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学位論文審査の要旨

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学位論文題名

Modulation of inflammatory responses by megalo-type isomaltosaccharides
（イソマルトメガロ糖による炎症反応の調節作用）

This dissertation consists of 84 pages, 23 figures, 6 tables, and 4 chapters with 1 accompanied publication.

Non-digestible saccharides modulate various immune responses depending on their structures, which may contribute to host health condition. Megalo-type isomaltosaccharides (M-IMs) are composed of 10 to 50 glucose units with α-1,6-glycosidic linkages. M-IMs display delayed absorption and may stay relatively long time in the gastrointestinal tract, which enables them to encounter host immune cells. It is possible that M-IMs regulate not only innate immunity, but also adaptive immunity in. To evaluate this possibility, we investigated the influence of M-IMs on immune system by using primary macrophages and experimental animal models.

1. M-IMs induce tumor necrosis factor α production in primary macrophages via toll-like receptor 4 signaling

Intestinal bacteria utilize M-IMs in a slow rate, suggesting that ingested M-IMs may encounter ileal Peyer's patches, which contain innate immune cells such as macrophages or dendritic cells. Macrophages are responsible for incorporation and presentation of antigens during the initial step of immune responses. We investigated whether M-IMs modulate macrophage functions such as cytokine productions, nitric oxide production, and phagocytosis. Primary macrophages collected from male WKAH/HkmSlc rats were cultured with M-IMs or lipopolysaccharides (LPS). M-IMs and LPS induced the production of tumor necrosis factor α (TNFα), interleukin 6 (IL6), and nitric oxide in the primary macrophages. The gene expression profile of inflammatory factors in the M-IM-stimulated cells was similar to those in the LPS-stimulated cells. However, the induction of the cytokine expression required quite high concentration of M-IMs than that of LPS. Stimulation with the M-IMs did not affect phagocytosis in the primary macrophages. The M-IM-induced TNFα production was suppressed in the cells treated with a toll-like receptor 4 (TLR4) inhibitor, TAK-242. The M-IMs have an ability to modulate cytokine expression via TLR4 signaling and may play a role in immune responses.

2. Ingestion of M-IMs ameliorates LPS-induced acute liver injury in rats

Interaction between innate and adaptive immune responses results in elimination of pathogen and contributes to maintenance of host homeostasis. Innate immune cells incorporate exogenous substances and present them as antigens to T cells. In other words, innate immune cells can activate adaptive immune cells. In our previous experiment, ingestion of the M-IMs dose not induce acute inflammation in a 2-week-study. However, in the cellular experiment shown above, the primary macrophages induce inflammatory cytokines in response to the M-IM stimulation. Obviously, there is a variety of immune cell population depending on organs and collaboration of such cells could decide the way of response to M-IMs. In addition, duration of exposure to M-IMs might alter cellular responses in innate as well as adaptive immune cells. To clarify in-vivo immune responses to M-IMs, we investigated whether a long-term ingestion of the M-IMs influences T-cell-dependent antibody productions and LPS-induced liver injury.

Male F344/Jcl rats (5 weeks old) were fed diet supplemented with or without M-IMs (30 g/kg diet) for 5 weeks. Keyhole limpet hemocyanin (KLH) was administered subcutaneously (1 mg/rat) at week 2 as an exogenous antigen. We measured the production of KLH-specific IgM and IgG in the blood serum on day 7 and day 18 after the administration, respectively. At the end of the experimental period, the rats were administrated with 4 mg/kg of LPS to induce acute liver injury. At 6 hours after the LPS administration, the aorta plasma was collected to measure alanine transaminase (ALT) and aspartate transaminase (AST) activities as well as factors related with acute inflammation such as TNFα, IL6, caspase-1, and interleukin 1 β (IL1β). The liver was collected to analyze gene expressions associated with immune functions with RT-qPCR. There was no significant difference between the groups in the serum concentrations of KLH-specific antibodies and plasma TNFα. Also, there was no significant difference between the groups in the inflammation-related cytokine expressions in the liver. However, plasma AST and ALT activities were attenuated accompanied by reduced plasma IL1β in the rats fed M-IMs. IL6 tend to decrease in the rats fed the M-IM diet. In a separate experiment in the rats without LPS injection, there was a reduction in hepatic expression of *Cd14*, one of the LPS receptors, in the M-IM-fed rats. These results suggest that long-term ingestion of the M-IMs suppress acute liver inflammation via alteration of gene expressions associated with recognition of endotoxins.

In conclusion, ingestion of M-IMs is considered to modulate innate immune responses such as macrophage functions rather than adaptive immune cell functions, which may contribute to prevention of acute inflammation induced by endotoxin.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.