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# Zymosan treatment of mouse mast cells enhances dectin-1 expression and induces dectin-1-dependent reactive oxygen species (ROS) generation

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## Abstract

Dectin-1 is a major β-glucan receptor expressed in the innate immune cells such as macrophages, neutrophils and dendritic cells. It can mediate pro-inflammatory mediator release and other cellular responses such as phagocytosis and respiratory burst in response to pathogens. Mast cells are sentinel cells of the immune system found in greater numbers at sites of pathogen exposure such as the skin and airways than in other body sites. Dectin-1 on human mast cells has been shown to mediate the production of leukotrienes in response to yeast zymosan. In this study, using RT-PCR and FACS analysis, we examined both mRNA and protein expression of dectin-1 on bone marrow-derived cultured mast cells (BMMC) from either C57BL/6 or TLR2 deficient mice. Low levels of surface dectin-1 were detected on the mast cell surface, which could be up-regulated by zymosan activation. Neither mouse plasma nor decomplemented plasma (56 °C, 30 min) induced altered dectin-1 protein expression although zymosan-activated C57BL/6 BMMCs expressed two dectin-1 mRNA isoforms. Addition of laminarin, a well-established dectin-1 inhibitor, significantly inhibited surface expression of dectin-1 ($p<0.05$), which further confirmed that dectin-1 surface expression was up-regulated by non-opsonized zymosan activation. Further studies showed that zymosan stimulated both C57BL/6 and TLR2(−/−) deficient BMMCs to generate intracellular oxidative burst. Pretreatment of BMMCs with laminarin inhibited ROS generation significantly ($p<0.05$) after 2 h zymosan activation. Therefore, intracellular ROS generation in murine mast cells in response to zymosan is dependent on dectin-1 receptors.


***Keywords:*** Dectin-1; Innate immunity; Reactive oxygen species; Mast cells; Zymosan

## Introduction

Dectin-1, an important β-glucan surface receptor, is expressed on a broad range of innate immune cells such as monocytes/macrophages, neutrophils, NK cells, fibroblasts and dendritic cells (Ariizumi et al., 2000; Kougias et al., 2001; Taylor et al., 2002; Williams, 1997). β-glucan is a major fungal cell wall component, and dectin-1 has been found to play an important role in the innate immunity against pathogenic fungi such as *Coccidiodies posadasii*, *Pneumocystis carinii* and *Aspergillus fumigatus* (Hohl et al., 2005; Steele et al., 2003; Viriyakosol et al., 2005). Recently, two different, functionally distinct dectin-1 isoforms were observed in murine macrophages. Either isoform can stimulate TNF production, but to different degrees (Heinsbroek et al.,

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2006). Dectin-1 is a type II transmembrane protein receptor with a C-type lectin-domain and a cytoplasmic tail with ITAM motif (Adachi et al., 2004) phosphorylation of which can stimulate pro-inflammatory mediators release and other cellular responses such as phagocytosis and respiratory burst in collaboration with TLR-2 (Brown et al., 2002; Gantner et al., 2003). However, recent studies showed that early inflammatory responses of mast cells to pathogens can often be attributed to complement receptors, not TLR-2 (Mullaly and Kubes, 2007). Several studies also showed that dectin-1 could interact with zymosan as well as yeast from *Candida albicans*, which stimulated phagocytosis and ROS generation in a TLR-independent manner (Rogers et al., 2005; Underhill et al., 2005). Little information is available concerning dectin-1 expression on murine mast cells and its role in regulating cellular responses to $\beta$-glucan.

Zymosan derived from the cell wall of *Saccharomyces cerevisiae* is rich in both $\beta$-glucan and mannan (Di Carlo and Fiore, 1958). It has been widely used as a model fungal particle to study immune responses conducted by different innate and adaptive immunity cells. Zymosan not only interacts with leukocytes, such as macrophages via non-opsonic dectin-1 receptors to stimulate pro-inflammatory mediator or cytokine release (Brown et al., 2002; Heinsbroek et al., 2006), but also can interact with complement or TLR-2 receptors in mediating inflammatory responses, such as phagocytosis (Gantner et al., 2003; McCurdy et al., 2003; Mullaly and Kubes, 2007). Both mast cells and other leukocyte populations have been shown to have important roles in the response to zymosan in models of peritonitis (Kolaczkowska et al., 2001, 2007). Recently, we described that fungal zymosan can stimulate human mast cells to produce $LTC_4$ in a dectin-1-dependent manner (Olynych et al., 2006).

Mast cells are distributed widely throughout the body tissues, which are commonly found at submucosal surfaces of skin, airway and the intestine. Mast cells function as sentinel cells in host defense, including enhancement of both innate and specific defenses, against pathogens. Mast cells are well known to release a large amount of pro-inflammatory and inflammatory mediators, which contribute to both immediate allergic reactions and inflammation (Marshall, 2004). They are also capable of phagocytosis of invaded pathogens, which plays an important role in innate immunity (Abraham and Malaviya, 1997). Following phagocytosis, the production of reactive oxygen species (ROS) is critical for pathogen killing (Forman and Torres, 2001; Henricks and Nijkamp, 2001; Malaviya et al., 1999, 1996). There are many substances such as IgE/Ag, calcium ionophore A23187, gold compound, D-penicillamine, compound 48/80, nerve growth factor and others (Brooks et al., 1999; Niu et al., 1996; Wolfreys and Oliveira, 1997) that can stimulate mast cells to generate intracellullar ROS. ROS generation has also been demonstrated to be frequently accompanied by degranulation (Swindle et al., 2002, 2004). Inhibition of ROS generation leads to decreased release of mediators such as histamine and $LTC_4$ in RBL-2H3 mast cells (Suzuki et al., 2003). Several studies showed ROS such as potassium superoxide or hydrogen peroxide could induce mast cells to degranulate (Akagi et al., 1994; Peden et al., 1994). In macrophage cell lines, dectin-1 mediates zymosan-induced ROS generation (Gantner et al., 2003). It is not yet known whether zymosan can induce intracellular ROS generation in mast cells after phagocytosis and the role of dectin-1 in ROS generation has not been determined in mast cells.

In this study, we examined murine mast cell expression of dectin-1 and intracellular ROS generation in response to zymosan as a model of fungal-induced ROS generation. Even though the level of dectin-1 expression on murine mast cells was far lower than that on the cell surface of macrophages, it was demonstrated that zymosan-induced intracellular ROS generation was dectin-1 dependent in mast cells. Furthermore, dectin-1 expression can be up-regulated by zymosan activation alone. To our knowledge, this is the first study to show the relationship between dectin-1 and ROS production in mast cells.

## Methods

### Reagents

Yeast zymosan from *S. cerevisiae* and laminarin were purchased from Sigma (St. Louis, MO). Dichlorodihydrofuorescein diacetate (DCFH-DA) was obtained from Molecular Probes (Eugene, OR). Zymosan suspensions were made in endotoxin free saline and vortexed immediately before use to ensure particles were equally distributed in suspension. Laminarin was also dissolved in endotoxin free saline and sonicated immediately prior to use. The primary Abs used in this study were 2A11, monoclonal rat anti-mouse dectin-1 receptors conjugated with biotin (ABD Serotec, Raleigh, NC). The secondary Abs were Streptavidin-PE obtained from BD pharmingen (San Diego, CA). IgG2b-biotin (eBioscience, San Diego, CA) was used as an isotype control.

### Mice

C57BL/6 (Jackson Labs, Bar Harbor, Maine) mice and TLR2-deficient mice on a C57BL/6 background (a gift from Dr. S. Akira, Osaka, Japan) were used in this study. All mice were 6–10 weeks of age and housed