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Mechanism of macrophage activation by (1,4)-α-D-glucan isolated from *Tinospora cordifolia*

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Abstract

The signaling mechanism of the novel (1,4)-α-D-glucan (RR1) isolated from the medicinal plant *Tinospora cordifolia* was investigated in macrophages to evaluate its immunostimulating properties. When RAW264.7 macrophages were incubated with RR1 at 4 °C, the novel glucan inhibited the phagocytosis of unopsonized zymosan A bioparticles in a dose-dependent manner. RR1 also inhibited the binding and internalization of opsonized zymosan A bioparticles, although at a lower level than laminarin. Incubation of macrophages with anti-CD11b mAb followed by RR1 failed to show any inhibitory effect on RR1-induced TNF-α synthesis confirming that complement receptor 3 (CR3) is not involved in the opsonic binding and internalization of RR1 in macrophages unlike zymosan A. The anti-CD11b mAb has significant inhibitory effect on the zymosan A-induced tumor necrosis factor (TNF)-α synthesis. RR1 induced TNF-α synthesis in macrophages in a dose-dependent manner which can be completely inhibited by the NF-κB inhibitor caffeic acid phenethyl ester (CAPE) or curcumin. RR1 activated NF-κB in a time- and dose-dependent manner and this modulation of nuclear NF-κB activity is associated with the degradation of I-κB α thus facilitating the translocation of NF-κB into the nucleus. RR1-induced NF-κB activity peaks at 8 h of RR1 stimulation while I-κB α degradation occurred within 1 h of stimulation. RR1-induced NF-κB activation occurred through TLR6 signaling as evidenced by the synthesis of IL-8 in TLR6-transfected HEK293 cells. These results show that the novel (1,4)-α-D-glucan from *Tinospora cordifolia* activates the immune system through the activation of macrophages that occurs through TLR6 signaling, NF-κB translocation and cytokine production.

Keywords: Immunostimulation; Macrophages; Toll-like receptor; TNF-α; NF-κB

Abbreviations: BRM, biological response modifier; CAPE, caffeic acid phenethyl ester; CR3, complement receptor 3; DMEM, Dulbecco's Modified Eagle Medium; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; I-κB, inhibitory protein-κB; IL, interleukin; IRAK, IL-1R-Associated Kinase; JNK, c-Jun N-terminal Kinase; LPS, lipopolysaccharide; mAb, monoclonal antibody; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TIR, Toll-interleukin receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor 6.

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1. Introduction

Innate immunity mediated by macrophages, neutrophils and natural killer (NK) cells is the first line of host defense mechanism against microbial invasion. The innate immune system targets the structurally conserved pathogen-associated molecular patterns (PAMPs) through specific germ-line encoded receptors called pattern recognition receptors (PRRs) [1]. The augmentation of the immune system with natural as well as synthetic immune stimulators may complement conventional therapies in managing infectious diseases especially when the immune system is weakened or when microorganisms develop antibiotic resistance. β-glucans are potent stimulators of the innate immune system in invertebrates, while in mammals they are potent activators of the complement system [2,3]. Several preclinical and clinical investigations have indicated the usefulness of β-glucans, a class of biological response modifiers (BRMs), for acceleration of wound healing and inhibition of the systemic inflammatory response syndrome and septic shock [4–6]. These polymers have therapeutic potential because of their effects on the immune system that may include anti-tumor and anti-infective activities as well as protection against fungal, bacterial, viral and protozoan infections [7,8]. Soluble and particulate β-glucans interact with cognate receptors on macrophages stimulating the synthesis of cytokines, chemokines and reactive oxygen intermediates [9]. The major receptors reported for β-glucan recognition/binding on macrophages are complement receptor 3 (CD11b/CD18 or CR3), Dectin-1 and Toll-like receptors (TLRs) 2 and 6. Although lactosylceramide and scavenger receptors are also identified in β-glucan recognition, their function is not well understood [10].

Even though several papers describing the immunostimulating properties of β-glucans are available in the literature, plant-derived α-glucans have been scarcely investigated to date. Bao et al. [11] reported on the biological activity of a (1→3)-α-D-glucan from the spore cell walls of the fungi *Ganoderma lucidum*. Also some of the modified derivatives of (1→3)-α-D-glucan showed potent immunostimulating effects on lymphocyte proliferation and antibody production in this study. The introduction of carboxymethyl group with low degree of substitution (DS < 0.28) was the best choice for the improvement of immunostimulating activity of α-D-glucans [12,13].

Toll like receptors (TLRs) are part of the large super family of Toll-interleukin (IL)-1 receptors (TIRs) possessing the cytoplasmic motif for the intracellular signaling function. These molecules provide a first line host defense and have been implicated in infectious and autoimmune diseases in a variety of organisms ranging from flies to mammals. It is now accepted that TLRs are the principal signaling molecules through which mammals sense infection [14]. In mammals 12 different TLRs, each recognizing distinct PAMPs have been identified [15] and the number is increasing. All TLRs, IL-1 receptor and other TIR domain containing receptors, with the exception of TLR3, share a common signaling pathway that depends on the adaptor myeloid differentiation factor 88 (MyD88) [9,16]. Besides MyD88, several adaptor molecules have recently been reported and the differential utilization of these adaptor molecules may provide the specificity for the TLR signaling [17]. Evidences for the physical and/or functional interactions among TLRs, and between TLR and other surface receptors are also available. Gantner et al. [9] have described the collaborative induction of dectin-1 and TLR in β-glucan stimulation and also the synergistic interaction between these two receptors on NF (nuclear factor)-κB activation.

TLR mediated cytokine production depends on its down stream mediators such as IL-1R-associated kinase (IRAK)-4 and TNF receptor-associated factor-6 (TRAF-6) that activate c-Jun N-terminal kinase (JNK) and NF-κB [10]. NF-κB is a ubiquitous transcription factor that regulates the cytokine gene expression in many immune effector cells. In most cells, NF-κB is usually present in cytoplasm as latent, inactive and bound to the inhibitory protein κB (I-κB) [18]. It is activated by a variety of stimuli such as pro-inflammatory cytokines, viral products, lipopolysaccharides, plant-derived compounds such as taxol, as well as pathogen and non-pathogen-derived β-glucans [19,20]. On stimulation, I-κB α is phosphorylated and rapidly degraded through proteasomal mechanisms which in turn release the active NF-κB so as to translocate to the nucleus and bind to DNA to initiate cytokine/chemokine gene transcription [18,21].

Immunostimulating properties of glucans have been ascribed to be due to the β-glycosidic linkages, degree of branching and solution conformation [22]. We have characterized and reported the immunostimulating properties of a novel polysaccharide-(1,4)-α-D-glucan (RR1)-from the medicinal plant *Tinospora cordifolia* [23]. This novel glucan is water soluble and has (1,4)-α-D-glycosidic linkages in the main chain and (1,6)-α-D-glycosidic linked side chains at an interval of 6,7 glucose units. It is non-cytotoxic to normal cells as well as tumor cell lines (CEM, CEM/VLB) even up to 1000 μg/ml and activates the human lymphocyte subsets at varying levels. The activation of NK cells, one of the major arms of innate immunity, was demonstrated by the increased level of killing of tumor cells by the RR1-treated lymphocytes in a