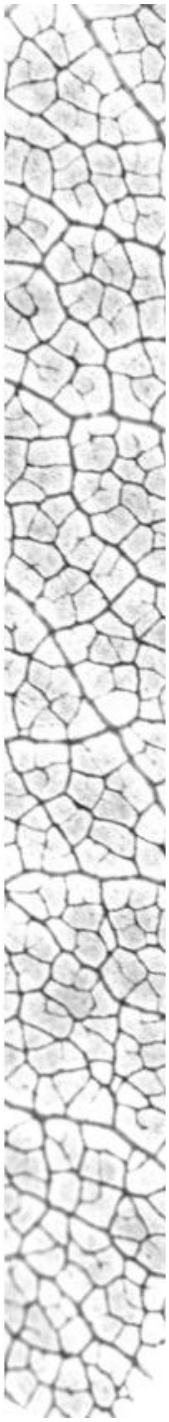

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The interaction of β-glucan on Dectin-1 receptor or TLR-2 might have the potency to activate function of Treg Cell and production of anti-inflammatory cytokine

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Abstract

Objectives: Atherosclerosis formed due to the accumulation of the fibrous tissues in the vascular wall and gradually become thrombus. *Agaricus brasiliensis* known as a traditional drug for lifestyle-related diseases, including obesity, hypertension, and diabetes. The aim of this research was to examine the potency of *Agaricus brasiliensis* to reduce the excessive inflammatory response that occurs in the process of atherosclerosis. The extract of *Agaricus brasiliensis* contains β-glucans and Agiritin that may bound to the Dectin-1 receptor and TLR-2. **Methods:** Therefore, we examine the interaction of the molecules by using bioinformatic. We have docked β-glucans and Agaritin to the active site of the Dectin-1 receptor and TLR-2. **Results:** The result of the analysis indicated the binding affinity of between TLR2/1 and 1,3-1,6 β-glucan or agaritin showed higher compared to positive control, Tri-Acylated Lipopeptide. Besides β-glucan 1,3-1,6 bound to Dectin-1 has similar binding affinity compared to the positive control, Beta-D-Glucose. **Conclusions:** It could be concluded that β-glucan 1,3-1,6 and agaritin might enable to activate the TLR2. However, Dectin-1 receptor might only be activated by 1,3-1,6. β-glucan.

Keywords: atherosclerosis, *Agaricus brasiliensis*, β-glucan, Dectin-1 receptor, TLR2/1

Key Messages: This study is critical to explore the potency of the fungus for preventing atherosclerosis process. This study showed that β-glucan 1,3-1,6 and agaritin might enable to activate the TLR2. However, Dectin-1 receptor might only be activated by 1,3-1,6. β-glucan.

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INTRODUCTION

Atherosclerosis is artery diseases, which occurs slowly and characterized by thickening of the tunica intima. This is because of fibrous accumulation that will gradually become a thrombus (Chistiakov et al. 2013). The formation of atherosclerosis starts with the lumen of the artery endothelial cell dysfunction. This condition occurs after endothelial cells injury or the other stimulus which increasing the permeability of the various components of plasma. Finally, it makes the accumulation of cholesterol and fat (Heriansyah et al. 2016). In general, the process of atherogenesis is divided into four stages, the formation of foam cells, the formation of plaques, rupture of the plaque, and the last is thrombosis.

Agaricus brasiliensis (*A. blazei* ss. Heinemann) has reportedly used for treatment lifestyle-related diseases, including obesity, hypertension, diabetes (Hsu et al. 2008), hypercholesterolemia, hepatitis, atherosclerosis, and heart disease (Firenzuoli et al. 2008). *Agaricus brasiliensis* have ability as anti-inflammatory, antitumor, inhibiting cancer, immune system modulators (Hsu et al. 2008, Karumuthil-Melethil et al. 2008, Ketelhuth and

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Hansson 2011, Rao et al. 2015, Khaled et al. 2015), and analgesic (Gonzaga et al. 2009).

Research of this fungus in cardiovascular still limited comparing with the research on the application of the fungus for anticancer (Reid et al., 2004). *Agaricus blazei* Murill has been proven for preventing the process of atherogenesis through the improvement of dyslipidemia (Reid et al. 2004, Niwa et al. 2011, Naso et al. 2010). Moreover, the fungus also has an activity for improving endothelial dysfunction associated dyslipidemia (Reid et al. 2004). The extract of fungus has activity as an antioxidant, which it contain several bioactive compounds such as β-glucan, phenolic and flavonoid (Chistiakov et al. 2013, Kim et al. 2005). Therefore we intend to examine the potency of the active compound of the *Agaricus blazei* Murill might have an activity to bind to Dectin-1 receptor and TLR-2 that may increase Treg cells to reduce the inflammatory response. This study is critical to explore the potency of the fungus for preventing atherosclerosis process.(Gopalakrishnan et al 2016).

MATERIALS AND METHODS

Retrieving and Modeling Protein

TLR2 was taken from protein data bank with code access 2z7x (Niwa et al. 2011, Kim et al. 2005, Lakshminarayanan et al. 2015) and human sequencing TLR2 from UNIPROT. Meanwhile, TLR-1 was taken from UNIPROT database (Takaku et al. 2001). Dectin human receptor structure was modeled based on mouse Dectin as a template (PDB code 2bpd) (Lakshminarayanan et al. 2015). Human protein amino acid sequence Dectin was taken from UNIPROT (Takaku et al. 2001). The 3D structure was visualized by the Discovery Studio. Agaritin compound was taken from PubChem and drawn by the Discovery Studio.

Docking

Active compounds β-glucan and Agiritin was docked to the active site of protein target, Dectin-1 receptor and TLR 2. Docking process was carried out using autodock vina in Pyrx 0.8 (Xu et al. 2011). It was aimed to identification the inhibition potency and to calculate the binding affinity between ligand (active compound) and substrate (protein target). The binding and molecular interaction were visualized by Discovery Studio.

RESULTS

Docking analysis was done to examine the binding affinity between TLR2/1 complex molecule (receptor) and four ligands (1,3 β-Glucan; 1,3/1,6 β-Glucan; Agaritine; β-Glucan; Positive control). The hydrogen bond, binding position and distance between the receptor and ligands were examined to evaluate potentiality of the ligands in the exciting receptor activity. The binding affinity used as a parameter to determine the ability each ligand bind to the receptor. The result showed that 1,3/1,6 β-Glucan has the highest binding affinity compare with the other four compounds (**Table 1**). While the binding affinity of 1,3 β-Glucan, agaritine, β-Glucan, and a positive control with the TLR2/1 were -7.6, -7.7, -7.5 and -7.7, respectively (**Fig. 1**).

Table 1. Binding affinity between tlr2/1 with its substrate

Substrate	Binding Affinity (Kcal/mol)
1,3 β-Glucan	-7.6
1,3/1,6 β-Glucan	-8.6
Agaritin	-7.7
β-Glucan	-7.5
Positive control	-7.7

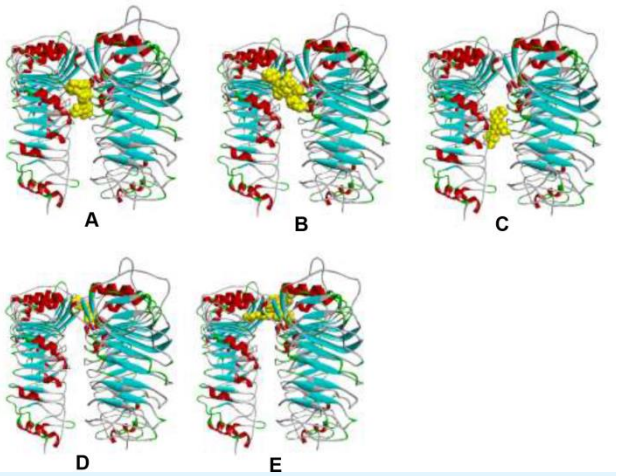

Fig. 1. The interaction between TLR2/1 with Small molecule (yellow). (A) the interaction position between TLR2/1 with 1,3 β-Glucan (B) 1,3/1,6 β-Glucan, (C) Agaritine, (D) β-Glucan, (E) Positive control.

The binding position between 1,3-1,6 β-Glucan with Human Dectin-1 has similarity with the complex between β-Glucan and mouse Dectin-1 (experimental data). This data suggested that the 1,3-1,6 β-Glucan might have a potency to activate the Human Dectin-1. Further analysis indicated that the complex between 1,3-1,6 β-Glucan with Human Dectin-1 has a binding affinity -52 kcal/mol, while β-Glucan with Human Dectin-1 has a binding affinity -59 kcal/mol (**Fig. 2**).

DISCUSSION

The data from the in silico analysis indicated that active compound of *Agaricus blazei* Murill (1,3-1,6 β-Glucan) bound to the TLR2/TLR1 and also Dectin-1. The binding might have an activity to stimulate the signal cascade to produce of anti-inflammation cytokine and the function of Treg Cell. This data correspond with the previous report that explains the fungi increased IL-10 cytokine in human peripheral blood mononuclear cells (hPBMC) (Xu et al. 2011, Jeurink et al. 2008). The main components in *Agaricus blazei* Murill a β-glucan and zymosan (yeast cell wall extract consisting of β glucan, mannan, chitin, proteins, and lipids targeted a specific Dectin-1 receptor (Brown 2006, Førland et al. 2011).

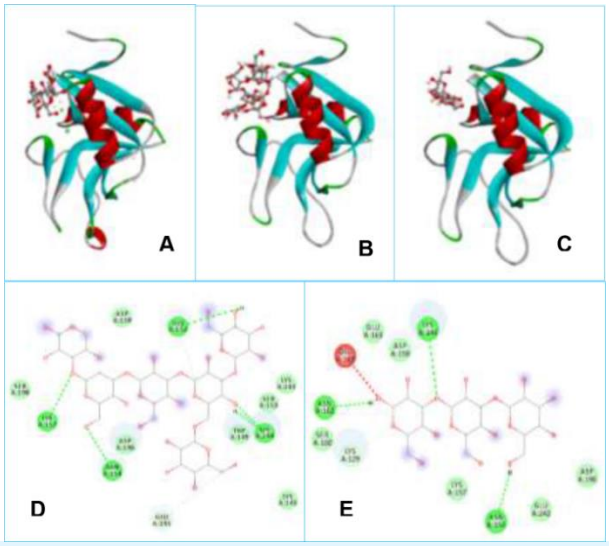

Fig. 2. Bounding of β-Glucan with Dectin. (A) Interaction between β-Glucan with Mouse Dectin-1, (B) 1,3-1,6 β-Glucan with Human Dectin-1, (C) β-Glucan with Human Dectin-1, (D) Molecular bond from 1,3-1,6 β-Glucan with Human Dectin-1 interaction, (E) Molecular bond from β-Glucan with Human Dectin-1 interaction

The Dectin-1 receptor is found in macrophages, monocytes, cells - PMN cells and a subset of T cells (Brown 2006, Førland et al. 2011, Taylor et al. 2002). Dectin-1 was significantly regulated cytokines that associated with Th2, especially IL-4 and IL-13, in which IL-10 (Førland et al. 2011, Taylor et al. 2002, Willment et al. 2003). The receptor is known to collaborate with TLR2 in inducing immune responses balance between the pro and anti-inflammatory (Goodridge and Underhill 2008, Underhill 2007). Signaling on Dectin-1 contributes to the expansion and function of Treg cells. Several studies using zymosan has been shown in simultaneous interaction with TLR-2 and Dectin-1 to induce a large number of suppressor cytokine (Dillon et al. 2006, Slack et al. 2007).

A number of bioactive from fungi including polysaccharides, small molecules, and protein complexes have the benefit of pharmacological (CFR Ferreira et al. 2010, Wasser 2010, Swain et al. 2015). Some study indicated that the zymosan is increased TGF-β1 and IL-10 production, and the suppressive function of $CD4^+CD25^+$ Tregs. The Tregs activation might be mediated through TLR2 and Dectin-1 (Ketelhuth and Hansson 2011). TLR2 recognizes lipoprotein/lipopeptide (LP) and peptidoglycan to form a heteromer with TLR1 or TLR6 to mediate intracellularly (Ketelhuth and Hansson 2011, Omueti et al. 2005, Farhat et al. 2008). Furthermore, TLR2 agonist demonstrates the potential to induce some cytokines suppressor significantly and increasing of $CD4^+CD25^+$ Treg (Zanin-Zhorov et al. 2006). Atherosclerosis is caused by stem activation of monocytes/macrophages in response to the accumulation of oxidized-LDL (Ox-LDL). Therefore upregulation T-reg by the active compound from *Agaricus blazei Murill* (1,3-1,6 β-Glucan) may have beneficial to normalize immune that may lead to preventing atherosclerosis (Sakaguchi et al. 2008).

CONCLUSION

The active compound from *Agaricus blazei* Murill, 1,3-1,6 β-Glucan, able to bind with TLR-2/1 and Dectin that might have the potency to activate Treg Cell and production anti-inflammatory cytokine. This result is a warrant for further study to elucidate the function of the fungi for developing anti-atherosclerosis.

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REFERENCES

Brown GD (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat Rev Immunol 6: 33-43.

CFR Ferreira I, Vaz A J, Vasconcelos MH, Martins A (2010) Compounds from wild mushrooms with antitumor potential. Anti-Cancer Agents Med Chem 10:424-36.

Chistiakov DA, Sobenin IA, Orekhov AN (2013) Regulatory T cells in atherosclerosis and strategies to induce the endogenous atheroprotective immune response. Immunol Lett 151: 10-22.

Dillon S, Agrawal S, Banerjee K, Letterio J, Denning TL, Oswald-Richter K, et al. (2006) Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. J Clin Investig 116: 916.

Farhat K, Riekenberg S, Heine H, Debarry J, Lang R, Mages J, et al. (2008) Heterodimerization of TLR2 with TLR1 or TLR6 expands the ligand spectrum but does not lead to differential signaling. J Leukoc Biol 83: 692-701.

Firenzuoli F, Gori L, Lombardo G (2008) The medicinal mushroom Agaricus blazei murrill: review of literature and pharmaco-toxicological problems. Evid-Based Complement Altern Med 5: 3-15.

Førland DT, Johnson E, Saetre L, Lyberg T, Lygren I, Hetland G (2011) Effect of an extract based on the medicinal mushroom Agaricus blazei Murill on expression of cytokines and calprotectin in patients with ulcerative colitis and Crohn's disease. Scand J Immunol 73:66-75.

Gonzaga ML, Bezerra DP, Alves AP, de Alencar NM, de Oliveira Mesquita R, Lima MW, et al. (2009) In vivo growth-inhibition of Sarcoma 180 by an α-(1→ 4)-glucan–β-(1→ 6)-glucan-protein complex polysaccharide obtained from *Agaricus blazei* Murill. J Nat Med 63: 32-40.

Goodridge HS, Underhill DM (2008) Fungal recognition by TLR2 and Dectin-1. Handb Exp Pharmacol: 183:87.

Gopalakrishnan, S. B., Kalaiarasi, T., & Gnanendra, S. (2016). Evaluation of phytochemical constituents of the fruits of cucumis sativus linn. for their hepatoprotective activity by molecular docking studies. Journal of Food Technology Research, 3(1), 12-22.

Heriansyah T, Siswanto BB, Santoso A (2016) The Effect of Darapladip on Lipid Profile, Insulin, Ox-LDL Serum Level and PVAT Thickness at Atherogenesis Development in DM Tipe 2 Rats Model. Int J Pharm Tech Res 9: 1-8.

Hsu CH, Hwang KC, Chiang YH, Chou P (2008) The mushroom *Agaricus blazei* Murill extract normalizes liver function in patients with chronic hepatitis B. J Altern Complement Med 14: 299-301.

Jeurink PV, Noguera CL, Savelkoul HF, Wichers HJ (2008) Immunomodulatory capacity of fungal proteins on the cytokine production of human peripheral blood mononuclear cells. Int Immunopharmacol 8:1124-33.

Karumuthil-Melethil S, Perez N, Li R, Vasu C (2008) Induction of innate immune response through TLR2 and dectin 1 prevents type 1 diabetes. J Immunol 181: 8323-8334.

Ketelhuth DF, Hansson GK (2011) Cellular immunity, low-density lipoprotein and atherosclerosis: break of tolerance in the artery wall. Thrombosis and Haemostasis 106: 779-786.

Khaled FM, Azza AA, Manal FS, Effat IS (2015) Encapsulation of Nano Beta-Glucan for Preservation of functionality and Targeted Delivery of Bioactive Food Component. Int J ChemTech Res 8: 587-598.

Kim YW, Kim KH, Choi HJ, Lee DS (2005) Anti-diabetic activity of β-glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei.* Biotechnol Lett 27: 483-487.

Lakshminarayanan V, Ponnuswamy R, Rengaraju B (2015) Screening, Purification and Characterization of β-Glucan from a Novel Strain *Bacillus cereus* LVK13 (KC 898956). Screening 8: 1156-62.

Naso D, Cangeri F, de Mello RN, Bona S, Dias AS, Porawski M, et al. (2010) Effect of Agaricus blazei Murill on the pulmonary tissue of animals with streptozotocin-induced diabetes. Exp Diabetes Res 201: 26.

Niwa A, Tajiri T, Higashino H (2011) *Ipomoea batatas* and *Agarics blazei* ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats. J Clin Biochem Nutr 48: 194-202.

Omueti KO, Beyer JM, Johnson CM, Lyle EA, Tapping RI (2005) Domain exchange between human toll-like receptors 1 and 6 reveals a region required for lipopeptide discrimination. J Biol Chem 280: 36616-36625.

Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM (2015) Herbal Medicine for Diabetes Melitus. Int J PharmTech Res 2: 1883-1892.

Reid DM, Montoya M, Taylor PR, Borrow P, Gordon S, Brown GD, et al. (2004) Expression of the β-glucan receptor, Dectin-1, on murine leukocytes in situ correlates with its function in pathogen recognition and reveals potential roles in leukocyte interactions. J Leukoc Biol 76:86-94.

Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133: 775-787.

Slack EC, Robinson MJ, Hernanz-Falcón P, Brown GD, Williams DL, Schweighoffer E, et al. (2007) Syk-dependent ERK activation regulates IL-2 and IL-10 production by DC stimulated with zymosan. Eur J Immunol 37: 1600-1612.

Swain RP, Subudhi BB, Mahapatra AK, Bolapareddi V (2015) Bridging Between Disease, Prevalence and Treatment of Diabetes Mellitus: A Review. Int J PharmTech Res 7: 212-228.

Takaku T, Kimura Y, Okuda H (2001) Isolation of an antitumor compound from Agaricus blazei Murill and its mechanism of action. J Nutr 131:1409-13.

Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomares L, Gordon S, Wong SY (2002) The β-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol 169:3876-3882.

Underhill DM (2007) Collaboration between the innate immune receptors dectin-1, TLRs, and Nods. Immunol Rev 219: 75-87.

Wasser SP (2010) Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms 12:1-16.

Willment JA, Lin HH, Reid DM, Taylor PR, Williams DL, Wong SY, et al. (2003) Dectin-1 expression and function are enhanced on alternatively activated and GM-CSF-treated macrophages and are negatively regulated by IL-10, dexamethasone, and lipopolysaccharide. J Immunol 171: 4569-73.

Xu X, Yan H, Chen J, Zhang X (2011) Bioactive proteins from mushrooms. Biotechnol Adv 29: 667-74.

Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR (2006) Heat shock protein 60 enhances CD4+CD25+ regulatory T cell function via innate TLR2 signaling. J Clin Inves 116: 2022.