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Physiology, injury and recovery of interstitial cells of Cajal: basic and clinical science

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Abstract

In the last 15 years, our understanding of the cellular basis of gastrointestinal function has been altered irreversibly by the discovery that normal gastrointestinal motility requires interstitial cells of Cajal (ICC). Research in this relatively short time period has modified our original concept that the core unit that controls motility is made up of nerves and smooth muscle, to one that now includes ICC. This concept has now expanded to beyond the gastrointestinal tract, suggesting that it may be a fundamental property of the regulation of smooth muscle function that requires rhythmic contraction. ICC are distributed throughout the gastrointestinal tract, have important functions in the control of gastrointestinal motility and are often abnormal in diseased states. Recently, significant steps forward have been made in our understanding of the physiology of ICC as well as mechanisms of injury and recovery. These advances will be the focus of this review.

The physiology of ICC

Unique motor patterns are intrinsic to every organ of the gastrointestinal tract, which suit their functions related to mixing, absorption and anally directed movement. ICC are an integral part of the control of these motor activities. The distribution of ICC throughout the musculature is associated with nerve structures. ICC surround the Auerbach's or myenteric plexus and are associated with nerve varicosities throughout the muscle layers, the so called intramuscular ICC (Figures 1,2). Other subpopulations of ICC are associated with non-ganglionated plexuses of nerve varicosities at the inner borders of the circular muscle layers in the intestine and colon (Figures 1,2). The best understood function is that of pacemaker activity in the stomach and small intestine where the ICC generate a periodic depolarization at a characteristic frequency in each of these organs that is called the slow wave or pacemaker activity. This involves rhythmic oscillations of intracellular calcium and activation of membrane ion channels that causes depolarization. Ion channels viewed as important in the generation of pacemaker activity include non-selective cat-ion channels [1] calcium-activated chloride channels [2–4] and sodium channels [5],

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Interstitial cells of Cajal are not unique to the gut; they are present in other rhythmically active structures, such as the portal vein [6] and the bladder [7]. This phenomenon, however, leads to a discussion on properties of cells that are essential to give them the identity of ICC [8]. Genetic abnormalities to the Kit receptor that lead to loss of ICC in the gut, may not equally influence ICC in other organs [9].

The rhythmic depolarization generated by pacemaker ICC propagates into the circular and longitudinal muscle layers, resulting in periods of low and high excitability of the smooth muscle cells at the pacemaker frequency. Under unstimulated conditions, i.e. much of the nocturnal period, this generally does not result in muscle contraction. However, under stimulated conditions, such as a meal, distention and/or neural excitation, smooth muscle cells will generate action potentials during the depolarized portion of the slow wave. Thus, the resulting characteristic motor patterns will have the unique frequency of the ICC pacemaker system. The pacemaker frequency decreases aborally, slow waves occur simultaneously along the circumference and spread in aboral direction, hence, excitation results in a ring of circular muscle contraction that propagates anally [10]. In the antrum this results in powerful peristalsis that also serves to mix and grind the stomach content. In the small intestine this results in peristaltic activity propagating over variable distances. Pacemaker activity also results in rhythmic pendular movements of the longitudinal muscle layer [11] that maximizes mixing of content, optimizing digestion and absorption.

Our current understanding of the role of ICC in peristalsis requires a distinction between peristalsis and the peristaltic reflex [10]. The peristaltic reflex is a specific motor pattern that is evoked by a bolus and involves excitation oral to and inhibition anal to the bolus, programmed by the enteric nervous system [12, 13]. There is a remarkable variety of motor activities, including peristalsis that are controlled to varying degrees by the central and the enteric nervous system, the ICC and hormonal and myogenic mechanisms. Expression of various motor patterns is generally not the consequence of independent actions of different control systems but rather, depending upon specific stimuli, varying domination of one or more of the control activities. This intermingling of control systems is probably the main reason why the exact role of ICC in gastrointestinal motor disorders has been controversial, perhaps unnecessarily so. It is the perspective of the authors that the question should not be whether a particular cell type is 100% responsible for a particular function; rather, it should be how different cell types integrate their functions to coordinate gastrointestinal function. Demonstration of the importance of the ICC pacemaker system can be found in the motor activity of the gastro-pyloro-duodenal junction where one finds a continuous musculature, but a discontinuity of the ICC pacemaker network [14]. Consequently, the stomach and duodenum have their own independent peristaltic activities and the pylorus can act independently and be controlled by the enteric nervous system to perform sphincter function. In the stomach, strong evidence exists that the intramuscular ICC provide secondary pacemaker activity and Purkinje fiber-like conduction pathways [15]. In the colon, ICC situated at the submucosal border of the circular muscle are carrying out pacemaker function as shown in the canine and human colon [16].

A dominant characteristic of ICC is their extensive innervation (Figure 2, table 1). While smooth muscle cells are innervated primarily through non-synaptic neurotransmission, ICC appear to have synapse-like contact with varicosities of the intrinsic nervous system. This is supported by the presence of proteins involved in neuro-vesicle docking to presynaptic membranes in nerve fibers in close apposition to ICC and the expression of postsynaptic density proteins by ICC [17]. There is substantial evidence for the notion that enteric motor neurons innervate ICC and regulate the slow wave frequency [18] and ICC excitability [19, 20], and thus, indirectly affect smooth muscle function. In the esophagus and fundus it has been demonstrated conclusively that ICC are associated with vagal afferent nerve endings [21]. The synaptic innervation of ICC brought forth the suggestion that ICC are a primary target of nerves and

possibly a preferred pathway for inhibitory and excitatory neural innervation of smooth muscle cells [22], but this remains controversial. Purinergic and peptidergic innervations, appear to follow diffusion of neurotransmitters directly to receptors on smooth muscle cells. For nitrergic and cholinergic innervation however, evidence for an intermediary function of ICC comes from apparent lack of smooth muscle responses to enteric nerve stimulation in the fundus, lower esophageal and pyloric sphincters and colon of mice with hypomorphic mutations in Kit protein (W/W^v) or Kit ligand (Sl/Sld mice) where ICC networks are disrupted [23, 24] and from the small intestines of mice where ICC networks had been depleted by injections of neutralizing anti-Kit antibodies 25. However, lack of nitrergic innervation in the absence of intramuscular ICC has not been found consistently by other investigators in the fundus or lower esophageal, pyloric and internal anal sphincters or the whole stomach of W/W^v mice and in Ws/Ws fundus [26–29]. Direct and indirect innervation of smooth muscle may exist side by side. In summary, smooth muscle activity is not the consequence of a single cascade of events but rather parallel streams of influences. The primary ones are the following: 1) Intrinsic activity of smooth muscle cells (secondary pacemaker activity, action potential generation, direct responses to depolarizing stimuli and distention); 2) Non-synaptic neurotransmission from excitatory and inhibitory motor neurons; and 3) Primary pacemaker activity from ICC that can be modified by synaptic innervation of the ICC.

ICC are situated ideally to monitor the contractile state of the musculature and transmit this to the extrinsic and/or intrinsic nervous system, hence function as mechanoreceptors by virtue of their distribution throughout the musculature and their multiple processes that contact many smooth muscle cells. There is good evidence for interactions between vagal afferent nerves and ICC in the esophagus and fundus [21]. Although the functional consequences are still to be elucidated, loss of ICC-IM is associated with loss of vagal afferent nerve endings, thereby suggesting a survival dependency [30]. One of the more advanced hypotheses is that mechanical distortion of human ICC activates a sodium channel that depolarizes the ICC and increases pacemaker frequency [5]. Mutations in this sodium channel macromolecular complex may lead to gastrointestinal symptoms [31] and may be associated with irritable bowel syndrome [32] and intestinal pseudoobstruction [33]. A very interesting hypothesis that has not yet been adequately explored is that mechanical interaction between ICC and smooth muscle cells involves dynamic creation of peg and socket junctions [34].

ICC and the pathophysiology of gastrointestinal motility disorders

Acknowledgment of the importance of ICC for the integrity of the motor function of the gastrointestinal tract prompted interest in the fate of ICC in gut motor disorders. Damage to ICC and/or reduction of its population has been described in almost every gastrointestinal motility disorder from the esophagus to the rectum. There is already a significant body of evidence for the involvement of ICC in the pathophysiology of gastroparesis and constipation, but ICC abnormalities are also present in acquired conditions such as achalasia, Chagas disease, intestinal pseudo-obstruction, and the inflammatory bowel disorders as well as congenital diseases such as Hirschsprung's and congenital hypertrophic pyloric stenosis (CHPS). All these conditions exhibit abnormalities of motor activity leading to impaired regional transit and symptoms. ICC loss or disruption is associated often with concomitant neuronal and smooth muscle changes suggesting a close interdependence between these cell types. It is still unclear for most of these motility disorders if the disruption in ICC networks is primary or secondary and it will be important to resolve this question.

Mechanisms underlying abnormalities in the populations of ICC are currently incompletely understood but various factors are likely to influence the fate of ICC: 1) a variable degree of regional obstruction and subsequent proximal dilation, 2) injury to the nervous system, 3) the immune system and 4) ICC plasticity.

Obstruction and plasticity

Studies in animal models show that ICC viability and function are compromised in dilated bowel segments proximal to an area of partial obstruction. The degree of disruption of the ICC network is a function of the distance from the obstruction and it is reversible after the obstruction is removed [35] (table 1), highlighting a remarkable degree of plasticity. This could explain the recovery of the pyloric ICC-IM population in patients with CHPS after pyloromyotomy, a procedure that resolves the mechanical and functional obstruction to gastric emptying associated with this condition. It could also explain the frequently observed lack of correlation between the degree of ICC loss and the duration of the disease. Timing and type of treatments varies between patients, influencing the degree of intraluminal distension. This intrinsic ICC plasticity is important as it opens a window for recovery of the ICC phenotype and function if the underlying insult is addressed, although it is still unclear if human ICC are as susceptible to distension as they are in smaller species.

Injury to the nervous system

ICC appear to develop independently from the enteric nervous system and an apparently normal ICC network was observed in a newborn without an enteric nervous system [36] as well as in mouse models. Nevertheless, one should not conclude that there is no interaction between nerves and ICC related to survival as demonstrated for intramuscular ICC in the stomach and afferent vagal nerves [30]. It is currently unknown if ICC and nerves, in diseased states, simultaneously are injured by the same mechanism or if injury to ICC can be secondary to the loss of neural structures. Interestingly, NOS containing nerves contain membrane bound stem cell factor but it is unclear if ICC have access to neuronally produced stem cell factor [37]. nNOS derived NO promotes ICC proliferation *in vitro* and nNOS knockout mice have altered ICC networks [38]. Thus, ICC could be particularly susceptible to damage to NOS containing nerves, particularly relevant for achalasia, diabetic gastroparesis and CHPS.

Relationship with the immune system

Damage to neural structures in Crohn's disease or ulcerative colitis is to a large part mediated by the inflammatory infiltrate, which also appears also to contribute to the development of achalasia and a subset of idiopathic intestinal pseudo-obstruction. The role of an inflammatory infiltrate in the fate of ICC in these conditions is currently unknown and the available information is limited to morphological descriptions on the spatial relationship between ICC and different inflammatory cells infiltrating the gut wall. Interestingly, susceptibility of the different ICC subpopulations appears to depend on upon their proximity to blood vessels that carry immune cells into the tissue, as deduced from animal models of gut inflammation. Membrane to membrane contacts have been observed between ICC and both macrophages and mast cells in Crohn's disease [39]; intimate contacts with the latter have also been described in achalasia [40]. Secretory products from macrophages can have a deleterious influence on ICC. iNOS derived NO produced by resident macrophages has been proposed in the disruption of ICC networks in the endothelin-B receptor null rat [41], considered a model for long-segment Hirschsprung's disease. By contrast, different types of macrophages appear to be cytoprotective to ICC, as in a mouse model of diabetic gastroparesis, likely due to upregulation of heme oxygenase 1 and subsequent reduction in oxidative stress [38].

Our knowledge about functional consequences of loss or injury of ICC is still rudimentary. This is in part due to the fact that ICC are rarely the only affected cell type in a motor disorder and furthermore, it is not known how much damage ICC networks can withstand before functional abnormalities develop. Studies are needed to assess the state of pacemaker activity, distension-induced peristalsis and neurotransmission in human diseases with abnormal ICC.

Although the assessment of ICC in gastrointestinal motility disorders has entered clinical practice, the published literature is conflicting. There are several reasons for this, including the inability to visualize all ICC from formalin fixed, paraffin embedded tissue despite optimal antigen retrieval [42], the need to correctly handle human tissue as ICC appear to be particularly susceptible to ischemia [43], the general lack of data that quantify the normal ICC population [44] and the reliance upon Kit antibodies as the sole method of identifying ICC. In this regard, antibodies to Ano-1 may be of use to determine or confirm abnormalities in ICC in human tissue, with the additional advantage that Ano-1, unlike Kit, is not expressed on mast cells [4]. Establishment of normal values and standardization of tissue collection, fixation and ICC visualization are required in order for the field to progress.

ICC injury, death and recovery

ICC are reduced or otherwise affected in several motility disorders as outlined above but resolution of these disorders in animal models (Table 1) has been demonstrated to result in repopulation of ICC networks underscoring their plasticity as described above. This occurrs in partial small bowel obstruction [35], inflammation [45 46], surgical transection and anastomosis [46, 47]. In health, ICC numbers are dynamic [48, 49]. Conservative estimates indicate human colonic ICC turn over completely in months and similar kinetics appear to be present in other regions of the gastrointestinal tract. Therefore, to maintain functional ICC networks, ICC turnover needs to be tightly controlled with processes that regulate both ICC loss and ICC replacement. Mechanisms shown to contribute to ICC loss include apoptosis [49] and trans/de-differentiation [50, 51], while repair from injury, proliferation from adult ICC [43, 48] and replenishment from ICC stem cell precursors [52] contribute to ICC replacement (Figure 3).

Recent data suggest that adult ICC proliferate [43, 48 53]. The Kit signaling pathway, activated by the Kit-ligand stem cell factor, was the first pathway associated with the control of ICC survival and proliferation. We now know that several other signaling pathways also contribute to ICC survival and proliferation, including neuronally derived nitric oxide [38], serotonin signaling through the 5-HT_{2B} receptor [54], interleukin 9 [55] insulin and IGF-1 signaling through stem cell factor [56] and heme oxygenase 1 [57]. 5-HT appears to play an unexpected role in the maintenance of ICC networks. ICC express several 5-HT receptors, including the 5-HT_{2B} receptor. Activation of the 5-HT_{2B} receptor causes an increase in ICC proliferation and activation of PKCγ. This pathway appears to be active *in vivo* as 5-HT_{2B} knockout mice have markedly reduced ICC networks. ICC appear also to be particularly susceptible to oxidative stress. Data from diabetic non-obese diabetic (NOD) mice reveal that maintenance of ICC in diabetes is dependent upon diabetes-induced upregulation of heme oxygenase-1 (HO-1), an enzyme that generates the cytoprotective gaseous mediator carbon monoxide in tissue macrophages [57]. Mice that had high levels of HO-1 had normal Kit expression while mice that could not sustain elevated levels of HO-1 had high levels of oxidative stress and lost Kit expression. These data suggest that HO-1 plays a central role in protecting ICC from oxidative damage. Future work needs to be directed towards determining if these diverse factors interact and whether there are common intracellular signaling pathways that may offer therapeutic targets.

ICC replenishment can also occur through local precursor cells. The discovery of a candidate ICC progenitor or stem cell [52] suggests the possibility that manipulation or transplantation of cells with stem-cell properties is a new therapeutic approach to diseases associated with ICC loss. These studies in postnatal murine gastric muscle have revealed rare cells that express up to 10 times less Kit on their surface than mature ICC. They also express CD44, CD34, Ano-1 and receptors for insulin and IGF-1 [52, 58]. Unlike adult ICC, which require membrane bound stem cell factor, their proliferation can be sustained with soluble stem cell factor and by the direct action of IGF-I. Interestingly, differentiation of these $Kit^{low}CD44^{+}CD34^{+}Insr^{+}Igf1r^{+}$

cells gave rise to not only ICC but also smooth muscle and other cell types, which may have the additional advantage of providing the correct milieu for survival and maintenance of mature ICC networks.

While a decrease in ICC replenishment from either adult ICC or ICC precursors is associated with several gastrointestinal motility disorders, an uncontrolled increase may result in gastrointestinal stromal tumors (GISTs). Most GISTs arise from ICC lineage as a result of activating mutations in Kit [59, 60], and occasionally in PDGFR [61, 62]. Treatment of advanced-stage GISTs with imitinab, a tyrosine kinase inhibitor, has increased survival but is rarely curative.

Different mechanisms have been proposed for ICC loss. Ultrastructural studies have suggested that in diseased states, ICC might dedifferentiate into an earlier developmental stage, more reminiscent of smooth muscle cells or fibroblasts from an ultrastructural viewpoint [50, 51, 63]. Whether this changed phenotype is reversible into ICC or is destined to be lost is not yet known. As outlined above, ICC can be lost through apoptosis. Apoptotic ICC, as detected by activated caspase-3 or TUNEL, can be detected in all layers of the human colonic muscle [49].

ICC, together with the enteric nervous system, provide a critical control system for gastrointestinal motility. ICC are affected by inflammation, oxidative stress and obstruction and their function and survival markedly influenced by various growth factors. Development of an understanding of the factors that regulate maintenance of ICC networks is well underway although much more remains to be discovered. The new paradigm (Figure 3) of a tightly controlled ongoing balance between pro-survival and pro-loss factors is of significance because it indicates that strategies to stop ICC loss or reverse loss can be directed to both sides of the equation, decrease ICC loss or increase ICC proliferation by specific interventions on ICC signaling pathways, and/or restoration from precursors.

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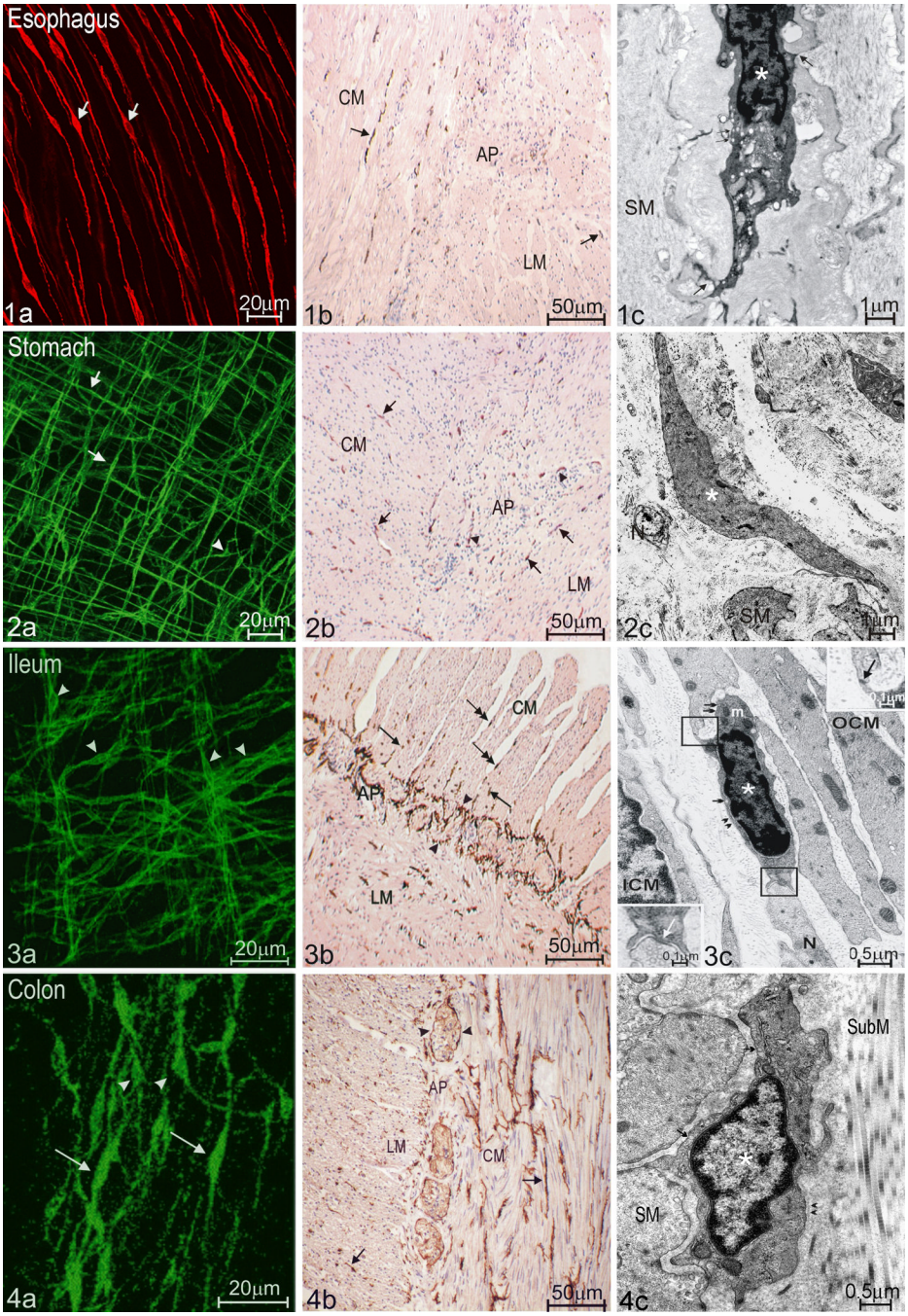

Figure 1. Subtypes and distribution of ICC along the GI tract as shown by various morphological techniques

a: whole mount light microscopy

b: cross sections light microscopy

c: electron microscopy

1. The esophagus has only ICC-IM (1**a** from mouse LES, 1**b**&**c** from human esophagus). These ICC-IM (arrows in 1a&b) exhibit a spindle shaped body with processes emerging from both cellular extremes (1**a**). Ultrastructural characteristics of ICC (* in 1c) are: electron dense cytoplasm, oval nucleus with heterochromatin distributed in the periphery and abundant

mitochondria. ICC in the esophagus can establish close connections (arrows in 1c) with nearby smooth muscle cells (SM). Small arrows: caveolae.

2. There are two main classes of ICC networks in the stomach: ICC-IM, including septal ICC in larger animals, and ICC associated with the myenteric (Auerbach's) plexus (2**a** from mouse corpus; 2**b**&**c** from human stomach). **2a** shows ICC-IM with typical spindle shaped bodies (arrows) and myenteric pacemaker ICC with characteristic stellar bodies (arrowheads) in the wholemount preparation. 2**b** demonstrates ICC (arrowheads) and ICC-IM (arrows) scattered among smooth muscle cells in both muscle layers. 2**c** shows a longitudinal section of a myenteric ICC (*). N: nerves; SM: smooth muscle cells.

3. There are three main ICC populations in the small bowel (3**a–c**, all from human): ICC-IM (arrows in 3**b**), myenteric pacemaker ICC (arrowheads in 3**a** and 3**b**) and ICC-DMP, which are ICC, associated with the deep muscular plexus located between the outer and inner circular muscle layer (OCM&ICM). ICC-IM are distributed both within the musculature (arrows) and in the septa (double arrows) in 3**a**. ICC-DMP show weak Kit staining compared to other ICC and can be better identified by electron microscopy (3**c**). An ICC-DMP (*) in 3**c** establishes simultaneous close contact with a nearby nerve terminal (N) and a neighboring outer circular smooth muscle cell (OCM). Upper right inset in 3**c** shows a contact with the smooth muscle cell and lower left inset shows a synapse like contact with the nerve terminal. ICM: inner circular muscle. Small arrows in 3c: caveolae; Arrowheads in 3c: basal lamina.

4. Three different subtypes of ICC are present in the colon (4**a–c**, all from human). ICC-IM (arrows in 4**a**&**b**), myenteric pacemaker ICC (arrowheads 4**a**&**b**) and ICC-SM (* in 4**c**), which are ICC, associated with the submuscular plexus. Arrows in 4**c**: caveolae along the membrane; Arrowheads in 4c: basal lamina.

CM: circular muscle cells; LM: longitudinal muscle cells; AP: Auerbach's plexus.

Acknowledgements: Figure 4c was obtained with permission from Shigeko Torihashi [64]. Figure 2c was obtained with permission from Simonetta Faussone-Pellegrini [65].

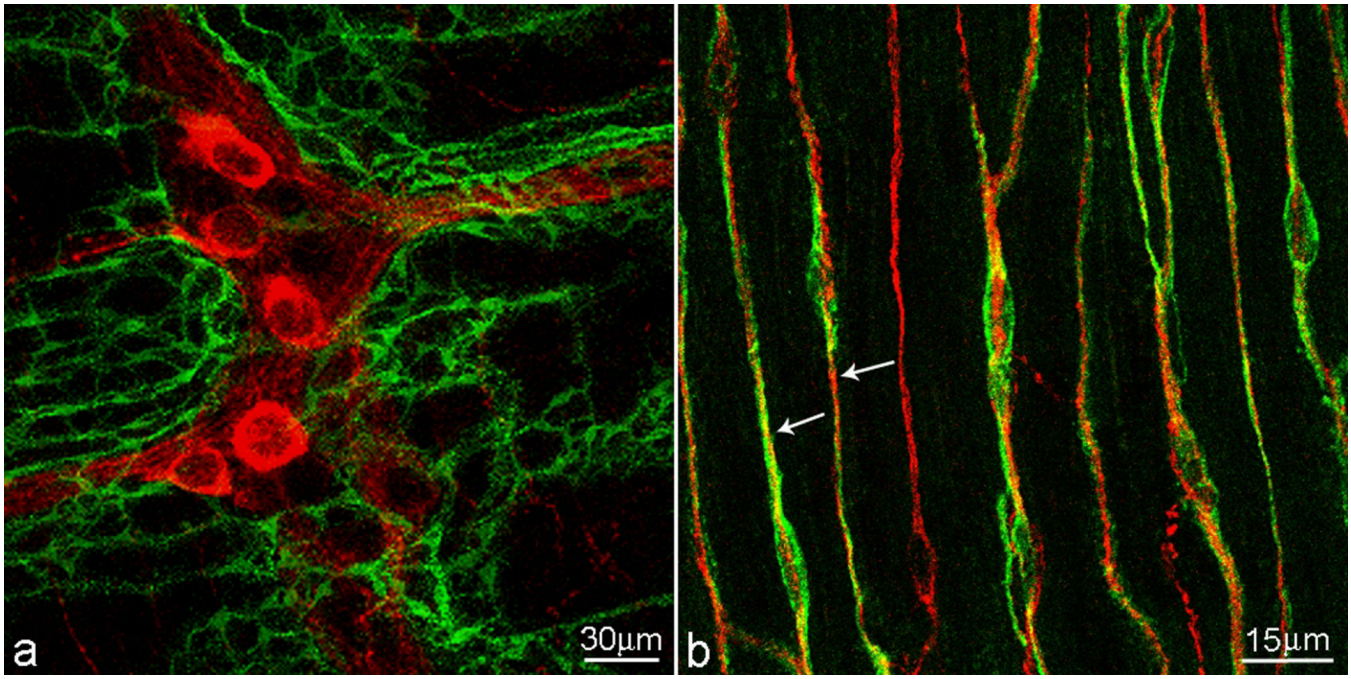

Figure 2. ICC are associated with the enteric nervous system

a. A dense network of myenteric pacemaker ICC surrounds the myenteric plexus ganglia in the small intestine. b. ICC of the deep muscular plexus in the small intestine are bipolar and aligned with smooth muscle cells. Many ICC are fully aligned with enteric nerves (shown here are nitrergic nerves). The confocal scanning thickness was 4 µm.

C-kit staining of ICC (green), neural plexus is stained with nNOS antibody (red).

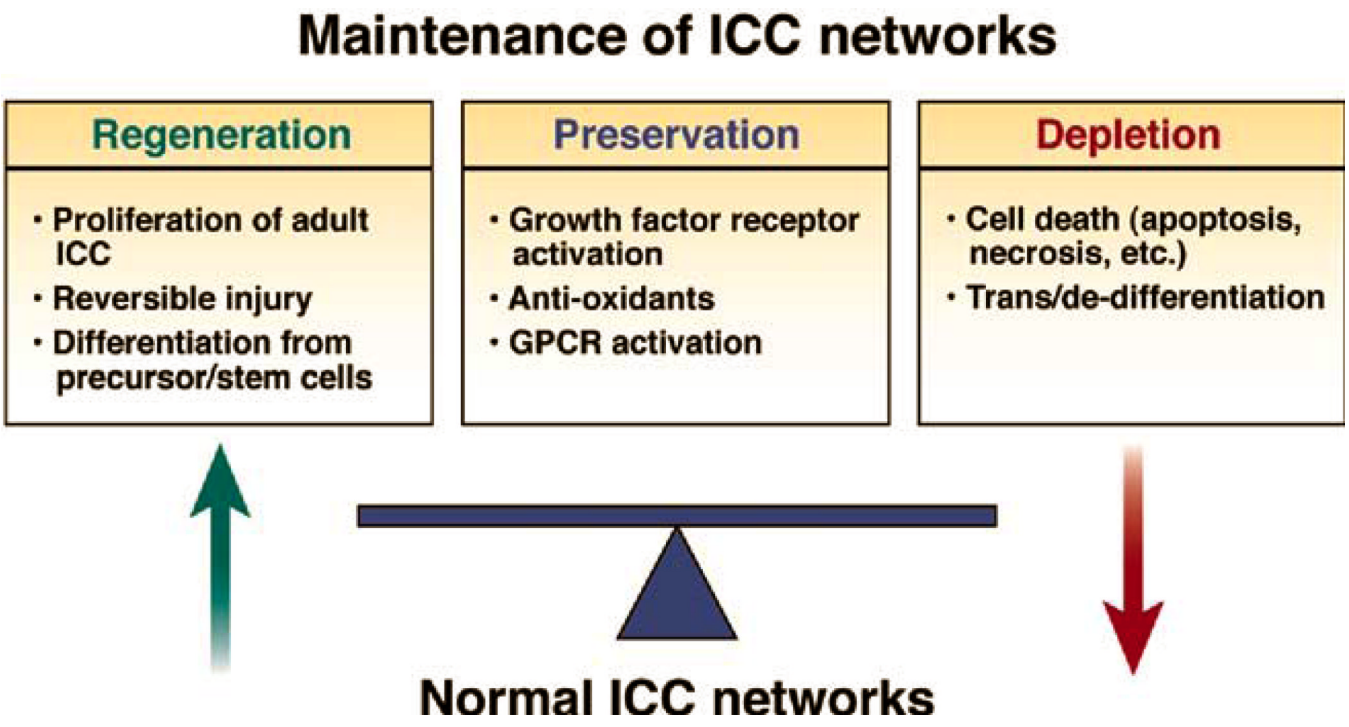

Figure 3. Proposed paradigm for the control of ICC networks

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Table 1

Animal models affecting interstitial cells of Cajal

Pacemaker ICC refers to ICC located in the myenteric plexus. Only a limited number of references are included because of space limitations.

Animal	Type	Name	Alteration	Status of ICC	Functional changes observed
Mouse	Models which primarily target the ICC population	W/W^v	Mutation of the proto-oncogene *c-kit* reduces tyrosine kinase activity	Loss of ICC-IM in stomach and sphincters[24] ; loss of pacemaker ICC in the intestine[78,79]; reduced ICC in the colon	Increased gastric compliance[24]; altered [24] or no change [28] in neurotransmission; increased fundic muscle excitability ; abnormal intestinal motility due to loss of pacemaker activity
		Sl^d/Sl^d	Mutation of the *Steel* locus produces an abnormal, ineffective membrane bound SCF, the ligand for Kit	Loss of ICC-IM in stomach and sphincters[66] ; loss of pacemaker ICC in the intestine[67]	Altered fundus neurotransmission[66], abnormal intestinal motility due to loss of pacemaker activity
		WZsGreen/+	Insertion of green fluorescent protein sequence, ZsGreen, into the first exon of the *c-kit* gene	Expresses a fluorescent tag in KIT-expressing cells[68]	Allows morphological identification of Kit-expressing cells
		Pharmacological blockage of tyrosine kinase activity	*In vivo* blockage of Kit receptor by i.p injection of antibodies	Severe disruption of ICC populations[53,69]	Intestinal ileus with loss of pacemaker activity and ineffective neurotransmission
		Murine partial bowel obstruction	Mechanical obstruction achieved by placing a clip on the small intestine	Disruption of ICC networks proximal to the obstruction site; recovery after clip removal [35]	Loss of electrical slow waves and responses to enteric nerve stimulation proximal to obstruction site; recovery after clip removal
	Diabetes models exhibiting ICC alterations	Db/db	Model of Type 2 diabetes	Reduced ICC population in antrum, small intestine and colon[70]	Gastroparesis, intestinal dysmotility, decreased whole gut transit
		NOD/LtJ	Model of human Type 1 diabetes	Reduction of antral and body myenteric ICC and ICC-IM; loss of close connection with nerve terminals; reduced SCF production [71]	Gastroparesis with impairment in pacemaker activity and altered neurotransmission
	Models of enteric agangliosis	Ls/ls	Homozygous for the lethal spotted (*ls*) allele display a loss of function mutation in the endothelin-3 gene. Model of short Hirschsprung's disease	Aganglionosis in the terminal regions of the large intestine. Distribution and density of ICC populations seems unaltered[72]	Loss of spontaneous electrical activity and postjunctional neuronal responses; unlikely to depend on changes in ICC
		GDNF−/− mice	Glial cell line-derived neurotrophic factor (GDNF) knockout mice. Model for long segment Hirschsprung's disease	Aganglionosis along most of the GI tract. Distribution and density of ICC populations seems unaltered[73]	Normal pacemaker activity
Rat	Model which primarily targets the ICC population	Ws/Ws	Deletion at the tyrosine kinase domain of Kit due to a mutation in the proto-oncogene *c-kit*	Loss of ICC-IM in the esophagus [27]; reduction or loss of ICC populations in the stomach and colon[74] ; loss of pacemaker ICC in the small intestine [75]	Neurotransmission relatively preserved, increased spontaneous contractile activity in esophagus[27] and colon; altered colonic pacemaker activity in the colon [76]

Animal	Type	Name	Alteration	Status of ICC	Functional changes observed
	Diabetes model exhibiting ICC alterations	STZ-DM	Streptozotocin-induced diabetes mellitus in Wistar rat. Model of human Type 1 diabetes	Reduced antral and colonic ICC-IM and ICC-SMP[77]	Gastroparesis; increased amplitude of stretch induced colonic contractions
	Models of enteric agangliosis	ETB receptor null rat	Endothelin receptor null rat possess an autosomal recessive gene (*sl*) that leads to aganglionosis. Model for long segment Hirschsprung's disease	Severe disruption of pacemaker ICC network[41]	Irregular spontaneous phasic contractile activity.