学位論文内容の要旨

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学 位 論 文 題 名

The regulation mechanisms of immune balance through the activation of innate immunity.

自然免疫を介した免疫バランス制御機構の解明に関する研究

【Introduction】T helper type 1 (Th1) cells regulate Type-1 immunity, has an essential function in the control of cellular immunity against tumor, intracellular bacteria and virus. On the other hand, Type-2 immunity, controlled by T helper type 2 (Th2) cells, plays a crucial role for humoral immunity against parasitic worms and allergens. Both Type-1/Type-2 immune systems, which are closely related with acquired immunity, inhibit the activation of each other and retain the homeostasis. It is thought that the increase of allergy, infectious diseases, tumor resulting from the excessively polarized Type-2 immunity and deteriorated Type-1 immunity. Many studies have reported that several types of Lactic acid bacteria (LAB) switch the Type-2 immunity-biased allergic patients towards a balanced Type-1/Type-2 immune profile, leading to amelioration of allergy. In this research, I attempted to elucidate how food-derived components, including LAB, regulated Type-1 immunity activation. I especially focused on innate immunity, which not only act as the first line of host defense but also play a critical role in the subsequent regulation of acquired immunity. Because dendritic cells (DCs), professional antigen presenting cells, have a critical role in innate and acquired immune responses, regulation of DC function is most important for Type-1/Type-2 immune balance. In addition, DCs effectively induce cytotoxic T lymphocytes (CTLs), which are essential for vaccination against virus, bacteria and tumor. Thus, I focused on regulation of DC function and investigated the precise mechanisms. Finally, I studied on application research for cancer immunotherapy by bone marrow-derived DCs (BMDCs) using a mouse tumor-implanted model.

【Methods and Results】Mouse spleen cells were stimulated with *Lactobacillus pentosus* S-PT84. After 12-48h incubation, I determined IFN-γ and IL-12 production levels in the supernatant by ELISA. As a result, S-PT84 strongly induced production of IFN-γ and IL-12 from spleen cells. Next, I analyzed IL-12 and IFN-γ producer using techniques of intracellular staining and cell-depletion or isolation. IFN-γ and IL-12 are produced by $NK1.1^+$ cells (NK and NKT cells) and $CD11c^+$ DCs, respectively. DC-derived IL-12 is completely required for production of IFN-γ from $NK1.1^+$ cells. Moreover, direct interaction between $NK1.1^+$ cells and DCs is essential in the IFN-γ production by $NK1.1^+$ cells. To elucidate which receptor recognized S-PT84, I isolated DCs from wild-type (WT), Toll-like receptor (TLR)$2^{-/-}$, $TLR4^{-/-}$ and $TLR9^{-/-}$ mice. The productions of both IL-12 from DCs and IFN-γ from NK cells were significantly decreased in $TLR2^{-/-}$ or $TLR4^{-/-}$ DCs compared with those from WT mice.

I screened 57 lactic acid bacteria (LAB), isolated from Hokkaido vegetable pickles, by the individual IFN-γ, IL-12 and IL-10 production by spleen cells after the stimulation. I identified a novel *Lactobacillus sakei* strain, which was named ‘Bio-S24’. Bio-S24 could stimulate spleen cells to induce the production of high levels of IL-12 and IFN-γ but negligible levels of IL-10. Bio-S24-mediated IL-12 induction is

completely dependent on TNF-α production. I examined IL-12 induction mechanisms by isolated spleen dendritic cells. As a result, DC-DC interactions through transmembrane TNF-α-TNFR-I/II and soluble TNF-α-TNFR-I signaling were required for maximum IL-12 production.

I examined whether the eight kinds of bean extract had a capability of inducing IFN-γ production in culture system with mouse spleen cells. Surprisingly, only the extract of *Glycine Max*, Kurosengoku, highly induced IFN-γ from spleen cells. Kurosenogku induced Type-1 cytokines through TLR2- and TLR4-signaling pathways as same as S-PT84. Finally, I revealed that Kurosengoku significantly enhanced IFN-γ production by human PBMCs stimulated with anti-CD3 mAbs.

Furthermore, I screened a new adjuvant from various extracts of agricultural products using BMDCs. As a result, I found that the extract of *Larix Leptolepis (Larix kaempferi)* (ELL) strongly activates BMDCs. Indeed, ELL induced antigen-specific CTLs in vivo through BMDC activation. I demonstrated that adoptive transfer of BMDCs with ELL and antigen remarkably inhibited tumor growth in the tumor-bearing mouse model. Thus, ELL would be useful for prevention of tumor and infectious diseases via effective induction of antigen-specific CTLs.

【Discussion】In this research, I firstly found that S-PT84 activated Type-1 immunity. TLRs on DCs are key regulator in activation of Type-1 immunity. As well as LAB asuch as S-PT84 and Bio-S24, I demonstrated that extract of Kurosengoku effectively elevated Type-1 immunity by DC activation through TLRs. Interestingly, although TLR2 and TLR4 recognized these components, mechanism of cytokine production was very different. In the present experiments, I confirmed that the IFN-γ production by the Kurosengoku-stimulated spleen cells was partially blocked in the presence of galactomannan, mannan, or galactose, and completely blocked by the addition of EDTA, whereas IFN-γ production by LAB-stimulated spleen cells was not blocked. These findings suggest that the extract of Kurosengoku has at least sugar-related compounds, binding with a C-type lectin.

I found that DC-derived IL-12 was essential for IFN-γ production by $NK1.1^+$ NK cells and NKT cells in the present investigation. This data indicated that several innate immune cells cooperatively acted during Type-1 immune activation. Moreover, I revealed that not only NK-DC interaction but also TNF-α-mediated DC-DC interaction was very important for Type-1 immune activation. I speculated that different DC subsets such as $CD4^+$, $CD8^+$ and $CD4^-8^-$ conventional DCs and plasmacytoid DCs, which respectively exhibit different phenotypes and functions, were contributed to the production of TNF-α and IL-12.

The present data suggest that Bio-S24 and Kurosengoku would be a promising tool for activation of Type-1 immunity via oral intake. In the future, it is important to perform in vivo animal study and the clinical study to confirm Type-1 immuno-improving activity of Bio-S24 and Kurosengoku, which might prevent Type-2 immunity-dependent immune diseases including allergy as well as infectious diseases and cancers. In addition, I revealed that ELL effectively induced antigen-specific CTLs in vivo model, which might be useful as a TLR-mediated novel adjuvant for prevention of tumor and infectious diseases.

【Conclusion】IL-12 production by DCs through TLR-signaling cascades is required for the subsequence induction of IFN-γ by $NK1.1^+$ cells. Moreover, cell-to-cell interaction among DCs through two types of TNF receptor signal cascades is essential for maximum IL-12 production. These data exhibited that hierarchical and successive reactions among innate immune cells play an important role in activation of Type-1 immunity.