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Editorial

Does Decreased c-KIT Expression in Myenteric Interstitial Cells of Cajal Cause Decreased Spontaneous Contraction in Murine Proximal Colon?

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Article: Alterations of colonic contractility in an interleukin-10 knockout mouse model of inflammatory bowel disease
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In the current issue of the *Journal of Neurogastroenterology and Motility*, an article entitled "Alterations of colonic contractility in an IL-10 knockout mouse model of inflammatory bowel disease" demonstrated that interleukin-10 (IL-10) knockout mice exhibited altered colonic contractility and damaged interstitial cells of Cajal (ICC) networks. It also exhibited a decreased cholinergic response, caused by lowered expression of muscarinic receptors in the proximal colon and decreased neuronal nitric oxide synthase expression in both proximal and distal colon.[1]

For the past decades, more than 60 different animal models have been applied to study inflammatory bowel disease (IBD), which can be divided into chemically induced, cell-transfer, congenital mutant and genetically engineered models.[2] Genetic techniques such as inducible knockout and knockin systems make it possible to characterize the mechanisms that are dysregulated in IBD.[2] Up to date, most IBD studies have focused on mucosal inflammation and healing or related pathomechanisms.[3] The lesions in most IBD models are limited to the mucosa or submucosa area and the observed lesions resemble those found in colitis; as such, no existing models satisfactorily recapitulate the human disease.[4] The animal model used in this study, however, showed inflammatory cell infiltration in all colonic layers (including the muscular layer) and exhibited lesions similar to those found in Crohn's disease.[1] This paper does take a novel approach by studying changes in smooth muscle and motility in an immunodeficiency model. The findings from this paper suggest that IL-10

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immunodeficient mice might be an attractive model for researching IBD including Crohn's disease.[1,5] In addition, this study's investigation of the function and expression level of muscarinic type 2 (M_2) receptors is worthwhile in that the M_2 receptors may play a greater role in the inflamed colon.[6,7] These considerations make the article a strong one, and its conclusions can serve as a stepping stone to investigate several unanswered problems in gastrointestinal (GI) smooth muscle biology as follows.

One such problem is the causality of the relationship between loss of c-KIT expression in specific ICC such as myenteric ICC (ICC-MY) or ICC associated with the submuscular plexus (ICC-SMP) and loss of spontaneous contraction in the proximal colon of mouse. Activation of *Kit* signaling is necessary for ICC development[8] and to date, antibodies against c-KIT have been used as the only reliable antigenic marker for labeling ICCs in murine and human tissue. However, it was recently reported that discrepancies between ANO1 and c-KIT staining were found in both wild-type and W/W^V colon, especially in ICC-SMP.[9] In W/W^V mice, c-KIT expression was reduced by 50% but ANO1 staining showed a normal network with rhythmic calcium transients similar to those in wild-type ICC-SMP.[9] The motor pattern observed in W/W^V colon was consistent with the pattern expected for ICC-SMP.[9] For these reasons, ANO1 is a more appropriate marker than c-KIT to investigate changes into ICC population.[9] Since it has also been shown that colonic slow wave generation is regulated by ICC-SMP,[9-11] it will be necessary to analyze this cell population in addition to ICC-MY. According to the previous report, spontaneous contraction in murine colon can be classified into 2 types by frequency and amplitude.[10,11] In mouse colon, ICC-SMP is probably responsible for high frequency low amplitude (HFLA) contraction during circular contraction. In contrast, low frequency high amplitude (LFHA) seems to originate from ICC-IM, ICC-MY or through a different mechanism.[10,11] In this paper, the spontaneous contraction in the colon of IL-10 knockout exhibits HFLA but not LFHA. This implies that the colon of IL-10 knockout in this study might conserve ICC-SMP while losing ICC-MY.

The article also allows smooth muscle biologists to begin clarifying which of the muscarinic receptors contributes more to contractility. Is type 2 more important than type 3 in the IBD mouse colon?

Despite abundant expression of the M_2 receptors in the GI tract,[12] M_3 receptors seem to have a more important role in smooth muscle contraction of the ileum and colon.[13-15] However, it has been reported that the number and sensitivity of the muscarinic receptors can be altered[14,15] and that M_2 receptors may play a greater role in some situations such as in the inflamed rat and canine colon.[6,7] Thus, inflammation seems to modulate M_2 receptor function[16] and to be associated with a shift in M_2 receptor potency, due chiefly to a decrease in receptor density.[7] In this paper, the decreased contractile response to carbachol in IL-10 knockout mice with the reduced expression of M_2 receptor in the inflamed proximal colon is different from some other literature.[6,7] To clarify this difference, further study on M_3 receptor is necessary.

Finally, concern can be raised about the electrical field stimulation (EFS) experiments reported in this article. Excitatory junctional potentials (EJPs) are mediated by acetylcholine followed by typical 2-component inhibitory junctional potentials (IJPs), fast hyperpolarization (fast IJP), and a slow hyperpolarization (slow IJP) which are driven by the release of nitric oxide, purine neurotransmitters, and peptides, such as vasoactive intestinal polypeptide (VIP) and pituitary adenylatecyclase-activating polypeptide (PACAP).[17] Especially, it has been reported that these neuropeptides can be released when EFS is applied at more than 10 Hz.[17] Fast IJP is reduced or blocked by apamin and is considered as purinergic responses in GI muscles.[18] The P2Y1 receptor has an important role in regulating this phenomenon, through purines such as ATP or β-NAD.[19] The second part of the inhibitory response is nitrergic, as it blocks slow IJP via inhibitors of neuronal nitric oxide synthase.[17,20]

Cholinergic EJP and nitrergic IJP mechanisms can be the main events of the EFS results in this paper, however it might be driven by other neurotransmitters or neuropeptides. In order to explain these points, excitatory and inihibitory peptidergic and purinergic responses using EFS should be studied together.

In conclusion, to identify the exact mechanism of colonic contractility changes in IL-10 knockout, more immunohistochemical and electrophysiological evidence is needed regarding the different types of ICC. The authors also need to show whether there is a change in M_3 receptors and in excitatory and inihibitory peptidergic and purinergic responses related to EFS.

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