主論文の要旨

Acceleration of Ileal Pacemaker Activity in Mice Lacking Interleukin 10

（インターロイキン 10 欠損マウスにおける回腸ペースメーカー活動の加速）

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Introduction:

Interstitial cells of Cajal (ICC) play a major role in gut motility by coordinating the electric activity of cellular members as well as generating pacemaker potentials. ICC cells are interposed between enteric neurons and smooth muscle cells in gastrointestinal muscles. Endogenous interleukin 10 (IL10) is a central regulator of the mucosal immune response as IL-10 deficient mice (IL-10$^{-/-}$) develop chronic inflammatory bowel disease (IBD). Epidemiological studies indicate the genetic association between irritable bowel syndrome (IBS) and IBD, including genetic mutations related with IL-10. Many studies focused on inflamed tissues specially colon regarding inflammatory signals in IBD whether histological changes, including a reduction of ICC, are associated with any immune cells, while little is known about functional modulations in other parts of the gut specially ileum which is apparently unaffected. We hypothesize that basal electric activity, including in ICC, may be accelerated in IL-10$^{-/-}$ mice in ileal musculature, thus spatiotemporal electrical activity was examined and compared with wild-type (WT) mice (C57BL/6J).

Methods:

Ileal musculatures containing the myenteric plexus and ICC were isolated from wild-type (WT) and IL-10$^{-/-}$ mice (8-10 weeks old). A microelectrode array (MEA) system was employed to simultaneously measure 8 × 8 field potentials over a ~1 mm^2 area to evaluate the spatial electrical activity of the ileal musculatures in mice. Digital band-pass filter (DBF), power spectrum and auto-correlation analyses (Fig.1) were performed on field potentials array data using commercial add-in software (Kyowa Electronic Instruments, Tokyo, Japan). Two-dimensional field potential images were constructed by using the MATLAB software package. Nifedipine and tetrodotoxin (TTX) were applied to predominantly evaluate ICC electric activity. Histological changes were also assessed by immuno histochemistry.

Result:

Potential mapping revealed that spontaneous electric activity was synchronized throughout the recording area in ileal musculature preparations of both WT mice and IL-10$^{-/-}$ mice, but rapid propagation was observed in the latter (Fig.2). The spectral power in the frequency range of 9.4 to 30.0 cpm ($Pw_{9.4\text{-}30.0}$) did not differ between these preparations, but the oscillation frequency estimated using auto-correlation analysis was significantly higher in IL-10$^{-/-}$ mice than in WT mice (22.16±4.10 vs 15.72±1.61 cpm). In immunohistochemistry, no significant changes were observed in ICC, macrophages and enteric neurons in the ileum of WT and IL-10$^{-/-}$ mice.

Discussion:

The electrical slow wave activity determines the characteristic frequency of phasic contractions of the intestine, direction and velocity of propagation of peristaltic activity. In the present study we observed a smaller number of potential mapping images (Fig.3) for propagating electric waves in IL-

$10^{-/-}$ mice than WT. Furthermore, in the power spectrum, the peak of the sum of all 64 recording channels, shifted significantly toward a high frequency direction (Fig.4) in IL-$10^{-/-}$ mice and in each channel, auto-correlation of recorded field potential reveals a shorter duration between the center and adjacent peaks (Fig.5) in IL-$10^{-/-}$ mice than WT. These results suggest that ICC pacemaker activity makes a major contribution to the difference of electric activity in IL-10-deficient mice. In IL-$10^{-/-}$ mice, the defect of intestinal permeability occurs prior to mucosal inflammation due to a dysregulated immune response to normal enteric microflora. It is reported that IL-$10^{-/-}$ mice show an increase in ileal and colonic permeability at two weeks of age in the absence of any histological injury. Colonic permeability remains elevated as inflammation processes, while ileal permeability is normalized by 6 weeks of age. This correlate well with our present experiment as in immune histochemistry, we found the distribution of ICC and macrophages are essentially the same in IL-$10^{-/-}$ and WT mice (~10 weeks old) (Fig.6), but the electric activity is hyper in the small intestinal tissue (ileal tissue) of IL-$10^{-/-}$ mice, in terms of oscillation frequency. Considering all the above observations, the present study reveals a significant difference between WT and IL-$10^{-/-}$ mice, suggesting that this could be a helpful tool to detect abnormalities of gut motility prior to the onset of any histological change in the gastrointestinal (GI) tract. Thus we can conclude a possible inter linkage relationship between IBS and IBD which commonly share a clinical course of psychiatric stress-mediated impairment. In addition recent epidemiological studies suggest the genetic association between IBS and IBD. IBS is significantly associated with several genes related with bowel inflamation like TNF superfamily 15 (TNSF15), IL-23 receptor, positive regulatory domain I-binding factor, and immunity-related GTPase family M protein, and Toll-like receptor 9. Thus, it is considered that at least a part of patients diagnosed as IBS and IBD may have the same genetic cuase with different phenotypes, such as functional and organic disorders in the gut, respectively.

Conclusion:

The study provides evidence for accelerated pacemaker activity in the ileum of IL-10-deficient mice, not accompanied by any significant histological changes, which could be accounted, as an example, by a genetic cross-link between IBD and IBS.