence for the neuromediatory function of such ICC was recently provided by immunohistochemical staining for synaptic molecules (Beckett *et al.* 2005).

A clear difference between the distribution patterns of immunoreactivity for Kit-positive cells (ICC) and of the gap junction protein connexin 43 (Cx43) was demonstrated in the muscle coat of the guinea-pig digestive tract (Seki *et al.* 1998). The dense staining for Cx43 in the circular muscle layer of the small intestine indicates that muscle cells were coupled well with each other by gap junctions, but were not intercalated by ICC, because Kit immunoreactive cells were rarely observed in the outer circular muscle layer. On the other hand, the colon contained a regular distribution of Kit immunoreactive cells, and had much less Cx43 immunoreactivity in the circular muscle layer. These data suggest that circular muscle cells of the colon are not well coupled with each other by gap junctions, but are interconnected by intercalated ICC. The reciprocal distribution of Kit and Cx43 immunoreactivity was also shown within the mouse stomach (Seki & Komuro, 2002). The thick circular muscle layer of the fundus had a dense population of ICC-CM, but only weak Cx43 immunoreactivity, whereas the thin muscle portion of the corpus was characterized by a sparse population of ICC-CM, but a dense Cx43 immunoreactivity. These observations suggest that muscle cells that are not well coupled to each other by gap junctions receive nerve signals via a rich network of ICC-CM, whereas muscle cells that are well coupled with each other to form large units of an electrical syncytium receive nerve signals

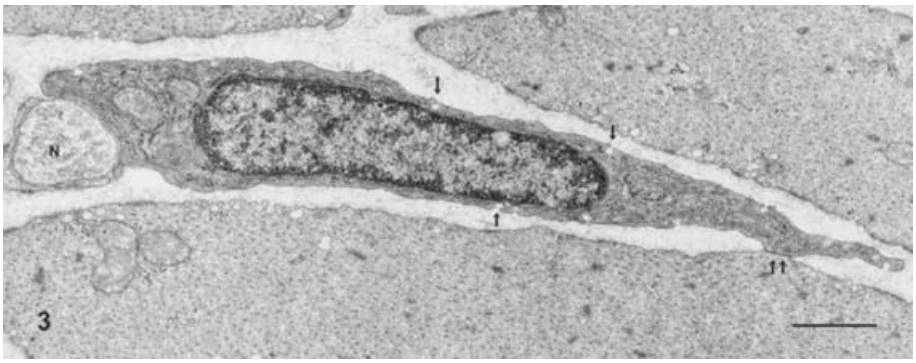

Figure 3. ICC-CM
ICC-CM in the rat stomach characterized by electron-dense cytoplasm, caveolae (arrows), a gap junction with a smooth muscle cell (double arrow) and a close contact with nerve terminal (N). × 16 000. (Reproduced with permission from Ishikawa *et al.* 1997.) Scale bar, 1 μm.

at fewer contacts with ICC-CM. Regarding coupling between ICC-MP and smooth muscle cells, ultrastructural observations failed to provide good enough evidence for their coupling by gap junctions hitherto, except one report in the rat small intestine (Komuro, 1989).

Ultrastructure and dependency on the Kit/stem cell factor system

ICC are generally characterized by ultrastructural features such as the presence of numerous mitochondria, abundant intermediate filaments, moderately developed Golgi apparatus, rough and smooth endoplasmic reticulum, close contacts with nerve varicosities and formation of gap junctions with each other and with smooth muscle cells (Komuro, 1999). However, ICC show a certain range of morphological heterogeneity ranging from features similar to the fibroblasts to those specific to smooth muscles cells such as caveolae, a basal lamina and subsurface cistern, depending on anatomical location and species (Table 1; Komuro *et al.* 1999; Mitsui & Komuro, 2003).

In spite of the fact that in normal development ICC express Kit and their cell maturation depends on signalling between the Kit receptor and its ligand, stem cell factor (SCF), many reports indicate that a certain population of ICC can survive in the absence of proper Kit/SCF signalling. Such ICC were observed in both *c-kit* and stem cell factor mutant animals. Those are ICC-MP in the pylorus of *W/Wv* mouse (Ward *et al.* 1998; Seki & Komuro, 2002), and ICC-MP and ICC-SM in the pylorus of *Ws/Ws* rat (Mitsui & Komuro, 2003). Other examples include the ICC-DMP of the *Ws/Ws* rat small intestine (Horiguchi *et al.* 1995; Horiguchi & Komuro, 1998), the ICC-DMP of the small intestine of the *Sl/Sld* mouse (Ward *et al.* 1995) and *W/Wv* mouse (Malysz *et al.* 1996) and ICC-SMP of the *Ws/Ws* rat colon (Ishikawa *et al.* 1997).

These ICC share common ultrastructural features and are classified into Type 3 ICC (Table 1), the most similar to smooth muscle cells, in respect of the presence of many caveolae and a distinct basal lamina (Komuro *et al.* 1999), regardless of the organ or tissue layer concerned. The evidence suggests that the most muscle-like Type 3 ICC can develop and mature cytologically independent of the Kit/SCF system, or that some other system can compensate in their cell maturation. These observations appear to raise important questions for future studies on why ICC show a fairly wide range of phenotypes and which factors determine the ultrastructural features of particular type of ICC, together with studies to demonstrate if ICC and smooth muscle cells are derived from the same mesechymal progenitor cells (Kluppel *et al.* 1998) and that blockade of Kit signalling induces a smooth muscle cell phenotype (Torihashi *et al.* 1999).

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