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学 位 論 文 要 旨
Dissertation Abstract

氏名：
Name　Agus Budiawan Naro Putra

学位論文題目：
Title of Dissertation　Studies on the stimulatory effects of jellyfish collagen on innate immune system
（クラゲ由来コラーゲンの自然免疫促進効果に関する研究）

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Nemopilema nomurai is one of the largest jellyfish in the world, attaining a bell diameter of about 2 m and a wet body weight of about 200 kg. It has been reported that more than 95% of jellyfish mass is water, while 40-60% of its dry weight consists of collagen. *N. nomurai* contains collagen up to approximately 88% estimated by its hydroxyproline content.

Jellyfish collagen is becoming a valuable marine substance for cosmetics and functional foods due to its versatile health benefits. More specifically, collagen isolated from *N. nomurai* has been known to show potential in improving the acquired/adaptive immune system. However, studies on the effects of jellyfish collagen on the innate immune system are not enough. Innate immune system is defined by its initial response that prevents and eliminates infections of the host caused by microbes or other foreign invaders. Hence, in order to understand another healthful potential of jellyfish collagen on immune system, and to reveal its immunostimulatory activities on innate immune response, this dissertation was concerned with the activation of macrophages and dendritic cells (DCs) induced by jellyfish collagen. Macrophages are phagocytes which internalize and kill microbes. Macrophages also produce proinflammatory cytokines which activate body defense system. DCs are antigen-presenting cells that play an important role to initiate several immune responses such as the activation of helper T cells.

At first, the effects of collagens from jellyfish and bovine Achilles' tendon on the innate immune response, especially on macrophages were evaluated. Macrophage functions were examined by the phagocytotic activity, cytokine production activity, and gene expression. The phagocytotic activity of mouse macrophage cell line, J774.1 cells was enhanced by both collagens. Collagens from jellyfish and bovine Achilles' tendon also stimulated the productions of tumor necrosis factor (TNF)-α and interleukin (IL)-6 by J774.1 cells and mouse primary peritoneal macrophages, as the result of elevated gene expression levels of these cytokines. Oral administration of collagens elevated cytokine production by peritoneal macrophages in mice. In addition, collagens suppressed gene expression of PPARγ1 in macrophages. PPARγ1 suppression leads to

the activation of nucleus factor-κB (NF-κB), a nuclear transcriptional factor which plays pivotal roles in inflammatory responses such as the production of TNF-α and IL-6. These findings suggest that collagens from jellyfish and bovine Achilles' tendon enhance not only the acquired immune response, but also the innate immune response through the activation of macrophages.

As indicated above, jellyfish collagen promoted cytokine production and phagocytotic activity of macrophages. These findings have become a stimulus to further determine the mode of action of jellyfish collagen on macrophage activation. As a result, cytokine production-stimulating activity of jellyfish collagen was canceled by treatment of macrophages with a Toll-like receptor 4 (TLR4) inhibitor. Moreover, jellyfish collagen stimulated phosphorylation of inhibitor of κBα (IκBα), promoted the translocation of NF-κB, and activated c-Jun N-terminal kinase (JNK). A JNK inhibitor also abrogated the cytokine production-stimulating activity of jellyfish collagen. These results suggest that jellyfish collagen facilitates cytokine production by macrophages through activation of NF-κB and JNK via the TLR4 signaling pathways.

In order to evaluate the stimulatory effects of jellyfish collagen on another innate immune cell, mouse bone marrow-derived dendritic cells (BMDCs) were used. Jellyfish collagen stimulated TNF-α, IL-6, IL-1β and IL-12 production by BMDCs, as the result of the elevation of gene expression level of these cytokines. In addition, jellyfish collagen-treated BMDCs had more wrinkles and longer pseudopodia on their cell surface compared with the control cells. Jellyfish collagen also stimulated cell-surface MHC-II expression level on BMDCs. Furthermore, jellyfish collagen downregulated phagocytotic activity of BMDCs, indicating the transition from antigen-capturing cell to antigen-presenting cell. These findings suggest that jellyfish collagen has the potential to activate DCs and thereby contribute to health promotion.

In summary, jellyfish exhibited an overwhelming potential as health promoting food by activating macrophages and DCs. Consuming jellyfish collagen might act as protective approach from infectious agents or diseases due to its capability to stimulate the activation of our innate immune response.