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Invited Review

Interstitial cells of Cajal in gastrointestinal inflammatory diseases

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Abstract

The gastrointestinal (GI) tract is a vital organ that digests food, absorbs nutrients, and excretes waste. Normal GI motility is the basis for these functions. The interstitial cells of Cajal (ICC) in the GI muscularis layer promote GI motility together with the enteric nervous system and smooth muscle cells. Since GI motility results from complex coordination of these heterogeneous cells, failure of any one of them can lead to GI dysmotility. Knowledge about ICC in physiological conditions has accumulated in recent decades, while the pathophysiology of ICC in GI inflammatory diseases, such as inflammatory bowel disease, is not well understood. In this review, we summarize the previous studies about the pathophysiological changes of ICC in inflammatory diseases and discuss the inflammatory mediators that induce ICC dysfunction.

Key words: interstitial cells of Cajal, gastrointestinal motility, inflammation

Introduction

Santiago Ramon y Cajal, a Spanish neuroscientist and pathologist, observed the small intestines of rabbits and discovered the presence of unique cells near the autonomic nerve endings (1). These cells were later termed "interstitial cells of Cajal (ICC)". After the first discovery of ICC, their physiological functions remained unidentified for many years, as methods to identify ICC did not exist (other than morphologic techniques). The first breakthrough in this field was the discovery of a molecular marker for ICC. Maeda et al. reported that the administration of a neutralization antibody against c-Kit, which was produced by Nishikawa et al., disrupted gastrointestinal (GI) motility (2, 3). Moreover, Torihashi et al. reported that the blockade of c-Kit by an antibody resulted in the disruption of ICC networks and electrical rhythmicity in the small intestine (4). Ward

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et al. and Huizinga et al. reported that animal models with a gene mutation in c-Kit had a reduced number of ICC and displayed abnormal intestinal electrical rhythmicity (5, 6). These findings accelerated the understanding of the ICC function. Now, we know that the functions of ICC vary among the different ICC subtypes. For example, ICC in the myenteric plexus region (ICC-MY) are a pacemaker of GI motility. ICC-MY shows spontaneous and rhythmic electrical activity, known as slow wave activity, which propagates into smooth muscle cells causing the contraction/relaxation of smooth muscle cells. In addition, ICC in the deep muscular plexus (ICC-DMP) are involved in the coordination of the segmentation motor pattern (7). Furthermore, intramuscular ICC (ICC-IM) are closely associated with enteric motor nerve terminals (8), and are considered to play an important role in neurotransmission signaling from neurons to smooth muscle cells (9, 10). Thus, ICC are an essential component in the generation of normal GI motility.

GI dysmotility is observed in inflammatory diseases such as inflammatory bowel disease (IBD) and sepsis. The pathophysiological changes and mechanisms underlying the dysfunction of smooth muscle cells and the enteric nervous system are relatively well explored for these diseases (11, 12). In contrast, the detailed mechanisms of ICC dysfunction are not well understood, although functional and morphological abnormalities of ICC have been reported in humans and experimental animals with inflammatory diseases. Elucidating the inflammatory changes in ICC would provide novel treatments for GI dysmotility targeting ICC.

This review outlines the pathophysiological changes in ICC during inflammatory diseases and discusses the inflammatory mediators that induce ICC dysfunction.

Pathophysiological Features of ICC in Inflammatory Disease

Inflammatory bowel disease

IBD is a group of inflammatory conditions in the small intestine and colon. The most common types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Common symptoms of CD and UC are abdominal pain, diarrhea, and rectal bleeding. Patients with IBD display abnormal motility of the small intestine (13–16) and colon (17–19). Figure 1 shows schematic overview of the pathophysiological changes of ICC in humans and animal models with IBD. Patients with UC show a reduced number of c-Kit$^+$ ICC-MY and ICC-IM in the colon (20) (Fig. 1). Rumessen et al. reported that the ultrastructure of ICC at the colonic submuscular border showed abnormalities in comparison to smooth muscle cells, indicating the involvement of ICC for GI dysmotility in UC (21). They also reported that colonic ICC-MY in patient with UC also showed ultrastructural abnormalities (22). Patients with CD also showed a reduced number of c-Kit$^+$ ICC and ultrastructural destruction of small intestinal ICC-MY, such as swelling of mitochondria, decreased electron density, autophagosomes, and partial depletion of the cytoplasm (23, 24). On the other hand, there are controversial reports. Villancci et al. reported that the number of colonic c-Kit$^+$ ICC-MY was not changed in patients with UC in comparison to controls (25). These differences may depend on the duration of symptoms, disease activity, reliability of staining methods, and genetic/environmental background of individuals. Horses with IBD also displayed a reduced number of c-Kit$^+$ ICC-MY in the ileum (26). Huizinga and Sarna's groups, for the first time, reported that ICC in an experimental dog model with colitis induced by mucosal exposure to ethanol and acetic acid showed dysfunction and an abnormal ultrastructure (27). They found a reduced amplitude of spontaneous phasic contractions, which was associated with the disruption of slow waves. They concluded that the amplitude and duration of slow waves may be related to ICC injury. We investigated an experimental rat. We also reported that colitis induced by 2,4,6-trinitrobenzene sulfonic acid and reported that the c-Kit$^+$ ICC-MY network was disrupted in accordance with the infiltration of macrophages and neutrophils into the inflamed muscularis layer (28). An

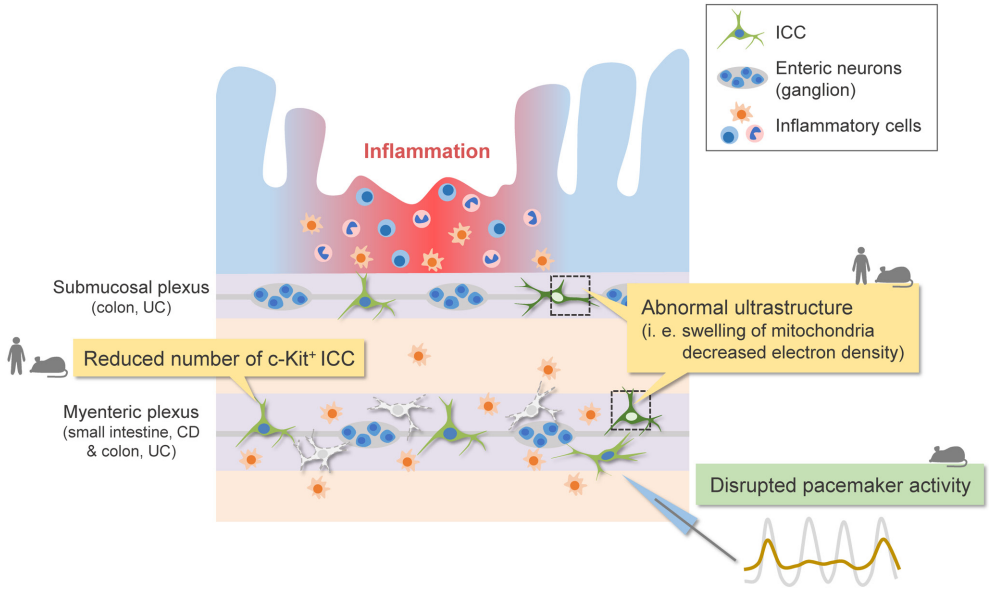

Fig. 1. Interstitial cells of Cajal in inflammatory bowel disease.
Schematic overview of the pathophysiological changes of interstitial cells of Cajal (ICC) in humans and experimental animals with inflammatory bowel disease (IBD). The ultrastructure of ICC at the colonic submuscular plexus and the small intestinal myenteric plexus (ICC-MY) showed abnormalities in patients with ulcerative colitis (UC) and Crohn's disease (CD), respectively. Experimental animals with IBD also showed ultrastructural destruction of colonic ICC-MY. Patients with CD and UC showed a reduced number of small intestinal and colonic c-Kit$^+$ ICC-MY, respectively. Experimental animals with IBD showed a reduced number of colonic c-Kit$^+$ ICC-MY and disrupted pacemaker activity. The human and rodent symbols in the scheme mean that the pathophysiological changes of ICC have been reported in humans and experimental animals, respectively.

ultrastructural analysis also revealed a decrease of ICC in this animal model. The muscularis externa from the inflamed colon showed the impairment of tetrodotoxin-resistant spontaneous contraction, suggesting ICC dysfunction. Interestingly, inflammatory mediators, such as TNF-α, IL-1β, and IL-6, were individually upregulated not only in the mucosal layer but also in the smooth muscle layer, indicating the probable existence of its own local muscularis immune system and suggesting a possible connection to inflammation-associated motility disorders, including ICC dysfunction. The reduced number and dysfunction of ICC was also suggested in a dextran sodium sulfate (DSS)-induced colitis model (29) and the IL-10 knockout mouse model of IBD (30).

Hirschsprung's disease and Hirschsprung-associated enterocolitis

Hirschsprung's disease (HD), which affects 1 in 5,000 human births, is the most prevalent congenital GI motility disorder (31). The most important gene associated with HD is *RET*, other associated genes include *EDNRB* and *NRG1*. The main symptom is colonic obstruction due to the absence of enteric neurons in the distal gut. Hirschsprung-associated enterocolitis (HAEC) is a complication of HD; its clinical manifestations include diarrhea, bloody stool, and abdominal pain. Figure 2 shows schematic overview of the pathophysiological changes of ICC in humans and animal models with HD. Many studies have reported the loss of colonic c-Kit$^+$ ICC-MY and ICC-IM in patients with HD (32). Ultrastructural injury of the ICC cytoplasm and processes, including swollen mitochondria, and dilated rough endoplasmic reticulum has also been reported (33). On the other hand, other studies found little the difference in ICC between HD patients and healthy subjects (34, 35). Thus, it is difficult to determine whether the loss of ICC is a cause or consequence of the disease process (32).

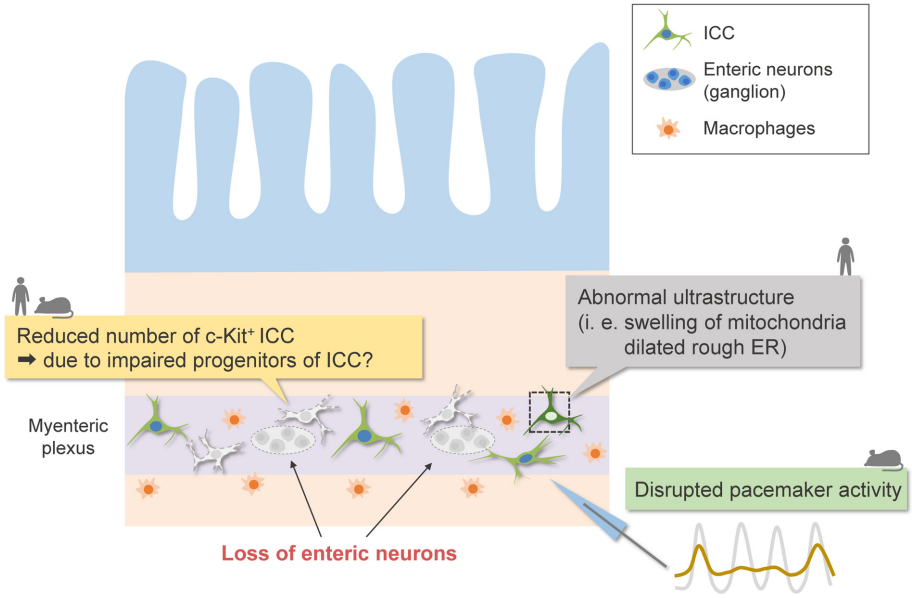

Fig. 2. Interstitial cells of Cajal in Hirschsprung's disease.
Schematic overview of the pathophysiological changes of interstitial cells of Cajal (ICC) in humans and experimental animals with Hirschsprung's disease (HD). In patients with HD, the ultrastructure of ICC at the colonic myenteric plexus (ICC-MY) showed abnormalities, including the swelling of mitochondria, and dilated rough endoplasmic reticulum (ER). Patients with HD and an animal model of HD showed a reduced number of colonic c-Kit$^+$ ICC-MY. A study suggested that progenitors of ICC may be injured in patients with HD, which resulted in disrupted c-Kit$^+$ ICC networks. Experimental animals with HD showed disrupted pacemaker activity of ICC. The human and rodent symbols in the scheme mean that the pathophysiological changes of ICC have been reported in humans and experimental animals, respectively.

In patients with HD, progenitor cells of ICC were present in the proximal segment of the obstructed colon, while mature and committed progenitor ICC dramatically decreased (33). In HD patients, mature ICC may change their phenotype to an earlier stage of progenitor ICC. *EDNRB*-deficient animal models showed marked distention of the proximal small intestine due to long-segment aganglionosis, with histological and pathophysiological features that were similar to those in HD patients (36–40). We found the loss of c-Kit$^+$ ICC-MY in the dilated ileum of *EDNRB* mutant rats (41, 42). The number of resident macrophages was dramatically increased in the ileal muscularis externa. In addition, CD14, a binding protein with LBP/TLR4 complex, was upregulated in those resident macrophages, suggesting that inflammatory cytokines released from activated macrophages may injure ICC. Similar phenomena were found in a model of small intestinal obstruction in rats (43). Furthermore, Chen et al. reported that TNF-α from muscularis-resident macrophages inhibited the c-Kit expression in colonic ICC and impaired the pacemaker function by activating the NF-κB/miR-221 pathway in HAEC (44).

Sepsis

Sepsis is the most common cause of in-hospital death worldwide. GI motility disorders are a common symptom of sepsis, which can further exacerbate sepsis by causing intestinal bacterial overgrowth and bacterial translocation (45). To the best of our knowledge, there have been no reports about pathophysiological changes of ICC in patients with sepsis. On the other hand, there several reports have shown the loss of ICC in animal models of sepsis, which are produced by the injection of lipopolysaccharide (LPS) and cecal puncture and ligation. Figure 3 shows schematic overview of the pathophysiological changes of ICC in animal models

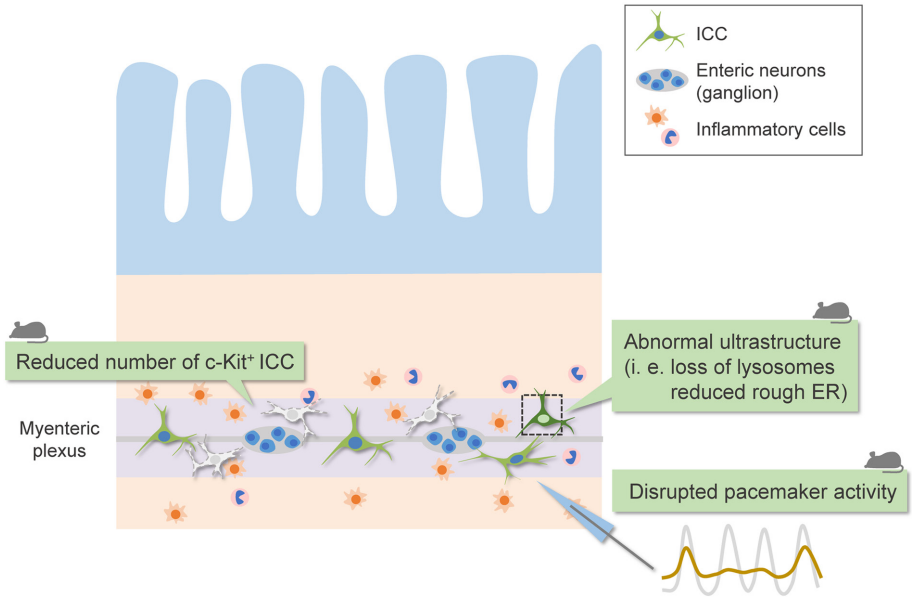

Fig. 3. Interstitial cells of Cajal in sepsis.
Schematic overview of the pathophysiological changes of interstitial cells of Cajal (ICC) in rodent models of sepsis. The ultrastructure of ICC at the small intestinal myenteric plexus (ICC-MY) showed abnormalities, including the loss of lysosomes and reduced rough endoplasmic reticulum (ER). An immunohistological analysis revealed a reduced number of c-Kit$^+$ ICC-MY in this animal model. A disruption of slow wave activity has been reported in this model animal.

with sepsis. Spontaneous rhythmic contraction and carbachol-induced sustained contractility were inhibited in these animal models of sepsis (46, 47). Animal models of sepsis showed a reduced number of c-Kit$^+$ ICC, disrupted slow waves, and their ICC showed an abnormal ultrastructure, including loss of lysosomes and reduced rough endoplasmic reticulum (46, 48, 49). In a rat model of sepsis, resident and monocyte-derived macrophages, and neutrophils in intestinal muscularis externa were shown to produce inflammatory mediators, including IL-1β, TNF-α, IL-6, MCP-1, prostaglandins, and nitric oxide (50). In the small intestine, prostaglandin E_2 (PGE_2), which is produced in resident macrophages by exposure to LPS, can directly activate EP2 and EP4 receptors in smooth muscle cells to accumulate cAMP, then inhibit the contractile ability of the cells at the early stage of peritonitis. At a later stage of peritonitis, PGE_2 activates the same receptors in macrophages in an autocrine manner, which in turn upregulates inducible nitric oxide synthase (iNOS) to produce nitric oxide (47). ICC expresses soluble guanylate cyclase, a nitric oxide receptor (51, 52). A previous study reported that nitric oxide suppresses the pacemaker activity of ICC via cGMP (53). Thus, pacemaker dysfunction of ICC in sepsis may be caused by nitric oxide. Ueshima et al. reported that iNOS inhibitor, but not neuronal NOS (nNOS) inhibitor, inhibited the reduction of c-Kit$^+$ ICC in mice with LPS-induced sepsis. Moreover, inactivation of macrophages with gadolinium prevented the reduction of the number of ICC, which indicates that nitric oxide from resident microphages via iNOS impairs ICC.

Surgical intestinal manipulation-induced GI dysmotility

Intestinal manipulation (IM), resection, and anastomosis during abdominal surgery often induce postoperative GI dysmotility due to inflammation in the muscularis externa of the GI tract.

Postoperative ileus (POI), a common complication of abdominal surgery, is a state of absence or reduced peristalsis, which causes abdominal distension, vomiting, and nausea. POI leads to prolonged hospitalization

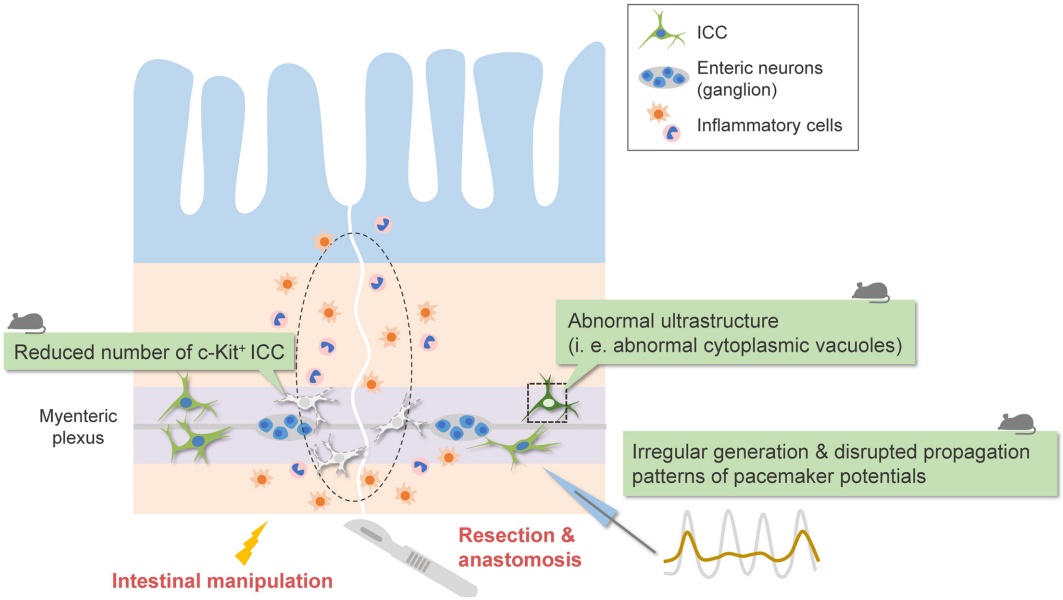

Fig. 4. Interstitial cells of Cajal after surgical manipulation.
Schematic overview of the pathophysiological changes of interstitial cells of Cajal (ICC) in rodents after surgical manipulation, including resection and anastomosis. The ultrastructure of ICC at the small intestinal myenteric plexus (ICC-MY) showed abnormalities including abnormal cytoplasmic vacuoles. An immunohistological analysis revealed a reduced number of c-Kit$^+$ ICC-MY in this animal model. A disruption of slow wave activity and propagation of pacemaker potentials has been reported in this model animal.

and increased treatment costs (54). However, there is currently no effective clinical therapy. The etiology of POI is considered to be multifactorial, including inflammatory cell activation, autonomic neuropathy, opioid receptor activation by narcotic analgesics, regulation of GI hormone activity, and electrolyte abnormalities (55). The involvement of inflammatory responses has been studied in animal models of POI induced by IM. The primary immune cells that activate inflammation in POI are muscularis-resident and monocyte-derived macrophages. In response to IM, muscularis-resident macrophages produce cytokines and chemokines, which promote the infiltration of monocyte-derived macrophages and neutrophils. Inflammatory cells also produce prostaglandins and nitric oxide, which cause disruption of smooth muscle contractility (56, 57). It is also reported that mast cells and T helper type 1 memory cells are involved in the development of POI (58, 59). In addition to smooth muscle dysfunction, it was reported that the prolonged impact of IM on the enteric nervous system contributes to POI (60). We reported ICC dysfunction in a mouse model of POI for the first time. Figure 4 shows schematic overview of the pathophysiological changes of ICC in animal models with surgical intestinal manipulation-induced GI dysmotility. In this model, the c-Kit$^+$ ICC network was disrupted, and the number of ICC was dramatically reduced at 24 h after IM (Fig. 4). The propagation of pacemaker potentials was also examined with microelectrode arrays, and irregular generation and reentry-like propagation patterns of pacemaker potentials were observed (61). Moreover, the prophylactic administration of an iNOS inhibitor significantly suppressed the IM-induced decrease in the number of ICC without affecting inflammation. This result suggests that nitric oxide may be a factor responsible for ICC failure in POI. On the other hand, it was reported that IL-6 and miR-19a disrupted the ICC function in a model of POI (62, 63); however, further studies are needed to determine whether the changes observed in animal models of POI occur in human patients.

Intestinal surgical resection and anastomosis also disrupt the ICC function (64). In the smooth muscle layer near the site of resection, phasic contractions and slow waves were dramatically diminished with a loss

of c-Kit$^+$ ICC at five hours after the operation. In addition, an ultrastructural analysis revealed a loss of cells with ultrastructural features of ICC at the level of the myenteric plexus and deep muscular plexus. This surgical damage to ICC was weakened in iNOS deficient mice, suggesting that nitric oxide produced by iNOS damaged ICC (65).

Mediators to Induce ICC Injury: Insight from *in vitro* Experiments

As described above, animal experiments suggest that pro-inflammatory cytokines and nitric oxide may be mediators that impair ICC. However, inflammation is a complex process, and it is difficult to identify responsible mediators by *in vivo* experiments alone. *In vitro* experiments have also been conducted to elucidate the mechanisms by which ICC is impaired in inflammatory disease.

We examined the effect of inflammatory cytokines on ICC using cultured small cell clusters obtained from jejunal muscularis (66). The cell clusters contain a group of cells that make up the GI muscularis externa, including smooth muscle cells, neurons, ICC, and macrophages. In our study, the treatment of clusters with IFN-γ and LPS induced disrupted Ca^{2+}-oscillation and the loss of ICC with the upregulation of proinflammatory cytokines such as TNF-α, IL-1β and IL-6. However, individual treatment with these inflammatory cytokines did not affect Ca^{2+}-oscillation of ICC. These results suggest that ICC may be less sensitive to cytokines. On the other hand, several reports showed that treatment of cultured ICC with TNF-α resulted in a decreased number of ICC (44, 67–70).

In our *in vitro* experiment, we suggested that nitric oxide and its related oxidative stress induced pacemaker dysfunction of ICC (66). In support of this, iNOS inhibitor ameliorated ICC failure in an animal model of inflammatory disease (49, 61, 65). On the other hand, it was also reported that nitric oxide is essential for the maintenance of ICC homeostasis (71). These findings suggest that low concentrations of nitric oxide produced by nNOS have a beneficial effect on ICC, whereas excessive amounts of nitric oxide derived from iNOS-positive activated macrophages have a negative effect on ICC. If nitric oxide is to be used as a therapeutic target molecule for ICC injury during inflammatory disease, it may be necessary to control nitric oxide levels.

We should note that these results were obtained from experiments with mixed cultures of cells from the intestinal muscularis externa and not isolated cultures of ICC. It is difficult to prove the direct effects of inflammatory mediators on ICC because these mediators act on many types of cells and promote the production of many kinds of mediators. To elucidate the pathophysiology of ICC in inflammatory diseases, it is necessary to generate animals with ICC-specific deficiencies of receptors for inflammatory mediators and to establish a method to isolate cultured ICC.

Phenotypic Changes in ICC

Although c-Kit is the most common marker of ICC, it is important to note that a decrease in c-Kit$^+$ cells does not necessarily mean the loss of ICC, because it has been suggested that there are ICC with no c-Kit expression (or a low level) and the loss of the pacemaker function (72, 73). Several experiments have shown that ICC may rapidly change their phenotypes in response to inflammatory conditions. In animals that have undergone intestinal resection and anastomosis, the number of c-Kit$^+$ ICC decreases at 5 h after the operation but recovers at 24 h (64). Functional and morphological recovery was accelerated by treatment with an iNOS inhibitor. Furthermore, in animal models of POI, c-Kit$^+$ ICC decreased at 24 h after IM but recovered at 48 h after IM, when inflammation had subsided (61). Further studies are needed to clarify which of the inflamma-

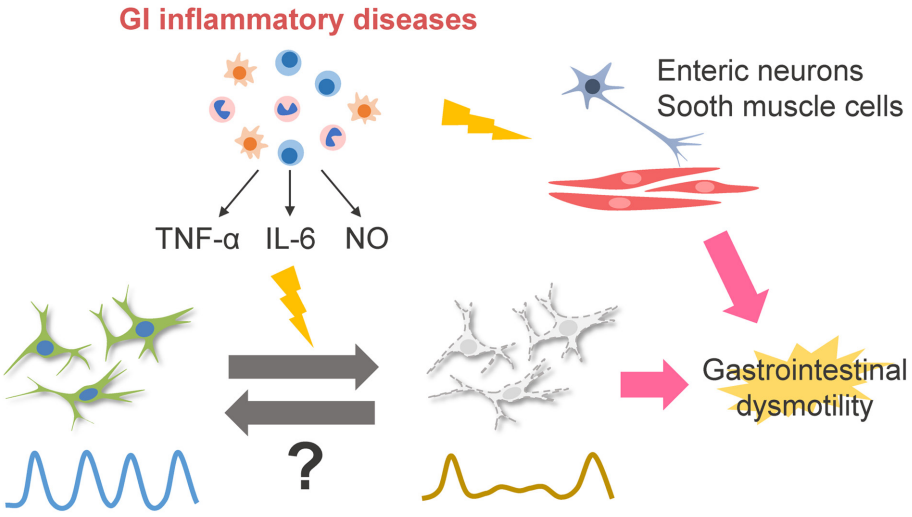

Fig. 5. Summary of pathophysiological changes of interstitial cells of Cajal in gastrointestinal inflammatory diseases.
Schematic overview of the pathophysiological changes of interstitial cells of Cajal (ICC) in gastrointestinal (GI) inflammatory diseases. Ultrastructural abnormalities of ICC, reduced number of c-Kit^{+} ICC, and pacemaker dysfunction of ICC were seen in these diseases. TNF-α, IL-6, and nitric oxide (NO) may be responsible for the pathophysiological changes of ICC seen in inflammation. Several experiments have shown that ICC may rapidly change their phenotypes in response to inflammatory conditions. It is not known whether ICC can redifferentiate into the pacemaker phenotype.

tory mediators causes the phenotypic change in ICC and what signals are associated with the redifferentiation of phenotypically changed ICC.

Summary

Figure 5 summarized the pathophysiological changes of ICC in GI inflammatory diseases. In inflammatory GI diseases, the number of ICC was decreased, and ICC showed an abnormal ultrastructure, and impaired pacemaker function, which strongly suggests that the GI dysmotility in these diseases is partly due to ICC injury. In vitro experiments have identified TNF-α, IL-6, and NO as factors that impair ICC. Although the pathogenesis of inflammatory GI diseases differs, these inflammatory mediators are responsible for causing the pathological changes in ICC. Inhibition of these signals is expected to lead to the development of therapeutic agents targeting ICC he treatment of GI dysmotility in patients with inflammatory diseases.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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