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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目： Studies on the stimulatory effects of jellyfish collagen on innate immune system
Title of Dissertation （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）

学位論文要約：
Dissertation Summary

Functional foods are known as foods providing additional physiological benefits in the modulation of our physiological systems such as the immune, endocrine, nerve, circulatory, and digestive systems. The prevalence of health problems such as cancer, cardiovascular disease, diabetes, digestive disorders and other diseases has triggered consumer demands for functional foods in recent years. Moreover, the need to maintain a healthy body has become a locomotive of increased public consumption of functional foods which are less-toxic and have no significant side effects. Hence, screening of foodstuffs in order to discover active substances possessing the immune-stimulating effects is very significant.

Data from Food and Agriculture Organization (FAO) of the United Nations showed that the global production of jellyfish for human consumption has increased year by year. In 1985, jellyfish production was approaching 100,000 tons and doubled ten years later. It was also estimated that jellyfish production was reaching 400,000 tons in 2005 (Purcell et al., 2007). From the turn of this century, the population of the giant jellyfish, *Nemopilema nomurai* Kishinouye 1922, began to explode in the Japan Sea. This species is one of the largest jellyfish in the world, attaining a bell diameter of ca. 2 m and a wet body weight of ca. 200 kg. The medusas are transported from the main habitat, i.e. the Bohai, Yellow and East China Seas, by the Tsushima Current to the Japan Sea, usually in small numbers, but sometimes in extreme abundances sufficient to seriously damage local fisheries. Such a mass occurrence took place in 1920, 1958 and 1995, about once per 40 years. Since 2002, however, damaging blooms have occurred almost every year (Kishinouye, 1922; Uye, 2008). Unfortunately, this abundant jellyfish has not been optimally utilized or sufficiently reported in the literature.

Jellyfish constitutes a very important foodstuff in Asia, particularly in China and Japan (Sugahara et al., 2006). In China, jellyfish has been consumed for more than a thousand years. As a natural diet food, jellyfish is low in fats and cholesterol but rich in minerals and proteins (Hsieh et al., 2001). More than 95% of jellyfish mass is water (Hsieh et al., 2001), while 40-60% of its dry weight consists of collagen (Nagai et al., 1999; Kimura et al., 1983). Collagen itself is known as an essential material of muscle tissue, cartilage and bone, and has a great medicinal efficacy, such as anti-fatigue and anti-oxidation (Ding et al., 2011) and consequently may exert anti-aging activities.

Amino acid analysis revealed that jellyfish *Nemopilema nomurai* fractions contained collagen up to approximately 88% which was estimated by its hydroxyproline content (Nishimoto *et al*., 2008). Sugahara et al. (2006) showed that jellyfish collagen stimulates production of immunoglobulins and cytokines by human peripheral blood lymphocytes. In addition, jellyfish collagen also facilitates production of IgM by the transcription-suppressed HB4C5 cells, a human hybridoma cell line, indicating that jellyfish collagen might stimulate not only transcriptional but also translational activities of HB4C5 cells (Nishimoto et al., 2008). These findings indicated that jellyfish collagen stimulates the acquired/adaptive immune system and thus had attracted many researchers to uncover another health benefit of jellyfish collagen. Nevertheless, few studies have been aimed to evaluate the stimulatory effects of jellyfish collagen on the innate immune system.

This dissertation, with three main objectives, was focused on investigating the potential immunostimulatory activities of jellyfish collagen on innate immune system, especially on macrophages and dendritic cells activation.

1. Evaluation of the stimulatory effects of jellyfish collagen on mouse peritoneal macrophages (P-Mac) and murine macrophage-like J774.1 cells.
2. Uncovering the underlying mechanisms of the stimulatory activities of jellyfish collagen on J774.1 cells.
3. Evaluation of the stimulatory effects of jellyfish collagen on mouse bone marrow-derived dendritic cells.

At first, the effects of collagen from jellyfish on macrophages were evaluated. Macrophages produce cytokines such as TNF-α and IL-6 and free radicals such as superoxide and nitric oxide (NO) (Maekawa et al., 2000). Results showed that jellyfish collagen significantly stimulates TNF-α production by J774.1 cells about 2.5-fold and by P-Mac cells about 3.4-fold against control treatment. Jellyfish collagen also stimulated IL-6 production by J774.1 cells and P-Mac 38- and 11-folds against control treatment, respectively. In order to investigate *in vivo* effect of jellyfish collagen, mice were orally administered with jellyfish collagen for 7 consecutive days. On day 8, P-Mac were collected to evaluate the cytokine production activity. Results showed that jellyfish collagen highly increased TNF-α and IL-6 productions by P-Mac. Jellyfish collagen stimulated TNF-α production about 16-fold and IL-6 production 3-fold against control treatment. It is supposed from these findings that jellyfish collagen may have a significant stimulatory effect on macrophages.

Many evidences have shown that macrophages are activated by cytokines such as IFNγ and by bacterial endotoxins such as lipopolysaccharide (LPS) (Meng & Lowell, 1997; Hambleton et al., 1996; Mosser, 2003). Because there was a possibility that jellyfish collagen was contaminated with bacteria, the endotoxin concentration in jellyfish collagen was evaluated by the *Limulus* amebocyte lysate assay. Result showed that the concentration of endotoxin in 900 μg/mL of jellyfish collagen was 0.0099 EU/mL. Although the prepared jellyfish collagen was free of endotoxin contamination, there was also a possibility that another component other than collagen in the prepared jellyfish collagen stimulates cytokine production by macrophages. Hence, the immunostimulatory activity of jellyfish collagen digested with collagenase was investigated. Result showed that the proinflammatory cytokine production-stimulating activity of jellyfish collagen was decreased by the treatment with collagenase. This finding suggested that collagen is the main active substance in jellyfish to

stimulate production of cytokines.

Toll-like receptor (TLR) 4 is renowned to activate the innate immune response by recognizing specific molecular patterns. Results exhibited that TLR4 inhibition suppressed the cytokine production-stimulating activity of jellyfish collagen, indicating that jellyfish collagen stimulates cytokine production by J774.1 cells via the TLR4 signaling pathway. Thus, the mode of action of jellyfish collagen especially on TLR4 signaling pathway to elucidate the mechanism underlying the immunoregulatory effect of jellyfish collagen was observed. NF-κB is an important transcription factor locating downstream of the TLR4-mediated signaling pathway. Activation of TLR4 is characterized by phosphorylation of IκBα, nuclear translocation of NF-κB, and subsequent activation of MAPKs. Those process initiates expression of genes associated with the innate immune responses and inflammation (Hua et al., 2007; Hoshino et al., 2002; Porter & Jänicke, 1999; Toshchakov et al., 2002). Present results are in agreement with studies showing activation of TLR4. We found that jellyfish collagen enhances phosphorylation of IκBα, translocation of NF-κB to the nucleus, and activation of JNK.

In order to evaluate the stimulatory effects of jellyfish collagen on another innate immune cell, mouse bone marrow-derived dendritic cells (BMDCs) were used. Dendritic cells (DCs), classified as part of the innate immune system, are antigen-presenting cells that play an important role to initiate several immune responses such as the activation of antigen-specific T cells (Steinman, 1991). The maturation process of DCs is associated with several harmonized events such as changes in morphology, upregulation of costimulatory molecules and MHC-II, downregulation of phagocytosis activity, and release of cytokines. After induction with rmGM-CSF for 8 days, bone marrow cells were differentiated into dendritic cells. On day 8, BMDCs were treated with/without jellyfish collagen for 24 h. Results showed that the surface of control BMDCs (no treatment with jellyfish collagen) appeared mostly in a smooth and relatively round shape, showing that the cells were mostly in an immature state. Otherwise, both jellyfish collagen- and LPS-treated BMDCs had more wrinkles on the cell surface compared with control cells. Flow cytometry analysis showed that the $CD11c^{+}MHC\text{-}II^{high}$ cells population increased from 10.79% in the control cells to 32.12% in the jellyfish collagen-treated cells. In addition, LPS-treated BMDCs had the highest number (34.18%) of the $CD11c^{+}MHC\text{-}II^{high}$ cells population among others. These results indicate that both jellyfish collagen and LPS are strong stimulators for DCs maturation.

In summary, jellyfish demonstrated a very tremendous potential as health promoting food by activating innate immune system, especially macrophages and dendritic cells. 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.

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