School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

The role of Allergin-1 in intestinal barrier function

（腸管上皮バリア機能における Allergin-1 の役割）

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Abstract

The intestinal barrier contains several layers, including the mucosal barrier, epithelial barrier, and immunological barrier. The mucosal layer contains mucus and several components derived from either host or intestinal microbiota. Many studies have shown that microbial metabolites can promote anti-inflammatory response and shape the immunological barrier function, nevertheless, microbial derivatives such as LPS can also be harmful to epithelium barrier or even cause intestinal inflammation. Intestinal inflammation is initiated by immune cells, which activated by invading pathogens. Intestinal inflammation can not only clearance the invading microbes but also induce the epithelial layer repairment, however, over or under inflammation can both increase the tissue damage or pathogen invasion and cause further severe diseases. Thus, in the intestinal barrier function, microbiota, physical barrier, and immune regulation work as an interdependent triangle to maintain the intestinal homeostasis. Among this interaction, how an immune receptor can affect the barrier homeostasis is poorly understood. Allergin-1, an inhibitory immune receptor, which was reported by our group previously can negatively regulate several myeloid cells activation in skin and lung diseases, however, Allergin-1 functions in intestinal regulation have not yet been elucidated. Therefore, in this study, I aim to analyze whether Allergin-1 in immune cells can regulate or maintain the intestinal barrier function and further elucidate whether the microbiota is involved in this regulation.

To investigate the Allergin-1 function in intestinal homeostasis, I first analyzed the intestinal permeability of WT and Allergin-1 deficient mice by oral administration of FITC-Dextran and measure the FITC-Dextran concentration after 4 hours. I found that KO mice showed a

significant increase in intestinal permeability, which indicated the decrease of barrier function in the KO mice. To further analyze whether the barrier function deficient is dependent on the microbiota composition, I analyzed the intestinal permeability of WT and KO mice before and after broad-spectrum antibiotics complex treatment and found that depletion of microbial can rescue the KO mice barrier deficient. This result indicated the possibility that Allergin-1 KO mice may develop dysbiosis, which leads to barrier dysfunction. To analysis whether Allergin-1 KO mice have dysbiosis, I collected the feces from both WT and KO mice, and subject to the 16S rRNA sequencing for the microbial composition. As a result, Allergin-1 KO mice showed a significant increment of several bacteria strains, which indicate that Allerin-1 KO mice have dysbiosis, which leads to the defect of barrier function. I further analyzed the epithelial and immunological barrier function of Allergin-1 KO mice by analyzing the colon tissue RNA expression. I first compared the epithelial cell proteins gene expressions, including tight junction proteins, occludin, ZO-1, and claudins, antimicrobial peptides and mucins. I found that there was no significance between WT and KO mice, in tight junction proteins gene expression, but mucin-1 showed significantly decreased in the KO mice. Which indicated a decrease in the physical barrier function of Allergin-1 KO mice. I also analyzed several cytokines gene expression in the WT and KO mice, and I found that IL-17 showed the tendency to increase in KO mice, and IL-22 showed the tendency to decrease in KO mice. Previous reports have shown that IL-17 and IL-22 are both important for the barrier function regulation, however, the detail relation between these cytokines and the barrier function is still controversial. Bring together, the Allergin-1 KO mice showed defective in barrier function in both mucosal barrier, epithelial barrier, and immunological barrier. Next, to analyze whether microbiota alone can effect on intestinal barrier function, I perform fecal microbiota transplantation (FMT) from WT and KO feces to germ-free (GF) WT mice and analyze the microbiota effect on intestinal permeability 4 weeks after FMT. KO feces transfer group showed a tendency to increase intestinal permeability compare to the WT feces transfer group. Tissue RNA analysis in physical barriers showed no difference in the tight junction and mucins expression. However, in cytokine gene expression, the KO feces transfer group showed an increment of IL-17 and decrement of IL-22, which is similar to the KO mice gene expression tendency. To ensure the microbiota from WT and KO mice were colonized successfully, fecal bacteria composition was monitored weekly, and compared with the donor microbiota. We found a surprising fact that although KO microbiota can transplant to the WT GF mice successfully, these bacteria cannot be maintained in the WT mice intestinal environment. This result indicated the inhibitory immune receptor in the immune cells might be important to maintain the intestinal homeostasis in mice. Finally, I investigated whether the defection of intestinal barrier function in Allergin-1 KO mice may cause mice more susceptible to intestinal disease. Dextran sulfate sodium (DSS)-induced colitis was performed by adding 3% of DSS in mice drinking water, and the mice's body weight and survival were

monitored daily to evaluate the severity of the disease. In comparison with WT mice, KO mice showed significantly increased weight loss as well as mortality. Taken together, my results demonstrated that Allergin-1 deficiency may alter the microbiota composition and immunological regulation, which can lead to intestinal barrier function deficiency, and further increase the susceptibility in DSS-induced colitis.

Our findings describe that Allergin-1 has an important role in the regulation of barrier function. By analyzing the signature feature of each layer of the barrier, we found Allergin-1 KO mice have abnormal microbiota, decreasing mucins and changing of cytokines, however, the interaction between these barriers is still unclear. Several previous reports have shown that epithelial cells can recognize commensal and pathogenic microorganisms and produce antimicrobial peptides, or upregulate the cytokine productions, such as IL-10 (interleukin-10), TGFβ (transforming growth factor-β), and IL-1 superfamily. These cytokines can directly act on the immune cells' activation and regulate the immune function. Further studies also demonstrated that irregular cytokine production from immune cells can also alter the behavior of the epithelial cell and further induced dysbiosis. Nevertheless, the regulation of microbiota by a single inhibitory immune receptor is poorly described. In my future study, I need to clarify which specific cell is regulated by Allergin-1 and how these immune cells can influence the microbiota composition.