Physiology and Pathophysiology of the Interstitial Cells of Cajal: From Bench to Bedside

IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal

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IN CONSIDERING THE PHYSIOLOGY and pathophysiology of GI motor activity, one is struck by the mysterious etiologies of many congenital and acquired motility disorders. For example, pseudoobstruction is generally believed to be due to neuropathies or myopathies, but the details of specific lesions are difficult to find in the literature. Diabetic gastroparesis is generally thought to be due to a neuropathy, but there are rather vague correlations between verified enteric neuropathies and gastric dysfunction in the literature. During the past 10–20 years, an appreciation has developed for the important role of interstitial cells of Cajal (ICC) in gastrointestinal (GI) physiology. It is now recognized that loss or damage to these cells could cause serious motor dysfunction, and this knowledge has generated exciting new hypotheses regarding the potential etiologies of several GI motor disorders. New reagents, coupled with immunohistochemical techniques, have allowed pathologists to evaluate the status of ICC in dysfunctional tissues, and numerous studies report loss or damage to ICC networks in a variety of motility disorders. These studies have reported lesions in ICC networks long after development of symptoms; so it is difficult to establish a cause-and-effect relationship and to conclude that the defects in ICC networks are the cause of motility disorders.

Animal models provide the opportunity to evaluate the status of ICC at various stages during the development of symptoms and might provide a means of determining whether ICC defects are fundamental to the etiology of motility disorders. Changes in ICC networks consistent with the changes observed in human disease have been observed in animal models. An important observation has been that loss of ICC does not result from cell death, but rather ICC appear to undergo redifferentiation. A genomic approach in future experiments may offer a means of determining the factors that regulate the ICC phenotype, and this in-
formation may provide new insights into treating motor disorders related to defective ICC. This article discusses the basic function of ICC, the anticipated functional losses resulting from defective ICC networks, and new animal models to assess the factors responsible for loss of ICC in GI muscles.

ANATOMICAL LOCATIONS OF ICC IN GI MUSCLES

OFFER INSIGHTS ABOUT THEIR FUNCTION

ICC have discrete distributions within the tunica muscularis of the GI tract. In some cases, they form interconnecting networks within thin planes of the muscularis, and in other cases, the cells are loosely distributed within bundles of smooth muscle cells. ICC are nearly ubiquitous within the region of the myenteric plexus (IC-MY), and these cells are closely associated with ganglia and smooth muscle cells of the circular and longitudinal muscle layers. ICC can also be found within the muscle layers from the esophagus to the internal anal sphincter. In each case in which careful examination has been performed, these “intra-muscular ICC” (IC-IM) are in very close contact with the varicose processes of enteric motor neurons. In the esophagus, stomach, and colon, IC-IM are distributed throughout the circular, and in most areas, the longitudinal muscle layers. Cells equivalent to IC-IM are more restricted in the small intestine and populate the region of the deep muscular plexus (IC-DMP). In the stomach and colon, a population of ICC also lies at the submucosal aspect of the circular muscle layer (IC-SM). ICC form gap junctions with surrounding smooth muscle cells. Although it is possible that the various populations of ICC are interconnected, this has not been rigorously demonstrated.

GENERAL DESCRIPTION OF THE ROLE OF ICC

The locations of ICC and their associations with smooth muscle and enteric neurons led early anatomiasts to make predictions about their function. It turned out to be very difficult to determine the actual physiological roles of ICC, but recent progress has demonstrated the importance of these cells as pacemakers, in propagation of slow waves, and as mediators of inputs from enteric motor neurons. Other functions, such as modulators of sensory inputs (e.g., stretch receptors), have also been proposed, but little physiological evidence for this function has been published. Other papers within this series of themes articles have dealt with the specific functions of ICC in some detail, but a short recapitulation of their functional roles is germane to the current article (i.e., the consequences of defective ICC networks or loss of ICC).

IC-MY in the stomach, small bowel, and colon and IC-MY and IC-SM in the colon have dual functions as pacemakers and conduction pathways for the regenerative (active) propagation of electrical slow waves (8, 14, 16, 22, 37). Loss of this class of ICC leads to loss of normal electrical rhythmicity in GI muscles. Because electrical slow waves are the signals that organize the contractile behavior in many regions of the GI tract, loss of these cells would be expected to produce abnormal motor patterns.

IC-MY and colonic IC-SM also provide a conduction pathway for regenerative spread of slow waves. Here again the structure and distribution of IC-MY and IC-SM networks gave hints about their function. For example, IC-MY are arranged into loose networks, spreading throughout most of the myenteric region of stomach, small bowel, and colon. If these cells were only pacemakers, then they could reside in discrete locations (as in the heart). However, the distribution around and along the GI organs suggests a role in communication as well as signal initiation. Regeneration of slow waves is critical for normal function of organs such as the stomach, where the dominant (i.e., highest frequency) pacemaker cells reside near the greater curvature of the orad corpus. Slow waves spread circumferentially and distally to the pyloric sphincter, creating gastric peristalsis. ICC express the mechanism for slow-wave generation, and this mechanism is also employed for the regenerative spread of slow waves. In years of studying isolated smooth muscle cells, no one has found the ionic apparatus that is necessary for generating electrical slow waves in these cells. Thus smooth muscle cells respond to the depolarization caused by slow waves, but they cannot actively regenerate slow waves. Removing ICC in regions normally paced by slow waves causes decay of slow waves within short distances (as determined by the cable properties of the smooth muscle syncytium; see Ref. 26). In small mammals with thin layers of smooth muscle, ICC with regenerative capabilities might be restricted to planar networks within the myenteric plexus region or along the submucosal surface of the circular muscle layer (as in the colon). However, in mammals with thicker-walled organs (e.g., >1 mm), if pacemaker ICC were restricted to a thin plane, nonregenerative spread of slow waves through the smooth muscle layers would result in decay of these events before conduction could occur through the thickness of the muscle layers. Many smooth muscle cells would be inaccessible to the coordinating influence of slow waves. Recent studies of the canine gastric antrum have demonstrated that in thicker-walled organs, ICC within the circular muscle layer are capable of regenerating slow waves, creating a regenerative pathway to distribute high-amplitude slow waves throughout the muscle (13).

In general, IC-IM and IC-DMP of the small intestine appear to be involved in neurotransmission. These cells, via close association with varicose nerve terminals and expression of pertinent receptors for enteric neurotransmitters, receive and transduce neural signals and pass these signals along to electrically coupled smooth muscle cells in the form of hyperpolarization or depolarization responses. The hyperpolarizations or depolarizations occur by regulating the openings of ion channels, and inputs to IC-IM can regulate the excitability of the ICC-smooth muscle syncytium. Thus neural inputs to IC-IM regulate the response of smooth muscle cells to slow-wave depolarizations. For exam-
ple, a region of smooth muscle cells dominated by inhibitory neural input (typically accomplished by opening potassium channels) is less depolarized by slow-wave events and less likely to yield openings of voltage-dependent Ca\textsuperscript{2+} channels and produce a contraction.

A caveat to the role of IC-IM and IC-DMP in enteric neurotransmission is that this hypothesis has only been tested in a functional manner in mice, because murine mutants are available that do not have IC-IM in specific regions of the GI tract (4, 35). It is possible that parallel innervation of ICC and smooth muscle cells occurs in some species or in some regions of the gut. The degree to which smooth muscle cells are directly innervated or whether neurotransmitters, especially in larger animals and humans, can reach the receptors expressed by smooth muscle cells in functionally significant concentrations, is not yet understood. It is interesting to note, however, that close associations between enteric neurons and IC-IM have been documented in several other species, including humans (6, 32, 34). Furthermore, the only rigorous morphometric study at the electron microscopic (EM) level detected far more numerous close associations between nerve varicosities and ICC than with smooth muscle cells (6).

Thus the morphology suggests that the contribution of ICC to neurotransmission may be a generalized phenomenon in GI muscle tissues.

The specific loss of IC-IM or IC-DMP, therefore, might interfere with enteric motor neurotransmission. This could lead to gross motor dysfunctions of many types depending on the specific region of the GI tract affected. Because ICC appear to have a serial role in enteric neurotransmission, disorders that include a lesion in IC-IM or IC-DMP could have symptomology (and physiological responses) suggestive of an enteric neuropathy.

ASSOCIATIONS BETWEEN ICC AND MACROPHAGES

In small mammals, the region of the myenteric plexus is heavily populated with resident macrophages. The cells occur in a regular pattern and form close associations with ICC (18). These cells are also present in the human gut, however, with a less regular pattern of distribution. Because resident macrophages can be activated by a variety of conditions, including endotoxemia, and secrete a large variety of bioactive substances, such as cytokines, prostaglandins, leukotrienes, and nitric oxide, the presence of these cells may greatly affect the performance of ICC. It has been proposed, for example, that products of macrophages produce intestinal ileus and motor responses to bacterial toxins (e.g., Ref. 9).

A recent study has examined osteopetrotic (op/op) mutant mice that have an inactivating mutation in colony-stimulating factor (CSF-1). These animals lack resident macrophages in the tunica muscularis, but other cells, including ICC, appear to be normal (19). This model may be useful in determining the role of macrophages in regulating motility in normal and pathophysiological conditions and the impact of activation of resident macrophages on the functions attributable to ICC.

DISORDERS IN WHICH ICC ARE DEFECTIVE OR REDUCED IN NUMBER

Research into the distribution and function of ICC was greatly stimulated by discovering that ICC express c-kit, and signaling via Kit protein is necessary for development and maintenance of the ICC phenotype (30, 31). Antibodies to Kit provided pathologists with the opportunity to evaluate ICC networks in GI muscles from patients with motility disorders. These studies have revealed that ICC are reduced in number or lost in a variety of cases with motor dysfunction, including pseudobstruction, achalasia, ulcerative colitis, infantile pyloric stenosis, Chagas’ disease, diabetes, slow-transit constipation, and idiopathic gastric perforation. Because the scope of this themes article does not permit a thorough discussion of these studies, the reader is referred to recent reviews on this subject (25, 33).

ANIMAL MODELS TO STUDY THE CONSEQUENCES OF LOSING ICC

The ability to label ICC with Kit antibodies has allowed evaluations of the status of ICC in animal models with motility disorders. We have investigated the hypothesis that, like human motility disorders, animals with defective motility may also experience loss of ICC. Another approach to this problem is to study the GI motility-related consequences of primary ICC depletion. With the use of such animal models, it may be possible to study why and how ICC disappear from GI muscles and perhaps to establish a cause-and-effect relationship between ICC loss and the development of GI motor dysfunction. Although several good animal models with defects in ICC networks exist, to date there have been relatively few whole animal evaluations of the motility defects present in these animals.

The first and most widely studied animal models of ICC-dependent GI motility dysfunction were animals with mutations in or pharmacological blockade of Kit. Kit is the gene product of the protooncogene c-kit and is a 160-kDa protein consisting of extracellular, membrane-spanning, and cytoplasmic domains. The ligand for Kit, stem cell factor, binds to the extracellular domain and activates tyrosine kinase activity in the intracellular domain. Recruitment of additional signaling molecules influences cellular functions including growth, differentiation, and cell migration (23). Signaling via this pathway is known to be essential for the development and maintenance of the ICC phenotype, and it is possible that defects in Kit signaling could be a common factor linking a variety of motility disorders that result in loss of ICC. One of the most useful spin-offs from research into the function of Kit was the development of antibodies directed toward Kit and specifically to the extracellular domain of Kit. These
antibodies can be used as neutralizing reagents to block activation of Kit receptors in vivo (e.g., ACK2; see Ref. 20).

In the original study in which Kit antibodies were used for immunohistochemistry in GI muscles, small elongated cells were labeled within the tunica muscularis (17). The anatomical locations of these cells suggested they could be ICC. When animals were given injections of a neutralizing Kit antibody for several days after birth, cells with Kit-like immunoreactivity (Kit-LI) disappeared. The abdominal regions of these animals displayed features indicative of abnormal GI motility, including gastric distension, poor gastric emptying, and pronounced ileus. The mechanical activities of muscles from these animals were also abnormal. There were irregular phasic contractions in the small intestine, and the muscles displayed increased sensitivity to physiological excitatory agonists, such as bradykinin, acetylcholine, and prostaglandin F2α (17, 27).

It was proposed that blocking Kit might interfere with the pacemaker system of the GI tract.

The expression of Kit in GI muscles and the importance of this signaling pathway in GI function were demonstrated by showing that ICC do indeed express Kit, and loss of these cells interferes with electrical pacemaker activity, slow-wave propagation, and enteric motor neurotransmission (22, 30, 38). The main importance of these studies was to create a way to manipulate ICC populations with genetic and developmental techniques, and these studies produced the first animals and tissues in which the role of ICC in GI motility could be tested.

**KIT MUTANTS**

Spontaneous c-kit mutants (mouse W locus and rat Ws locus) have been useful for studies of the physiological role of ICC. Homozygote W mice die before birth due to severe anemia, so animals with less severe W mutations have been used for most studies. Compound heterozygotes from crosses of W+/ and W+/W+ heterozygotes have been used by many investigators. W is a point mutation at amino acid 660 in Kit that reduces but does not abolish tyrosine kinase activity. W/Wv animals survive through adulthood, although they have motor disturbances that appear to be attributable to loss of specific classes of ICC. It is interesting to note that the fractional activity of Kit left in W/Wv animals appears to be sufficient to allow relatively normal development of some populations of ICC, whereas other types of ICC are largely missing in these animals. The main ICC affected in W/Wv animals are IC-MY and IC-IM in the stomach and sphincters. The reasons for the difference in the sensitivity of the various classes of ICC to W mutations are not understood.

As with animals treated with neutralizing Kit antibody, small intestinal muscles of W/Wv mice were found to be electrically quiescent (14, 37). The loss of slow waves in the small intestine of W/Wv mice was associated with a severe reduction in the numbers of IC-MY and disruption of the IC-MY network. These observations further confirmed the importance of IC-MY as pacemakers. It was also noted that IC-DMP and enteric neurotransmission were not apparently affected in W/Wv animals. Later studies showed that IC-IM were missing from the stomach and sphincteric regions of W/Wv mutants. These tissues are not normally rhythmic, but loss of ICC from these tissues resulted in defects in inhibitory and excitatory neurotransmission (4, 35). These observations suggested a “division of labor” between different classes of ICC in the mouse. The fact that W/Wv mice have specific types of ICC missing in different regions provides interesting animal models to investigate the consequences of losing ICC on different aspects of motility.

A study on isolated muscles from the lower esophageal sphincter (LES) showed that nitric oxide-dependent neurotransmission is reduced in tissues from W/Wv mice (39). These animals have greatly reduced numbers of IC-IM in the LES, and these data suggest that IC-IM, as in the stomach, may be important mediators of enteric motor neurotransmission in the LES. A recent study tested the relaxation of the LES in response to swallowing (28). The normal response in the murine esophagus following a swallow is a wave of contraction that spreads down the body of the esophagus and relaxation of tone in the LES. The LES of W/Wv mice were hypotensive, having less than one-half the normal resting tone. Swallowing initiated a relaxation response that was partially blocked by inhibitors of nitric oxide synthesis. These observations suggest that although IC-IM may be involved in enteric motor neurotransmission, loss of these cells in the W/Wv model does not totally block the inhibitory response to swallowing. There may either be adequate inhibitory neurotransmission to smooth muscle cells to yield relaxation or there may be developmental compensation for loss of IC-IM in W/Wv mice. The hypotensive nature of the LES in W/Wv mice may have been due to loss of neurogenic tone, however, this hypothesis was not tested.

**STEM CELL FACTOR MUTANTS**

The binding of stem cell factor (SCF) activates the tyrosine kinase activity of Kit. There are soluble and membrane-bound forms of SCF, and, as discussed below, it is the membrane-bound form of the ligand that is effective in stimulating the Kit-signaling cascade in ICC. Thus cells next to ICC must express SCF. Studies have shown that both a subclass of enteric neurons and smooth muscle cells express SCF, and it appears that smooth muscle cell expression is sufficient to stimulate growth and development of the ICC networks, because functional ICC develop in mice that lack enteric neurons (40).

The SCF gene is syntenic with the Steel (Sl) locus on murine chromosome 10. Spontaneous mutations of steel have been identified, and nonlethal mutations, such as the Steel-Dickie (Sld), are commercially available. Sld is a 4-kb deletion of the genomic sequence that encodes the transmembrane and cytoplasmic portions
of SCF (3). Compound heterozygotes, such as Sl/Sld, survive and can be used for investigations of the significance of signaling via SCF in development of ICC.

As with W/WV mutants, Sl/Sld muscles had partial lesions in ICC (36). At day 10, Kit-positive cells were identified in the myenteric region, but the IC-MY networks were highly diffuse and of abnormal structure. By adulthood (day 30), IC-MY were not observed and ileal and jejunal tissues were not capable of generating slow-wave activity. Muscle cells in Sl/Sld muscles were capable of generating Ca$^{2+}$ action potentials, and neural responses were intact due to the apparently normal appearance of IC-DMP. The presence of action potentials in muscle cells and neural responses suggests that these animals, similar to W/WV animals, are capable of motility of some form. SCF mRNA levels are similar in tissues of Sl/Sld and wild-type animals (41), suggesting that similar amounts of SCF might be synthesized in mutant and wild-type tissues. However, membrane-bound SCF at the extracellular surface of cells is missing in Sl/Sld tissues, and this leads to lesions in ICC networks. This observation demonstrates the importance of the membrane-bound form of SCF for proper Kit signaling in GI muscles. The whole organ motor function of this interesting model has not been evaluated.

FATE OF ICC WHEN KIT SIGNALING IS BLOCKED

At present, the fate of ICC in motility disorders is unknown. The question of what happens to ICC when Kit signaling is blocked was investigated in animals treated with neutralizing Kit antibodies (29, 30). After treatment with neutralizing antibody, the distributions of ICC were evaluated in small intestine and colon, and ICC in both organs were greatly reduced in number. Ultrastructural examination revealed no evidence of cell death in regions formerly populated with ICC. This suggested that ICC might redifferentiate rather than die, and further tests failed to reveal evidence of apoptosis. EM confirmed that cells with the ultrastructural characteristics of IC-MY disappeared in animals treated with neutralizing antibodies. As the loss of ICC progressed, cells with residual Kit expression in the myenteric plexus region developed myofilaments and began to express desmin (29). These are features typical of smooth muscle cells, suggesting that when Kit signaling is blocked, cells with an ICC phenotype redifferentiate toward a smooth musclelike phenotype. How far the cells go toward becoming smooth muscle cells is difficult to assess, because, at present, the changes in redifferentiating ICC cannot be followed past the point when Kit expression ceases to be resolved. It is possible that in many of the motility disorders in which ICC are lost, blockade or loss of Kit signaling occurs. This could be the common feature that links otherwise disparate disease conditions to motility dysfunction. Further investigation into the fate of ICC when Kit signaling is blocked and studies to determine whether redifferentiated ICC can be recovered and the ICC phenotype restored are potentially very important directions for research in this field.

DIABETIC GASTROPATHY

Patients with diabetes mellitus for many years frequently develop gastric neuromuscular dysfunction that can cause symptoms from postprandial bloating to recurrent vomiting (15). In an advanced form, the gastropathy can develop into impairments in receptive relaxation and gastric emptying, which, in turn, can lead to poor glycemic control. Generalized autonomic neuropathy is commonly believed to be responsible for diabetic gastropathy, but this hypothesis has not been consistently supported by clinical observations (15). For example, diabetic gastropathy is often accompanied by impairment in electrical pacemaker activity, yet this activity does not require inputs from autonomic or enteric nerves (40). Therefore, it is difficult to understand how a neuropathy could lead to abnormalities in the pacemaker. Due to the important role of ICC in gastric motility, we tested the hypothesis that damage to ICC networks could be involved in the development of diabetic gastropathy using NOD/LtJ (nonobese diabetic) mice (Fig. 1; see Ref. 21).

NOD/LtJ mice spontaneously develop T cell-mediated, autoimmune insulinitis and have been used as a model of human type 1 (insulin dependent) diabetes mellitus (1). Approximately 2 mo after the development of diabetes (defined by glycosuria or blood glucose levels that exceeded 250 mg/dl), gastric emptying was assessed (21). Time-course experiments in nondiabetic mice demonstrated that emptying of a solid meal was completed within 3 h. Diabetic NOD animals emptied less than one-half the meal during the 3-h test period, demonstrating gastroparesis in this experimental model (Fig. 1A). Because gastric peristaltic contractions are initiated by electrical slow waves, we monitored electrical activity in diabetic mice. Electrical activity in the corpus was normal, but most regions of the antrum were electrically quiescent (Fig. 1B). Some cells in antral tissues had slow waves of normal rhythm, and other cells displayed arrhythmic activity. Immunohistochemistry and EM revealed that the abnormal antral electrical activity was accompanied by focal losses of ICC (both IC-MY and IC-IM) from the antrum (Fig. 1, C and D). EM examination of antral tissues failed to reveal signs of apoptosis, necrosis, or infiltration by mononuclear phagocytes. We also detected a microscopic lesion in the relationship between ICC and enteric neurons. These cell types are usually tightly associated with small (<20 nm) intercellular gaps. ICC remaining in the gastric tissue were not closely associated with enteric neurons, and there were large spaces filled with extracellular matrix proteins between ICC and neural processes. Together, these lesions in diabetic mice could lead to impaired enteric neurotransmission, faulty receptive relaxation, loss of slow waves in the distal stomach, weakened antral contractions, and delayed gastric emptying. All of these are symptoms of diabetic gastropathy. Thus these studies have suggested an interesting new hypothesis about the etiology of diabetic gastroparesis. The relevance of our findings in the NOD/LtJ mice to human...
pathology is supported by a recent case study reporting a decrease in ICC in the jejunum of a patient with long-standing type 1 diabetes and gastroparesis (10).

**BOWEL OBSTRUCTION**

Many disorders of motility result in partial or complete obstructions in which nutrients cannot move through the disabled region. One of the responses to obstruction is hypertrophy of the muscle proximal to the site of obstruction. Although the structural changes and expression of neurotransmitters in these regions have been carefully studied in the past, few functional studies have been performed on tissues altered by obstruction. A recent study evaluated the

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**Fig. 1. Effects of diabetes mellitus on gastric emptying of solids (A), electrical slow-wave activity (B), and interstitial cells of Cajal (ICC; C, D) in nonobese diabetic (NOD) mice.**

A: gastric emptying of solid food after 3 h in normal BALB/c, control NOD, and long-term diabetic (>1.5 mo) NOD mice. Data are expressed as %solids emptied. Gastric emptying in long-term diabetic mice was significantly reduced relative to both control groups \( P < 0.02 \), Kruskal-Wallis 1-way ANOVA on ranks; different lower-case letters above bars indicate statistically significant differences between groups \( P < 0.05 \) by all-pairwise multiple comparisons, Student-Newman-Keuls method. B: representative recordings of spontaneous activity in the corpus and antrum of nondiabetic (left) and diabetic (right) NOD mice. In control animals, rhythmic slow waves were recorded throughout the corpus and antrum. In diabetic animals, the majority of cells impaled in the antrum displayed no slow-wave activity. Slow waves in the corpus of diabetic mice were unaffected in most animals. Time scales in corpus and antrum traces are the same and denoted by the black bar (1 min). C: confocal images of Kit-like immunoreactivity in the distal (top) and proximal antrum (bottom) of nondiabetic (left) and diabetic (right) NOD mice. Note the profound reduction in ICC number in the diabetic animals. Scale bars in diabetic images apply to corresponding control images. D: transmission electron micrographs of the circular muscle layers (top) and the myenteric regions (bottom) of the antrums of nondiabetic (left) and diabetic (right) NOD mice. ICC were identified by ultrastructural features (electron-dense nucleus with heterochromatin distributed toward the periphery of the nuclear envelope, electron-dense cytoplasm, numerous mitochondria, membrane caveolae, well-developed rough endoplasmic reticulum, Golgi complexes, and an incomplete basal lamina). In nondiabetic muscles, intramuscular ICC (IC-IM; top left) were closely associated with nerve fibers (*) in nearly every section. In diabetic animals, enteric nerve fibers and bundles were not usually associated with IC-IM in either the circular or longitudinal muscle layers (top right; circular muscle layer is shown). In the myenteric region, the ICC remaining in the diabetic muscles (bottom right) were frequently separated from enteric neurons by wide extracellular spaces (ecm; G, enteroglial cell). Perinuclear cytoplasm was markedly reduced in IC-MY. Scale bars in control images apply to corresponding images from diabetic tissues. Reproduced from Ref. 21 with permission.
changes in electrical activity and responses to nerve stimulation of the small intestinal muscles above and below a site of partial mechanical obstruction (5).

To produce a model of partial obstruction, a loop of murine small intestine was exposed and a small polyethylene clip (6 mm in length, 4 mm interior diameter) was inserted over a segment of intestine 30–50 mm from the ileocecal sphincter. After the clip was installed, the abdomen was closed and the animals were allowed 2 days to 2 wk to recover. No significant changes were noted after 2 days, but within 2 wk, the region proximal to the clip underwent significant distension and hypertrophy (Fig. 2). Electrical activity 1–10 mm above the obstruction was characterized by depolarization of resting potential, reduced or no slow-wave activity, and decreased postjunctional responses to nerve stimulation. The severity in the functional loss decreased with distance above the obstruction, and tissues 75–100 mm proximal to the obstruction were essentially normal. Tissues immediately below the obstruction were also essentially normal.

The loss of electrical activity above the obstruction was consistent with the changes that might occur if ICC networks were damaged. Therefore, immunohistochemistry and EM were performed to assess the status of ICC. IC-MY and IC-DMP were not observed with Kit immunohistochemistry in tissues up to 25 mm above the site of obstruction. Above this region, we observed a gradient in cells with Kit-LI, and at ~100 mm, ICC networks were indistinguishable from the networks in control animals. Below the site of obstruction, cells with Kit-LI also appeared to be normal. EM confirmed the absence of typical ICC in the region of intestine immediately above the site of obstruction. In the spaces typically occupied by ICC were found cells with morphology “intermediate” between smooth mus-
cell and ICC. Cells with typical ICC morphology were found in regions in which ICC networks were observed with Kit immunohistochemistry.

In regions in which ICC networks were decreasing, there was no evidence of cell death, and it was reasoned that the intermediate cells found in these regions might be redifferentiated ICC (as in the case in which Kit signaling was blocked with neutralizing Kit antibodies). If this was true, then it might have been possible to restore ICC networks by removing the chronic obstruction. This was performed in a second laparotomy on a series of animals that had been exposed to the partial obstruction for 14 days. All of these animals had the gross symptoms typically observed in response to the partial obstruction. After recovery for 30 days, electrical parameters and ICC networks were partially restored in the region immediately above the site of the obstruction. Cells with morphological features typical of IC-MY and IC-DMP were observed with EM.

The development of an animal model in which ICC phenotype disappears (morphologically and functionally) and can be recovered is an extremely important advance in beginning to understand the factors that regulate the ICC phenotype. The bowel obstruction model provides the opportunity to study both the temporal (the damage in ICC networks occurs between 2 and 14 days) and spatial (severe lesion 1–10 mm above the obstruction, no apparent defect 100 mm above the obstruction) factors that contribute to loss of ICC. It may be possible to identify the molecular or genetic changes in these tissues that regulate the ICC phenotype in mice, and by comparison with human homologues, it might be possible to determine the factors that control the loss of the ICC phenotype in human patients. Understanding the molecular signals that lead to recovery of the ICC phenotype after removal of the obstruction might provide new therapeutic opportunities for treatment of motility disorders in humans.

INFLAMMATORY BOWEL MODELS

There have been reports of the changes in ICC ultrastructure in the submucosal pacemaker region of the colon in patients with severe ulcerative colitis (24). An animal model of inflammation-induced motor disorders produced by *Trichinella spiralis* infection was recently studied to evaluate the structure of IC-MY networks and changes in motor patterns in the murine small intestine (7). Structural damage in the IC-MY network was noted for 2 wk after infection. This seemed to be concentrated in the connections between IC-MY and smooth muscle cells. The structural changes were associated with aberrant pacemaker activity, including loss of slow waves or abnormally high-frequency slow waves. The motor patterns of the small bowel were also altered from peristaltic contractile activity in control animals to periods of quiescence and both oral and anal propagating contractions during inflammation. Motility and ICC networks recovered to normal activity within 60 days after the infection. The authors concluded that alterations in ICC contribute significantly to the motor disturbances in inflammatory bowel disease.

TRANSFORMATION OF ICC TO MALIGNANT CELLS

Gastrointestinal stromal tumors (GIST) are mesenchymal tumors of the human GI tract that have recently been associated with mutations in Kit (see Ref. 11). The most common mutations are found in exon 11, which encodes the juxtamembrane domain (i.e., the region between the membrane-spanning and tyrosine kinase domains), and cause Kit to be constitutively active. The authors proposed that GISTs may originate from ICC. Transfection of murine lymphoid cells caused malignant transformation, confirming that these mutations of Kit might be capable of transforming ICC into malignant cells. Additional examination of GISTs has also revealed mutations in exon 9 of Kit, which encodes the extracellular domain (12). This mutation also caused constitutive autophosphorylation in the absence of stimulation by SCF. In an extensive analysis of 133 GISTs, 5% were found to have the same mutations in exon 9.

A new compound, STI-571 (Gleevec; Novartis, Basel, Switzerland), is specifically targeted to blocking the tyrosine kinase activity of Kit receptors. STI-571 has been shown to have therapeutic value in some types of leukemia, and, in early clinical tests, it may also be effective in treating GIST (2). Because Kit signaling is required for maintenance of the ICC phenotype, one wonders if long-term inhibitors of Kit, such as STI-571, will also have deleterious effects on ICC in the GI tract. No evidence of trials to test this hypothesis was found in the literature.

GENOMICS APPROACH TO DETERMINING WHAT CONTROLS THE ICC PHENOTYPE

It is very difficult to clarify the cause-and-effect relationship between the loss of ICC and the development of GI motor dysfunction in human patients. Pathologists are left with the task of making cell counts of ICC in tissues removed from patients with advanced symptoms. The possibility of evaluating the status of ICC in full-wall biopsy samples has been considered, but this may be very difficult to accomplish due to lack of patient-specific controls and the focal nature of some lesions to ICC networks that have been noted (see Ref. 21). The diagnosis of damage to ICC networks would be greatly aided by the development of molecular tests that could pick up pathological changes more reliably and at earlier time points.

Although these are early days in our studies of ICC pathology, a picture is beginning to emerge that many disparate motility disorders result in loss of ICC. Admittedly, this could be a result of other pathophysiological events, but one must at least consider the fact that with the known physiological functions of ICC, losing these cells could result in a variety of motor dysfunctions. Many of the disorders in which ICC have been reported to be reduced have symptoms compatible
with the loss of ICC. Now, thanks to the genomics era, we have the ability to make large-scale screenings of genetic changes that occur in tissues undergoing loss of ICC. We can ask the question of whether there are common genetic fingerprints that either produce or result from loss of ICC. Just as animal models have provided unprecedented opportunities to discover the role of ICC in GI motility, pathophysiological models coupled with genomics analyses may offer the opportunity to discover why ICC are vulnerable in apparently disparate motor disorders and how to recover ICC networks in diseased organs.

There are at least three important general benefits that might come from genomic analysis of animal models of ICC loss. If there are common endpoints for the fate of ICC in a variety of motility disorders (e.g., redifferentiation), then it may be possible to determine specific gene profiles that occur in tissues that have suffered ICC loss or in tissues in the process of losing ICC. From this information, it may be possible to design specialized tests that can reliably evaluate the status of ICC networks from biopsy material. Second, if there are common serial changes in gene expression during the onset of a variety of motility disorders, it may be possible to halt the redifferentiation of ICC to minimize the damage and loss of function. Third, understanding the signals that cause reemergence of ICC networks in GI tissues might provide interesting new therapeutic approaches to treating these disorders in patients with advanced motor symptoms.

SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

It seems evident that further evaluations of the role of ICC in GI motor pathology is a promising new direction for research in gastroenterology. More immunohistochemical work must be performed on tissues from patients with motility disorders for us to fully appreciate the degree to which ICC are involved in a variety of disorders. Hopefully, GI pathologists will be able to develop standards for ICC populations in various parts of the normal GI tract and then develop means to rigorously count cells in diseased tissues. As we begin to understand more about the genetic changes that occur in animal models with loss of ICC, we can begin to compare the murine genes that are up- or downregulated to human homologs in diseased human tissues. As discussed above, this may help refine clinical tests for ICC loss. The ideal result of all this work is to discover prophylactic approaches to halt loss of ICC in patients with disorders known to result in ICC-dependent motor pathology (e.g., diabetes) and to restore functional populations of ICC in patients that have already suffered pathological loss of ICC.

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