Human Immunology 76 (2015) 146–154

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

β-Glucan enhances cytotoxic T lymphocyte responses by activation of human monocyte-derived dendritic cells via the PI3K/AKT pathway

Jun Ding [a,b,1], Tongbao Feng [a,1], Yongling Ning [a], Wenjing Li [a], Qiyong Wu [b], Keqing Qian [a], Yong Wang [a,b,*], Chunjian Qi [a,*]

[a] Oncology Institute, The Affiliated Hospital of Nanjing Medical University, Changzhou No. 2 People's Hospital, Changzhou 213003, China
[b] Deparment of Cardiothoracic Surgery, The Affiliated Hospital of Nanjing Medical University, Changzhou No. 2 People's Hospital, Changzhou 213003, China

ARTICLE INFO

Article history:
Received 16 April 2014
Accepted 14 January 2015
Available online 27 January 2015

Keywords:
β-Glucan
Immune response
Dendritic cells
Signal pathway

ABSTRACT

Purpose: To investigate the effects of β-(1,3/1,6)-D-glucan on dendritic cells (DCs) maturation, cytotoxic T lymphocyte responses and the molecular mechanisms of its transition.
Methods and results: Human monocyte-derived DCs were matured using yeast-derived particulate β-glucan (WGP) or a mix of TNF-α, IL-1β and IL-6 ("Conv mix"). Multicolor flow cytometry was used to study the DCs phenotype and cytotoxic T-lymphocyte priming and differentiation. ELISA and RT-PCR assays were used to evaluate cytokine production. Western blot was used to investigate the signal pathways. WGP-matured DCs functions were compared with those of Conv mix-matured DCs. WGP-matured DCs expressed higher levels of CD11c, CD86, CD40 and HLA-DR; produced higher levels of pro-inflammatory cytokines; and elicited more CTL priming and differentiation than Conv mix-matured DCs. The PI3K/AKT signaling pathway was involved in WGP-induced dendritic cell maturation. Furthermore, WGP-matured DCs significantly increased tumor-specific CTL responses.
Conclusion: Excellent ability of yeast-derived particulate β-glucan to induce DCs maturation and tumor-specific CTL responses explains, in part, its clinical benefits and emphasizes its utility in ex vivo maturation of DCs generated for therapy.

1. Introduction

Recently, new approaches in immunotherapy offer hope of prolonged survival in cancer patients [1]. There is great interest, in this regard, in the use of anti-tumor vaccines, in particular DC-based vaccines [2]. Vaccines operate through the activation of DCs that eventually stimulate antigen-specific T and B lymphocytes. Adjuvants promote and enhance immune responses to vaccine components. Adjuvants derived from microorganisms stimulate DCs directly, leading to the up-regulation of cytokines, MHC class II, and co-stimulatory molecules and to their migration to the T cell area of lymph nodes. Among all adjuvants, toll-like receptor (TLR) agonists and cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF) have been tested in both preclinical and clinical settings for cancer treatment. In addition to TLR agonists and cytokine adjuvants, ligands for the RIG-I-like, the NOD and the dectin-1 receptors have been demonstrated to activate DCs and to promote innate and adaptive immune responses. β-Glucan has been recognized as a major fungal pathogen-associated molecular pattern (PAMP), which can strongly influence natural and adaptive host immune responses, mostly through engagement of the C-type lectin receptor dectin-1 [3]. β-Glucan, as a biological response modifier, was first reported 45 years ago and has been extensively investigated for both its anti-tumor and anti-infective activity [4–6]. Recent studies also demonstrate that β-glucan can function as a potent adjuvant to stimulate innate and adaptive immune responses [7,8]. Most β-glucans are derived from yeast, bacteria, barely or fungi and have a backbone structure of linear β-1,3-linked D-glucose molecules (β-1,3-D-glucan). They also have β-1,6-linked side chains of β-1,3-D-glucan of varying sizes that occur at different intervals along the backbone [9]. There are at least four β-glucan receptors that have been identified: complement receptor 3 (CR3, CD11b/CD18, $\alpha_M\beta_2$-integrin, Mac-1) [10], lactosylceramide (LacCer) [11], selected scavenger receptors (SRs) including SR CD36 [12], and dectin-1 [8,13].

* Corresponding authors at: Oncology Institute, The Affiliated Hospital of Nanjing Medical University, Changzhou No. 2 People's Hospital, Changzhou 213003, China. Fax: +86 519 88115560.
E-mail address: qichunjian@gmail.com (C. Qi).
[1] J.D. and T.F. contributed equally to this work.

http://dx.doi.org/10.1016/j.humimm.2015.01.009
0198-8859/

Dectin-1 was originally reported as a DC-specific molecule with a T cell co-stimulatory capacity, but later, its expression was found more strongly in monocytes, macrophages and neutrophils and weakly in a subset of T cells, and in humans, B cells and eosinophils. The receptor dectin-1 expressed on polymorphonuclear leukocytes, mononuclear cells, macrophages, and DCs mediates the biological effects of β-glucan, including glucan-dependent anticancer immune responses. In murine tumor models, we found that orally administered β-glucan elicits potent anti-cancer immune responses, leading to delayed tumor progression [8,14]. Stimulation with β-glucan leads to DC maturation and cytokine secretion, enabling DCs to elicit T lymphocyte activation. Dectin-1 signaling in human monocyte-derived DCs triggers NF-κB activation through two independent signaling pathways: one through Syk [15,16] and the second through the kinase Raf-1 [17,18]. Both signaling pathways converge at the level of NF-κB activation and regulation to control antifungal adaptive immunity. Engagement of dectin-1 by β-glucan can trigger a series of intracellular signal transduction pathways through Syk kinase and Raf-1 signaling pathways, activating the cells and inducing a variety of cellular responses, such as cytokine production.

In this study, we demonstrated that whole β-glucan particles (WGPs) could activate and maturate human monocyte-derived DCs, up-regulate co-stimulatory molecules, increase pro-inflammatory cytokine production, and lead to augment tumor-specific cytotoxic T lymphocyte (CTL) responses. Furthermore, we showed that the PI3K/AKT pathway was involved in activation of human monocyte-derived dendritic cells by β-glucan.

2. Materials and methods

2.1. Preparation of β-glucans

WGP β-glucan (kindly provided by Prof Jun Yan, University of Louisville) was purified from the cell walls of *Saccharomyces cerevisiae*. A series of alkaline and acid extractions yielded hollow yeast cell wall "ghosts" composed primarily of long β-1,3 glucose polymers with 3–6% of the backbone glucose units possessing a β(1,6) branch (β-(1,3/1,6)-D-glucan). WGPs were hydrated in distilled water and sonicated to produce a single-particle suspension. To remove any trace amounts of LPS contamination, the WGPs were suspended in 200 mM NaOH for 20 min at room temperature (RT), washed thoroughly and re-suspended in LPS-free water as described previously. The endotoxin level was 0.06 EU/ml as tested by the Gel-clot method (Associates of Cape Cod, East Falmouth, MA).

2.2. Dendritic cell cultures

PBMCs were isolated from the blood of healthy donors (in compliance with the protocol approved by the ethics committee of Changzhou No. 2 People's Hospital) by Ficoll Paque Plus (GE Healthcare Biosciences, Shanghai China) separation using centrifugation for 30 min at 650g. The cells were then washed three times in PBS (Life Technologies), quantified, and labeled with CD14+ MACS microbeads (Miltenyi Biotec, Auburn, CA) as per the manufacturer's protocol. CD14+ cells (with greater than 95% purity) were cultured for 5 days in complete RPMI 1640 medium (Gibco Life Technologies, Breda, The Netherlands) containing L-glutamine, sodium pyruvate, nonessential amino acids and 2-mercaptoethanol supplemented with 10% FCS, 100 U/ml rHu GM-CSF (PeproTech) and 50 U/ml rHu IL-4 (PeproTech) to generate immature DCs (Mo-iDCs). Mo-mDCs were harvested at Day 7, after receiving stimuli at Day 5 with particulate β-glucan WGPs (100 μg/ml) or conventional maturation cocktail (50 ng/ml TNF-α, 25 ng/ml IL-1β and 10 ng/ml IL-6). Simultaneously, Mo-iDCs were cultured for an additional 2 days without stimuli as a control. The resulting DCs were thoroughly washed and used for phenotypical (flow cytometry) and functional characterization (co-culture assays), whereas supernatants were frozen for cytokine evaluation by ELISA.

2.3. Phenotype analysis of monocyte-derived DCs by flow cytometry

Cells (4×10^5) in 100 ml PBS were blocked with Fc-blocking monoclonal antibody for 15 min on ice. Cells were stained with fluorochrome-tagged monoclonal antibodies against CD11c, CD40, CD80, CD86, HLA-DR or isotype controls (eBioscience, San Diego, CA) on ice for 30 min, washed with ice-cold staining buffer, fixed with 2% paraformaldehyde in PBS, and analyzed by flow cytometry (FACSCanton II, BD Biosciences, San Jose, CA).

2.4. Enzyme-linked immunosorbent assay

After treatment, cell culture medium was collected and centrifuged at 10,000 rpm for 5 min. IL-2, IL-6, TNF-α, IL-10, IL-12p40 and IFN-γ concentrations were determined by enzyme-linked immunosorbent assays (ELISA, Biolegend, San Diego, CA) according to the manufacturer's instructions.

2.5. Western blot analysis

Western blotting was performed as described previously [19]. A total of 1×10^7 cells were washed with PBS and resuspended in 1 ml ice-cold RIPA buffer (1 × PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with freshly added protease inhibitors (10 mg/ml PMSF, 30 μg/ml aprotinin, and 100 mM Na_3VO_4). The preparation was transferred to microcentrifuge tubes and centrifuged at 10,000g for 10 min at 4 °C. The supernatant was subjected to 10% SDS–PAGE and transferred to nitrocellulose membranes according to the manufacturer's protocols (Bio-Rad Corp, USA). After blocking, the membrane was incubated with primary antibodies against p38 MAPK, phospho-p38 MAPK, ERK1/2, phospho-ERK1/2, JNK1/2, phosphor-JNK1/2, AKT, and phosphor-AKT (Cell Signaling Technology, Beverly, MA). Proteins were detected with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG and an enhanced chemiluminescence detection system (GE Life Sciences, Pittsburgh, PA).

2.6. Allogeneic mixed leukocyte reaction (MLR)

Effector T cells were obtained from non-adherent cells from the dendritic cell cultures and were labeled with 2.5 μM CFSE for 10 min at 37 °C in PBS supplemented with 1% human serum albumin and then thoroughly washed. Subsequently, the CFSE-labeled allogenic T cells (1×10^6 cells/well) were cultured with WGP-matured DCs, Conv-matured DCs or Mo-iDCs at a 10:1 ratio in 1 ml complete RPMI-1640 supplemented with 10% FCS in 24-well plates, and IL-2 (50 U/mL) was added to the cultures. T cells were cultured alone as a control. Fresh medium were added on days +3 and +5. After 7 days, CFSE-labeled T cell cultures were stained with anti-CD8 and anti-IFN-γ mAbs. Flow cytometry was performed to assess intracellular IFN-γ expression among CTLs. At least 50,000 events were acquired/antibody and analyzed by FlowJo software Version 7.2. For intracellular staining, CFSE-labeled lymphocytes were stimulated with leukocyte Activation Cocktail, with BD GolgiPlug processed in accordance with the available technical instructions (BD Pharmingen). After 5 h, cell surface was stained with anti-CD8 mAbs, fixed, permeabilized, and then intracellular stained with anti-IFN-γ (Biolegend) mAb.