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The effects of chitosan oligosaccharide on the activation of murine spleen CD11c$^+$ dendritic cells via Toll-like receptor 4

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ABSTRACT

To investigate the effects of chitosan oligosaccharide (COS) on dendritic cells (DCs) and the role of Toll-like receptor 4 (TLR4) in this immune process, Murine spleen CD11c$^+$ dendritic cells (SDCs) were isolated and cultured with S-COS and B-COS with polymeration degree of 3–7 and 7–16, respectively. The results showed that B-COS up-regulated the expressions of MHCII and CD86 on SDCs, promoted the secretion of TNF-α from SDCs. SDCs treated with B-COS stimulated the proliferation of the CD4$^+$T cells. However, these effects were not observed on SDCs treated with S-COS. Importantly, silencing the TLR4 expressions on SDCs by RNA interference approach attenuated the expression of CD86, MHCII on SDCs, and the secretion of TNF-α from SDCs, and the stimulating CD4$^+$T cells proliferation capacity of SDCs induced by B-COS. These results suggest that B-COS, but not S-COS, promotes the activation of SDCs and TLR4 plays a bridge role in this process.

1. Introduction

Chitin, a homopolymer of β-1,4 linked N-acetylglucosamine, is the second most abundant polysaccharides. Chitosan is the deacetylated derivative of chitin, and previous works revealed that chitosan plays a role in immune responses for plant and animal cells (Porporatto, Bianco, & Correa, 2005; Villiers et al., 2009). Chitosan oligosaccharide (Chitooligosaccharides, COS), derived from chitosan by enzymatic hydrolysis (Zhang, Du, Yu, Mitsutomi, & Aiba, 1999), is a new kind of biofunctional material which exhibits improved biological activities when compared with chitosan (Cho et al., 2008; Moon et al., 2007; Nam, Kim & Shon, 2007; Palma-Guerrero, Jansson, Salinas, & Lopez-Llorca, 2008; Rahman et al., 2008; Yoon, Moon, Park, Im, & Kim, 2007), such as inhibiting growth of bacteria and fungi, exerting anti-tumor activity, and acting as immunopotentiating effectors.

Dendritic cells (DCs) are central players in the process of the immune response. DCs are present in an immature state in peripheral tissues as sentinels to detect pathogens, influencing both innate and adaptive immunity immediately upon invasion. DCs are equipped with a range of pattern recognition receptors (PRRs), such as Toll-like receptor 4 (TLR4). Upon activation through PRRs, DCs turn mature and up-regulate the expressions of surface molecules that are essential for T cells activation, such as CD80, CD86 and MHCII, as well as the secretion of cytokines (Kadowaki et al., 2001; Michelsen et al., 2001; Qi, Denning & Soong, 2003). DCs activate T cells, resulting in specific acquired immunity. During T-cell activation, DCs also provide the T cells with signals which direct T cells to polarize and to develop into T helper1 (Th1) cells, T helper2 (Th2) cells, regulatory T cells (Treg) or IL-17 producing T helper cells (Th17). Thus, maturation of DCs is essential for the appropriate initiation of the subsequent adaptive immune response.

Like DCs, lymphocytes and macrophages are the antigen-presenting cells (APC) which perform as bridges in the immune response. The effects of COS on the activation of lymphocytes and macrophages have been reported by previous studies (Feng, Zhao & Yu, 2004; Maeda & Kimura, 2004). Most recently, it was reported that Chitosan activated DCs (Villiers et al., 2009). However, there is less report about the effect of COS on dendritic cells activation. The mechanism of COS activates DCs is not clearly addressed thus far. In the present study, the effects of COS with different polymeration degree on the SDCs activation and the role of TLR4 in this process are investigated.

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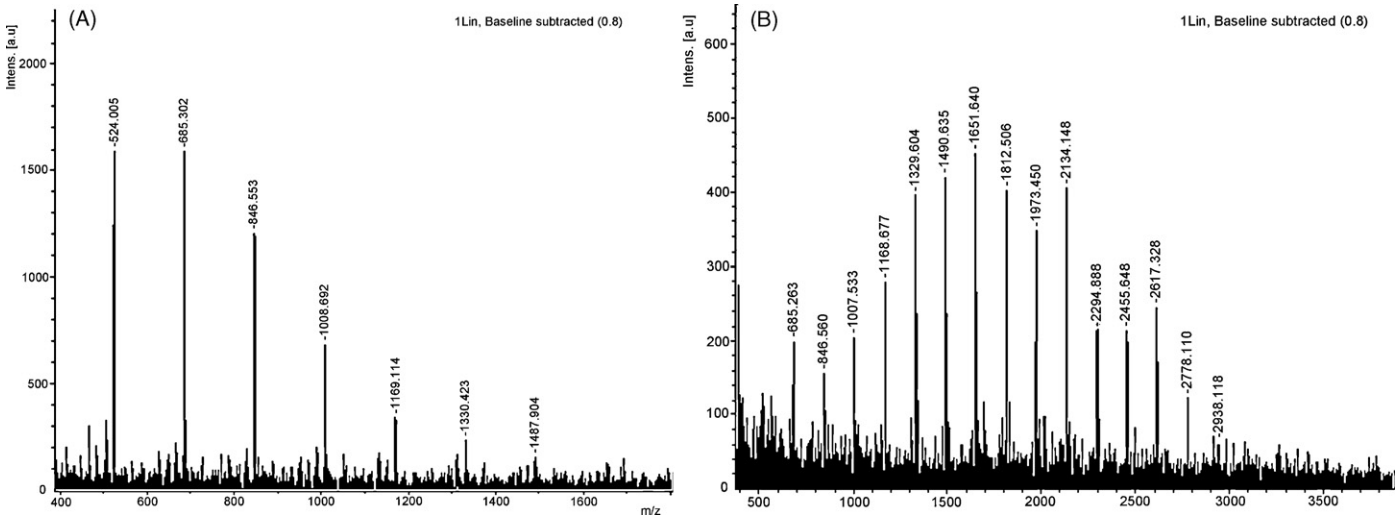

Fig. 1. Mass spectrogram of S-COS sample (A) and B-COS sample (B).

2. Materials and methods

2.1. Animals

Female BALB/c mice and wild-type C57BL/6 mice at age of 4–6 weeks were purchased from Dalian Medical University and allowed to acclimate to the animal facility for 1 week prior to any procedures. Mice were maintained on a 12 h light/dark schedule with the lights on at 6 a.m. Aggressive intruders were individually housed. All animal operations have been approved by Dalian Medical University Animal Ethic Committee.

2.2. Preparation of chitosan oligosaccharide (COS)

COS was prepared from enzymic hydrolysis chitosan according to our previous methods (Zhang et al., 1999). In brief, chitosan (5 g) was dissolved in 2% AcOH (100 ml), and then the pH of the solution was adjusted to 5.6. Enzyme mixture (5 mg) in 0.05 mol/L acetate buffer was added and the mixture was incubated for 30 min at 40 °C. The reaction was stopped by boiling for 10 min. The hydrolyzates were filtered on a hollow-fiber membrane. The pH of the COS solution was adjusted to 8 by slowly addition NH_4OH. The insoluble precipitate was filtered, washed and lyophilized, assigned as B-COS. The filtrate COS solution was lyophilized, assigned as S-COS. MALDI-TOF mass spectrometry analysis indicated that S-COS and B-COS are of polymeration degree of 3–7 and 7–16, respectively (Fig. 1).

2.3. Isolation of SDCs

SDCs were isolated from female BALB/c mice by using CD11c (N418) Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) (Pulendran et al., 1997) according to the manufacturer's instruction. Briefly, the spleens were gently teased and incubated with 2 mg/ml Collagenase D (Roche Diagnostics, Germany) for 30 min to obtain single cell suspension. The cells were harvested and washed with phosphate-buffered saline (PBS) containing 0.5% BSA and 2 mM EDTA, followed by incubation with mouse CD11c antibody-labeled microbeads at 4 °C for 30 min. Labeled cells were positively selected by magnetic separation using MS magnetic antigen cell separation (MACS). PE-CD11c antibody staining and Flow cytometry analysis indicated that the ratio of $CD11c^+$ SDCs in total cells isolated in this manner was up to 90 and 95% cells were alive. SDCs were adjusted to 1×10^6 cells/ml with complete medium (RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin) and seeded into 12-well plates at 2 ml/well. The cells were incubated at 37 °C in 5% humidified CO_2.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the surface molecule expression of SDCs treated with B-COS and S-COS

SDCs were cultured with B-COS and S-COS at different concentrations (0, 40, 80, 160, 320 μg/ml), respectively for 24 h. Total RNAs were isolated from SDCs using Trizol (Invitrogen, USA). cDNA was synthesized using RT-PCR kit (TaKaRa, Japan) according to the manufacturer's instruction. The cDNA was amplified by PCR using the specific primers set for mouse TNF-α, IFN-γ, IL-10 and GAPDH as an internal control. Primers used were listed in Table 1. PCR analysis was performed under the following conditions: denaturation at 94 °C for 5 min, and then 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 60 °C, extension for 30 s at 72 °C. The amplified products were analyzed by agarose gel electrophoresis, followed by ethidium bromide staining.

2.5. Enzyme-linked immunosorbent assay (ELISA) of the cytokine secretion of SDCs treated with B-COS and S-COS

SDCs were cultured with B-COS and S-COS at different concentrations (0, 40, 80, 160, 320 μg/ml), respectively for 24 h. The culture supernatants of SDCs were collected, centrifuged at 300 × *g* for 5 min and frozen at −20 °C. The contents of TNF-α, IFN-γ and IL-10 in culture supernatants were analyzed by using mouse cytokines ELISA kits (R&D Systems, USA).

Table 1
Primer sequences for RT-PCR.

Gene	Primer sequence
TNF-α	Forward: 5′-AGAAAGAAGCCGTGGGTTGG-3′ Reverse: 5′-CATGCCTAACTGCCCTTCCT-3′
IFN-γ	Forward: 5′-AGCGCTGACTGAACTCAGATTGTAG-3′ Reverse: 5′-GTCACAGTTTTCAGCTGTATAGGG-3′
IL-10	Forward: 5′-GGTTGCCAAGCCTTATCGGA-3′ Reverse: 5′-ACCTGCTCCACTGCCTTGCT-3′
GAPDH	Forward: 5′-GGCCGTGAAGTCGTCAGAAC-3′ Reverse: 5′-GCCACGATGCCCAGGAA-3′