Clinical and Medical Research and Studies

www.alcrut.com

Research Article **Clinical and Medical Research and Studies** **Open Access**

An Adverse Association Between Warmer Air Temperatures and Death Rates for Cancer or Heart Disease

Prasad Garrepally [1*], Swathi Chilukala [1]

Jangaon Institute of Pharmaceutical Sciences, Depot. Of Pharmaceutics, Jangaon, India.

***Corresponding Author:** Prasad Garrepally, Jangaon Institute of Pharmaceutical Sciences, Depot. Of Pharmaceutics, Jangaon, India.

Received: 08 August 2023 | **Accepted:** 24 August 2023 | **Published:** 28 August 2023

Citation: Prasad Garrepally. The Upregulation Expression of Cell Surface Markers. J. Clinical and Medical Research and Studies. 2(3) Doi: 10.59468/2836-8525/034

Abstract

Agaricus blazei Murill (AbM) is an edible Bacidiomycota phyllum mushroom used in traditional medicine as a remedy against a wide range of diseases, including infection and cancer. It is rich in β-glucans and antitumor protein-glucan complexes, which have been shown to have stimulating effects on cells involved in innate immunity, such as monocytes, NK cells and dendritic cells. The present report shows that stimulation of monocyte-derived dendritic cells (MDDC) with an AbM-based extract, AndoSan.

Keywords: Agaricus blazei Murill; monocyte-derived dendritic cells (MDDC); cell surface markers; CD1, CD11, CD14, CD40, CD69, CD80, CD86

Introduction

Mushrooms are rich in β-glucans, which are a broad class of bioactive polysaccharides with strong immunomodulating properties, found in the cell wall of yeast, mushrooms and fungi [1]. Generally, immune stimulation by medicinal mushrooms occurs via antigen-presenting cells (APC) in the innate immune system, i.e. monocytes, macrophages and dendritic cells (DC). The cellular response is triggered by the detection of conserved microbial derived molecules, named pathogen-associated molecular patterns (PAMP), by pattern recognition receptors (PRR) on immune cells [2] such as Toll-like receptors (TLR).

Methods and Meterials

Cells

Monocyte derived dendritic cells

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats from blood donors at the Blood Bank of Oslo University Hospital,Ulleval, using a modified version of Sallusto's procedure. Shortly, PBMC were isolated by centrifugation of a buffy coat through a Lymphoprep® layer (Axis-shield PcC AS), and washed in Hank's Balanced Salt Solution (HBSS) before resuspension in RPMI 1640 containing 10% AB Rh^+ serum.

Promonocytic cells

Cells of the human promonocytic cell line THP-1 were purchased from ATCC (Middlesex, UK). The cells were maintained in RPMI 1640 with 10mM HEPES, 50 mM β-mercaptoethanol, 1 mM sodium pyruvate, 2.5 mg/ml D-glucose, penicillin/streptomycin, 0.7 mM L-glutamine and 10% FCS. The cells were split every 3-4 days and kept at 5% saturated atmosphere at 37°C.

Reagents

The commercial mushroom extract AndoSan™ and the pure AbM extract contained therein were both obtained from ACE Co Ltd., Gifu-ken, Japan, via Immunopharma AS, Oslo, Norway. According to the producer, the AndoSan™ extract is constituted of 82% AbM, 15% *Hericium erinaceum* (He) and 3% *Grifola frondosa* (Gf), all *Basidiomycetes* mushrooms.

Experiments

The MDDCs were stimulated with the AbM-based extract AndoSan™ or with PBS (negative controls) for 24h and 48h and the expression of a range of cell surface markers associated with MDDC maturation, activation or function (see below), were examined by flow cytometry. MDDCs were also stimulated with LPS 1.5 µg/l (positive controls) and the expression of the cell surface markers CD69 and C86 were examined by flow cytometry after 24h.

Flow cytometry analysis was performed with a Becton Dickins FACSCalibur Canto II flow cytometer and the software, CellQuest (BD Bioscience, San Jose, CA, USA). MDDC cells were seeded into 6-well plates (Nuncleon) with AbM extract AndoSan™ in concentrations of 0% (=10% PBS control) and 10 % for 24h and 48h. Cells were stained by incubation for 15-20 minutes with FITC- or PE-labeled antibodies, including isotype controls, before washing with PBS, resuspension in a PBS-EDTA-BSA-glucose buffer, and examination in the flow cytometer.

The following FITC- or PE-labeled mouse monoclonal Abs were used in flow cytometry analyses. CD1a (cat.no. 555806), CD11b (347557), CD11c (IM1760), CD45/CD14 BD Leucogate™ (342408), CD40 (555588), CD69 (555531), CD80 (557227), CD83 (556910), CD86 (555657), and appropriate IgG1, k (555748) and IgG2b, k (555742) isotype controls, were all purchased from Beckman Coulter Co., Marseille, France.

THP-1 cells were seeded in 6 well plates at a concentration of $0.7x10^6$ cells/ml and incubated with 10% of AbM or PBS for 1h. Then $0.5x10^6$ cells were stained with TLR2 (cat.nr. MCA2152PE, AbD Serotec) or TLR4 (cat.nr. MCA2061PE, AbD Serotec) and unstained cells were used as control. The samples were analysed on a Becton Dickinson FACSCalibur flow cytometer.

Western Blotting was performed in THP-1 cells stimulated with 10% AbM for 1h. Nuclear extract kit (cat. Nr. 40010, Active Motif) was used to isolate nuclear and cytoplasmatic extract from the THP-1 cells. The

protein extracts were applied to the wells in a 12-15% acrylamide gel and separated by electrophoresis. The Precision Plus Kaleidoscope Standards (cat.nr. 161-0375, BioRad) were used to detect protein size, and NF-κB control cell extract (cat.nr. 9242, Cell Signaling) was used as a positive control (not shown in blot). TransBlot SD Semi Dry Transfer cells from Bio Rad was used to transfer the proteins to an Immuno-Blot polyvinyliden difluorid membrane (cat.nr. 162-0177 BioRad). The blot was blocked overnight in a 5% solution of dried milk before further incubation with primary and secondary antibodies. NF-κBp65 (22B4) Rabbit mAb (cat. nr. 4764 Cell Signaling) was used to detect the levels of the house keeping protein α-tubulin (11H10). Anti-rabbit HRP-linked antibody (cat.nr. 2125, Cell Signaling) were used as a secondary antibody. The blot was incubated in Immuno-star HRP substrate (cat.nr. 170-5040 BioRad) before blot images were taken in a Molecular Imager ChemiDocXRS+ system. All experiments were performed minimum twice.

Results

Stimulation of MDDCs with AndoSan™ 10% for 24h resulted in neoexpression of CD69, a strong upregulation of CD1a, CD14, CD40, CD80, CD83 and CD86 and a downregulated expression of CD11c. No changes in these markers were found in MDDC incubated with PBS. Stimulation with 0.5 µg/ml of *E.coli* LPS for 24h led to a similar degree of upregulation of CD86 and neoexpression of CD69 (not shown) as seen with AbM. For most of the upregulated markers there was a further increase of the expression after 48h incubation (data not shown). No changes in these markers were seen after PBS stimulation.

Discussion

The main purpose of the study was to investigate if the AbMbased extract, AndoSan™ activates MDDC as seen from modulation of cell surface marker expression. We detected neoexpression of CD69 and increased expression of CD1a, CD14, CD40, CD80, CD83 and CD86 after stimulation with AndoSan™ compared to incubation with PBS. Interestingly, stimulation with this mushroom extract induced a similar upregulation of CD69 (associated with activation) and CD86 (associated with antigen-presentation) as did stimulation with *E.coli* LPS. These findings are in line with the results from a previous investigation, where stimulation with AndoSan™ on MDCC was found to increase production of cytokines in a similar manner as stimulation with LPS, and, in some cases, in even higher quantities than did stimulation with LPS. The β (or leukocyte)- integrins, i.e. the CD11/CD18 complex, including CD11b and CD11c receptors are expressed on both macrophages and MDDC and play a role in cell-to cell adhesion.

Conclusions

The mushroom extract AndoSan™ upregulated CD1a, CD14, CD40, CD80, CD83 and CD86, neoexpressed CD69, and down-regulated CD11c in MDDC. The upregulation of these markers shows that AndoSan™ indeed activates MDDC *in vitro*.

Competing Interests

The authors declared that they have no competing interests.

References

1. Wasser SP and Weis AL. **Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective**. *Crit Rev Immunol.* 1999; **19**:65-96.
2. Brown GD. **Dectin-1: a signalling non-TLR pattern-recognition receptor**. *Nat Rev Immunol.* 2006; **6**:33-43.
3. Roeder A, Kirschning CJ, Rupec RA, Schaller M, Weindl G and Korting HC. **Toll-like receptors as key mediators in innate antifungal immunity**. *Med Mycol.* 2004; **42**:485-98.
4. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R and Liu YJ. **Reciprocal control of T helper cell and dendritic cell differentiation**. *Science.* 1999; **283**:1183-6.
5. Hemmi H and Akira S. **TLR signalling and the function of dendritic cells**. *Chem Immunol Allergy.* 2005; **86**:120-35.
6. Seya T, Funami K, Taniguchi M and Matsumoto M. **Antibodies against human Toll-like receptors (TLRs): TLR distribution and localization in human dendritic cells**. *J Endotoxin Res.* 2005; **11**:369-74.
7. Levitz SM. **Interactions of Toll-like receptors with fungi**. *Microbes Infect.* 2004; **6**:1351-5.
8. Kasai H, He LM, Kawamura M, Yang PT, Deng XW, Munkanta M, Yamashita A, Terunuma H, Hirama M, Horiuchi I, Natori T, Koga T, Amano Y, Yamaguchi N and Ito M. **IL-12 Production Induced by Agaricus blazei Fraction H (ABH) Involves Toll-like Receptor (TLR)**. *Evid Based Complement Alternat Med.* 2004; **1**:259-267.