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Jellyfish collagen stimulates maturation of mouse bone marrow-derived dendritic cells

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ABSTRACT

Jellyfish has recently attracted much attention for various applications, including functional foods due to its abundance of collagen. Jellyfish collagen, extracted from *Nemopilema nomurai*, was found to stimulate murine macrophage-like J774.1 cells. However, few reports have determined the immunostimulatory effects of jellyfish collagen on the innate immune response. We herein demonstrate the effect of jellyfish collagen on mouse bone marrow-derived dendritic cells (BMDCs). 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 have more wrinkles and longer pseudopodia on the cell surface compared with the control cells. Jellyfish collagen also stimulated cell-surface MHC-II expression level. Furthermore, jellyfish collagen downregulated phagocytosis capacity of BMDCs. Thus, our findings suggest that JC has the potential to activate DCs and thereby contribute to health promotion.

1. Introduction

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 one millennium. As a natural diet food, jellyfish is low in fat, cholesterol, and calorie, but rich in minerals and protein (Hsieh, Leong, & Rudloe, 2001). More than 95% of jellyfish mass is water (Hsieh et al., 2001), while 40–60% of its dry weight consists of collagen (Kimura, Miura, & Park, 1983; Nagai et al., 1999). Collagen is an essential component of muscle tissue, cartilage and bone, and has a great health benefit, such as anti-fatigue and antioxidation (Ding et al., 2011) and consequently may exert anti-ageing activities.

Collagen isolated from a giant jellyfish *Nemopilema nomurai* has favourable effects on the enhancement of immune

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Abbreviations: BMDCs, mouse bone marrow-derived dendritic cells; ELISA, enzyme-linked immunosorbent assay; FBS, foetal bovine serum; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; TNF, tumour necrosis factor; rmGM-CSF, recombinant murine granulocyte-macrophage colony-stimulating factor; SD, standard deviation

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response. 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). Moreover, the immunoregulatory function of jellyfish collagen has been highlighted *in vivo*, resulting in the enhancement of the antigen-specific immune response (Morishige et al., 2011). Jellyfish collagen also highly stimulated production of tumour necrosis factor (TNF)-α and interleukin (IL)-6 by macrophages (Putra et al., 2012), through activation of NF-κB and c-Jun N-terminal kinase via the Toll-like receptor 4 signalling pathway (Putra, Nishi, Shiraishi, Doi, & Sugahara, 2014). However, studies on the effects of jellyfish collagen on the innate immune response have rarely been reported. Hence, we investigated the immunostimulatory effects of jellyfish collagen on mouse bone marrow-derived dendritic cells (BMDCs).

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). Immature DCs capture an antigen and subsequently migrate to the lymphoid organs, where they select antigen-specific T cells. DCs present the antigen to $CD4^+$ helper T cells, which in turn regulate the immune effectors, including antigen-specific $CD8^+$ cytotoxic T cells and B cells, as well as non-antigen-specific macrophages, eosinophils, and natural killer cells. Mature DCs are present within secondary lymphoid organs while expressing high levels of major histocompatibility complex (MHC)-II and costimulatory molecules permitting antigen presentation (Banchereau et al., 2000). These features indicate DCs as a bridge which links the innate and adaptive immune responses.

Myeloid and lymphoid pathways of DCs development have been identified in mice. Lymphoid DCs are localized in the T cell-rich areas of the periarteriolar lymphatic sheaths (PALS) in the spleen and lymph nodes. In contrast, myeloid DCs are in the marginal zone bridging channels of the spleen but can be induced to migrate to the PALS under the influence of proinflammatory signals such as LPS or parasite extracts (De Smedt et al., 1996; Leenen et al., 1998; Pulendran et al., 1997; Steinman, Pack, & Inaba, 1997). All DCs ultimately derive from haematopoietic stem and progenitor cells in the bone marrow which give rise to several distinct progenitors that can differentiate into one or more DC subsets (Alvarez, Vollmann, & von Andrian, 2008; Fogg et al., 2006; Naik et al., 2007; Onai et al., 2007). Murine myeloid DCs, also known as conventional DCs, can be generated *in vitro* by stimulation of bone marrow progenitor cells using granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 (Lutz et al., 1999; Sallusto & Lanzavecchia, 1994; van de Laar, Coffer, & Woltman, 2012; Yi & Lu, 2012).

This present *in vitro* study gives beneficial insight and a better understanding of jellyfish collagen as a potential health promoting food. Consuming jellyfish collagen might act as protective approach from infectious agents or diseases due to its capability to stimulate the activation of our immune response and thus promotes a better body defence system.

2. Materials and methods

2.1. Materials

Materials used were as follows: RPMI 1640 medium and foetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA); recombinant murine granulocyte-macrophage colony-stimulating factor (rmGM-CSF; R&D Systems, Minneapolis, MN, USA); BD IMag Mouse Dendritic Cell Enrichment Set – DM (BD Bioscience, San Jose, CA, USA); WST-8 assay kit (Kishida Chemical, Osaka, Japan); Fc Receptor Blocker (Innovex Biosciences, Richmond, CA, USA); a fluorescein isothiocyanate (FITC)-conjugated hamster anti-mouse CD11c and a phycoerythrin (PE)-conjugated rat anti-mouse MHC-II (I-A/I-E) antibodies (BD Pharmingen, San Diego, CA, USA); Texas Red-conjugated zymosan A (*Saccharomyces cerevisiae*) BioParticles (Invitrogen, Carlsbad, CA, USA); TNF-α (R&D Systems), IL-12 p70 (R&D Systems), IL-6 (Biolegend, San Diego, CA, USA), and IL-1β (eBioscience, San Diego, CA, USA) ELISA kits; Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan); M-MLV reverse transcriptase (Promega, Madison, WI, USA); and an oligo-dT_{20} primer (Toyobo, Osaka, Japan).

2.2. Preparation of jellyfish collagen extracts

Jellyfish collagen was prepared as described previously by Sugahara et al. (2006) with slight modifications as follows: the exumbrella (5 g) from the jellyfish *N. nomurai* was cut into small pieces, extracted with 50 mL of dilute hydrochloric acid (pH 3.0) for 12 h, and heated at 121 °C for 20 min. Insoluble substances were removed by centrifugation at 8000× *g* for 20 min, and the acid-soluble substances were collected. The collected supernatant was neutralized using 1 M NaOH until the pH of the solution reached 7.0 and then sterilized by filtration. Cation-exchange chromatography and SDS-PAGE analysis revealed that the purity of the active collagen fragments in the extracts was 88% (Nishimoto et al., 2008). Protein concentration in the jellyfish extract was measured with a DC protein assay kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instruction with bovine serum albumin as a standard. The DC protein assay is a colorimetric assay for protein concentration following detergent solubilization. The reaction is similar to Lowry assay, which is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. The absorbance was measured at 655 nm with a microplate reader (Model 550; Bio-Rad).

2.3. BMDCs acquisition

BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). Mice were kept in a temperature-controlled environment (24 °C) and acclimated to their housing environment. Bone marrow cells were collected from femurs and tibias of 6- to 8-week-old male BALB/c mice according to Onishi et al. (2014). In brief, mice were sacrificed by cervical dislocation, and femurs and tibias were gained after cutting their hind legs. Muscles and