การตอบสนองที่แตกต่างกันของเดนไดรติกเซลล์ต่อแมนแนนในผนังเซลล์ของเชื้อรา

นายสิรวิชญ์ จิรวรรณาภรณ์

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DIFFERENTIAL DENDRITIC CELL RESPONSE TO FUNGAL CELL WALL MANNANS

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สิรวิชญ์ จิรวรรณาภรณ์ : การตอบสนองที่แตกต่างกันของเดนไดรติกเซลล์ต่อแมนแนนในผนังเซลล์ของเชื้อรา (DIFFERENTIAL DENDRITIC CELL RESPONSE TO FUNGAL CELL WALL MANNANS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: พัชรี ฤทธิ์ประจักษ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อรนาฎ มาตังคสมบัติ{, หน้า.

แมนแนนในผนังเซลล์ของเชื้อรามีบทบาทสำคัญในการกระตุ้นภูมิคุ้มกันโดยกำเนิด อย่างไรก็ตามยังไม่มีการศึกษาเกี่ยวกับผลของโครงสร้างแมนแนนที่แตกต่างกันต่อการตอบสนองของภูมิคุ้มกันโดยกำเนิด ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาบทบาทของโครงสร้างแมนแนนที่แตกต่างกันในผนังเซลล์ของเชื้อราแซคคาโรไมซิส เซเรวิซีอี ต่อการตอบสนองของเดนดริติกเซลล์ โดยทำการกระตุ้นเดนดริติกเซลล์ที่พัฒนามาจากไขกระดูกด้วยยีสต์สายพันธุ์ปกติ และยีสต์ที่ไม่มียีนส์ที่เกี่ยวข้องกับการเติมแมนแมนโครงสร้างต่าง ๆ ในผนังเซลล์ ซึ่งยีสต์ดังกล่าวจะถูกทำให้ตายด้วยความร้อนและนำมากระตุ้นเดนดริติกเซลล์ที่พัฒนามาจากไขกระดูก จากนั้นทำศึกษาระดับการแสดงออกของไซโตไคน์ยีนส์ที่เกี่ยวข้องกับการเหนี่ยวเฮลเปอร์ทีลิมโฟไซต์ชนิดต่างๆ ด้วยการวิเคราะห์การแสดงออกของสารพันธุกรรมเชิงปริมาณ โดยใช้เทคนิคปฏิกิริยาลูกโซ่โพลีเมอเรสในสภาพจริง

จากผลการวิจัยพบว่ายีสต์ที่ไม่มียีนส์เอ็มเอ็นเอ็น 1 ซึ่งทำให้ขาดโครงสร้างแมนแนนแอลฟา 1,3 สามารถเพิ่มการแสดงออกของยีนส์อินเตอร์ลิวคิน-12 แอลฟา และอินเตอร์เฟียรอนแกมมาอย่างมีนัยสำคัญในเดนไดรติกเซลล์เมื่อเทียบกับยีสต์สายพันธ์ปกติ และยีสต์ไม่มียีนส์เอ็มเอ็นเอ็น 2 ซึ่งทำให้ขาดโครงสร้างแมนแนนแอลฟา 1,2 และ 1,3 สามารถเพิ่มการแสดงออกของยีนส์อินเตอร์เฟียรอนแกมอย่างมีนัยสำคัญในเดนไดรติกเซลล์ โดยอินเตอร์ลิวคิน-12 และอินเตอร์เฟียรอนแกมมาเป็นไซโตไคน์สำคัญต่อการแปรสภาพของทีเฮลเปอร์ 1 นอกจากนี้ยังพบว่าการกระตุ้นเดนไดรติกเซลล์ด้วยยีสต์ที่ไม่มียีนส์โอซีเฮช 1 ซึ่งทำให้ขาดโครงสร้างเอ็นลิงค์แมนแนน มีผลทำให้มีการแสดงออกที่เพิ่มขึ้นอย่างมีนัยสำคัญของไซโตไคน์ยีนส์ชนิดอินเตอร์ลิวคิน 1 ซึ่งมีความสำคัญในการแปรสภาพของทีเฮลเปอร์ 17 และอินเตอร์ลิวคิน 23 ซึ่งทำหน้าที่ช่วยเพิ่มจำนวนและควบคุมการทำงานของทีเฮลเปอร์ 17 จากผลการศึกษาดังกล่าวแสดงว่าโครงสร้างแมนแนนที่แตกต่างกันในผนังเซลล์ของเชื้อราแซคคาโรไมซิส เซเรวิซีอีมีผลต่อการกำหนดทิศทางการตอบสนองของเดนไดรติกเซลล์ ซึ่งอาจมีอิทธิพลต่อการแปรสภาพของทีเซลล์ได้ ผลงานวิจัยนี้จะสามารถนำไปต่อยอดเพื่อการพัฒนาสารเสริมฤทธิ์ชนิดคาร์โบไฮเดรตได้ในอนาคต

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KEYWORDS: : PHOSPHOMANNAN, DENDRITIC CELL, SACCHAROMYCES CEREVISIAE, CYTOKINES, ADJUVANT

SIRAWIT JIRAWANNAPORN: DIFFERENTIAL DENDRITIC CELL RESPONSE TO FUNGAL CELL WALL MANNANS. ADVISOR: PATCHAREE RITPRAJAK, D.D.S.,Ph.D., CO-ADVISOR: ASSOC. PROF. ORANART MATANGKASOMBUT, D.D.S.,Ph.D.{, pp.

Mannan in fungal cell wall plays a crucial role in the activation of innate immunity, however it has not been studied how different mannan structure involves in innate immune responses. Thus, this study aims to investigate the effects of *Saccharomyces cerevisiae* possessing different cell wall mannan structures on dendritic cells (DCs) responses. Bone marrow-derived dendritic cells (BM-DCs) were stimulated with heat-killed *S. cerevisiae Wt* and mutants that lack of the single gene involving in each step of mannosylation. Then, mRNA expression of T helper lymphocyte (Th)-inducing cytokines was determined by quantitative real-time PCR.

The results demonstrated that *S. cerevisiae mnn1Δ*, lacking of α-1,3 mannan, notably enhanced the expression of Th-1 inducing cytokines, *Il12a* and *Ifng*, while *mnn2Δ*, lacking of α-1,3 and α-1,2 mannan, significantly increased *Ifng* expression in BM-DCs when compared to *Wt*. Furthermore, BM-DCs stimulated with *och1Δ*, which N-linked mannan was absence, showed the significant increase in Il1a, Il1b, and Il23a, the cytokines involving in Th17 induction and amplification respectively. The data suggested that the different mannan structures affected DC responses, which may consequently influence Th differentiation. This knowledge will be beneficial for the development of carbohydrate adjuvants in the future.

Field of Study:	Medical Microbiology	Student's Signature	.....
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CHAPTER I
INTRODUCTION

Adjuvants are immunological substances used to improve vaccine efficacy by several ways, such as recruiting immune cells to the injection site, boosting the adaptive immune responses, and directing the immunity to protect against infectious diseases. The major mechanism of adjuvant is to act as pathogen-associated molecular patterns (PAMPs), which bind to pathogen recognition receptors (PRRs) on antigen presenting cells (APCs), especially dendritic cells (DCs). The interaction between PAMPs and PRRs results in the maturation, migration and cytokine production of DCs, which consequently directing the immune response (1, 2). Nowadays, there are only five licensed adjuvants, alum, MF59, AS093, virosomes, and AS04. However, these adjuvants still lack the ability to induce adequate cell-mediated immunity (1, 3-7). Thus, it is important

to develop new formulations of adjuvants and vaccines simultaneously.

Carbohydrate is a natural compound that can bind with various PRRs, such as C-type lectin receptors (CLRs) and Toll-liked receptors (TLRs), resulting in the induction of immunity (8). There are 4 major types of carbohydrate adjuvants; glucans, fructans, chitins and mannans, and each type possesses distinct ability to interact with PRRs (9). *Saccharomyces cerevisiae* is a non-pathogenic yeast which has been used for vaccine delivery. The cell wall of *S. cerevisiae* comprises mannans, glucans and chitins (10, 11). Mannan is a polymer of mannose that can be recognized by its specific PRRs e.g. TLR-2, TLR-4, dectin, DC-SIGN, mannose recptor, mincle, FcγR, and langerin, depending on the sugar structures (12).

By taking the advantage of the availability of single-gene deletion mutants of various genes involved in the synthesis of mannan structures of *S. cerevisiae*, this study tested the effects of different mannan structures on DCs and immune responses.

Our findings will be beneficial for the development of carbohydrate adjuvants in the future.

CHAPTER II
OBJECTIVES

To study the responses of bone marrow-derived dendritic cells to different mannan structures of *Saccharomyces cerevisiae in vitro.*

CHAPTER III
LITERATURE REVIEW

1. Vaccine

Vaccines are the most cost-efficient tools to promote individual and public health by particularly preventing serious infections. The aim of vaccine is to stimulate a specific or an acquired immunity, which possesses a memory ability that responds more vigorously when repeatedly exposes to the same pathogen (13). Vaccination induces specific immunity via the potent antigen-presenting cell, a dendritic cell (DC). When DC is activated, it maturates and migrates into peripheral lymphoid organs. Antigen presentation to T cells by DC leads to T cell activation, proliferation and differentiation (13, 14). Vaccines are classified as the follow;

1) Live attenuated pathogen is the weakened pathogen that cannot cause the diseases. It can be made by growth under abnormal culture conditions or genetic engineering. The

advantages are long live immune memory, single immunization, and induction of strong immune responses. However, this kind of vaccine can be a double-edged sword because it can revert into the pathogenic form in some condition and it induces the complex immune response due to many PAMPs (5).

2) Inactivated pathogen vaccine is made by using physical or chemical agents to kill the pathogen to prevent the ability to cause the disease. The advantage of this vaccine is that it could be safer than the live attenuated vaccine. However, the immunogenicity is also decreased, so multiple doses is required. Furthermore, it is difficult to choose the method of inactivation due to the risk of important antigen denaturation (5).

3) Subunit vaccine is the vaccine that contain purified antigens from pathogen instead of using the whole organism. Therefore, the advantages include safety and less complex immune response due to the use of a single antigen. However, the disadvantages are that the immunogenicity is very poor, and the antigens may not retain their native conformation due to

extraction protocol which could mislead the immune responses (15).

4) Synthetic antigen vaccine is a vaccine containing mainly synthetic peptides, carbohydrates, or antigens. The advantages are similar to subunit vaccine. However, since synthetic antigen vaccine has no native structure, it may not bind to PRRs on antigen presenting cells, such as DCs, which promote pathogen uptake and direct the adaptive immune response (16).

5. DNA vaccine uses genes that encode important pathogen antigens inserted into a vector, usually a virus that has a very low virulence. It provides a safe way of vaccination and could induce prolonged immune memory. However, there is a risk of interfering with host genes that control important functions, such as cell growth, and that it may induce immunological tolerance of the antigen (17).

The ideal vaccine is the vaccine that has high level of safety and efficacy. Thus, to develop the ideal vaccine we may

need the helper called “adjuvant”. Among the types of vaccines listed above, almost all vaccines need adjuvant except the live attenuated vaccine and some inactivated vaccine.

2. Adjuvants

2.1 Adjuvant classification

2.1.1 Particulate adjuvant is the adjuvant that forms particles for better induction of the immune responses (1, 18).

2.1.2 Aluminium salts is the adjuvant that compose of aluminium hydroxide or alum with the size between 100-1000nm (4, 18).

2.1.3 Water-in-oil emulsion is the adjuvant that composed of water in mineral oil phase, such as Freund incomplete adjuvant (FIA) (18)

2.1.4 Oil-in-water emulsion is the adjuvant that composed of mineral oil in water phase (18).

2.1.5 Immune stimulating complex (ISCOM) is the adjuvant that looks like the open-cage with the size around 40

nm and composed of cholesterol, lipid, immunogen, and some mineral salts, such as saponins (18).

2.1.6 Liposome is the adjuvant that composed of lipid bilayer with the size around 20nm to 3µm (18).

2.1.7 Nano and microparticles is the adjuvant with very small size 1-100µm for microparticles and 10-100nm for nanoparticles (18).

2.2 Adjuvant mechanisms of action

2.2.1 **Immunomodulation Adjuvant** can regulate the direction of adaptive immune response by binding to the PRRs in antigen presenting cell and inducing different T-cell differentiation cytokines secretion. For example, flagellin purified from microbes can bind to Toll-like receptor 5 (TLR-5), resulting in secretion of IL-12p70 cytokine, a T-helper 1 differentiation cytokine, from antigen presenting cells (1, 3, 18).

2.2.2 **Presentation** Some adjuvants can enhance antigen presentation by MHC molecules, which can be the result

of many mechanisms such as the interaction between adjuvant and PRRs (1, 3, 18).

2.2.3 **Enhanced antigen uptake** by antigen presenting cells by regulating the danger signal and inflammation (1, 3, 18).

2.2.4 **Immune cell recruitment** by inducing inflammation or danger signal (1, 3, 18).

2.3 Currently licensed adjuvants

There are several classes of adjuvants e.g. mineral salts, emulsion, microbial derivatives, cytokines, immune stimulating complexes (ISCOM), particulate compounds, and carbohydrate (19, 20), which possesses the different immune induction properties. Thus, the mixture of vaccine with appropriate adjuvant is crucial for the efficient immune induction. At present, there are five licensed adjuvants used in human vaccines (Table 1).

2.3.1 Alum, which is composed of aluminium salts, can induce good antibody response and T helper 2 response, but poor T helper 1 response (3, 5, 21).

2.3.2 MF59, which is composed of squalene, polysorbate 80, sorbitan trioleate with oil-in-water form, can induce good antibody response and balanced T helper 1 and T helper 2 response. (3, 5, 21).

2.3.3 AS03, which is composed of squalene, Tween 80, and α-tocopherol with oil-in-water form, can induce good antibody response and T helper 1 response (3, 5, 21).

2.3.4 AS04 is composed of Aluminium hydroxide and monophosphoryl lipid A. It is an Alum-absorbed TLR-4 agonistic adjuvant that induces good antibody response and increase immune memory (3, 5, 21).

2.3.5 Virosome, which is composed of lipids and hemagglutinin with liposome form, induces antibody response and cytotoxic T lymphocyte (3, 5, 21).

Table 1 Currently licensed adjuvants

Adjuvant	**Classification**	**Immune induction**	**Reference**
Alum	Mineral salts	Ab Th 2 poor Th1	(3, 5, 21)
MF59	Oil-in-water emulsion	Ab Balanced Th1 and Th2 response	(3, 5, 21)
AS03	Oil-in-water emulsion	Ab Th1	(3, 5, 21)
AS04	Alum-absorbed TLR-4 agonists	Ab Increase immune memory	(3, 5, 21)
Virosome	Liposomes	Ab CTL	(3, 5, 21)

2.4 Current problems of adjuvants

Most of the currently licensed adjuvants stimulate humeral-mediated immune response, but not satisfactory cell-

mediated immune responses which are required in many infectious diseases. For example, the protective immunity against Tuberculosis requires the T-helper 1 responses, and the protective immunity against HIV infection required responses from CTL (1, 3-7).

2.5 Current new adjuvant development

The current new adjuvant development begins to use part of the pathogens or non-pathogens, such as Flagellin (TLR-5 ligand), Pam3Cys (TLR-2 ligand), or CpG (TLR-9 ligand) (5).

3. Carbohydrate adjuvant

Carbohydrate is one type of adjuvant that has the ability to bind with many PRRs and results in various direction of immune responses.(8) There are four major types of carbohydrate adjuvants.

3.1 Glucans are composed of repeating units of D-glucose binding together by glycosidic bonds in various conformations. Alpha-glucans (α-glucans), such as Dextran from *Streptococcus bovis,* can induce protective antibody. β-glucans, such as

Zymosan from yeast cell wall, induces nonspecific resistance against some bacteria and fungi (8, 9).

3.2 Fructans are composed of repeating units of Fructose binding together by glycosidic bonds, such as ɣ-Inulin, which induces both humoral and cellular immunity (8, 9).

3.3 Mannans are composed of repeating units of Mannose binding together by glycosidic bonds, such as N-linked mannan that induces various immune responses (8, 9).

3.4 Chitin is linear β-1–4-linked polymer of D-glucosamine and *N*-acetyl-D-glucosamine. It can induce inflammatory cytokines including IL-12, IL-18, TNF-α and IFN-γ and enhanced antibody production (8, 9).

4. Carbohydrate constituents of yeast cell wall and immune responses

4.1 Fungal cell wall

Fungal cell wall is mostly composed of polysaccharides, which are mannan, glucan, and chitin. The differences in

structures and proportion of cell wall polysaccharides have been shown to influence the type of immune responses (10, 22).

4.2 Immune induction by carbohydrate constituents in fungal cell wall

The innate recognition of fungal cell wall can be recognized by various groups of PRRs (Table 2). For example, C-type lectin receptors (CLR) can recognize N-linked mannan, α-Glucan, β-Glucan and chitin, while toll-like receptors (TLRs) can recognize O-linked mannan, RNA and DNA.

Table 2: Innate recognition receptors for fungal cell wall components

PRRs	Family	Ligands	References
DC-SIGN	C-type lectin	N-linked mannan, α-Glucan, β-Glucan and chitin	(2, 22, 23)
Dectin-1	C-type lectin	N-linked mannan, α-Glucan, β-Glucan and chitin	(2, 22, 23)
Dectin-2	C-type lectin	N-linked mannan, α-Glucan, β-Glucan and chitin	(2, 22, 23)
Mincle	C-type lectin	N-linked mannan, α-Glucan, β-Glucan and chitin	(2, 22, 23)
Galactin-3	C-type lectin	Β-mannosides	(2, 22, 23)

CR3	Complement receptor	C3b or C3d	(2, 22, 23)
CD36	Class B scavenger receptor	B-glucan	(2, 22, 23)
TLR-2	Toll-like receptor	O-linked mannan, RNA and DNA.	(2, 22, 23)
TLR-3	Toll-like receptor	O-linked mannan, RNA and DNA.	(2, 22, 23)
TLR-4	Toll-like receptor	O-linked mannan, RNA and DNA.	(2, 22, 23)
TLR-6	Toll-like receptor	O-linked mannan, RNA and DNA.	(2, 22, 23)
TLR-9	Toll-like receptor	O-linked mannan, RNA and DNA.	(2, 22, 23)

4.3 The role of mannan in immune responses

Mannan is an outer cell wall component that initially interacts with immune cells(10, 22). Previous report showed that ablation of *OCH1* gene, which leads to defect in N-linked mannan elongation, shifted specific immune response from Th1 to Th17 (24). In addition, deletion of β-1,2 mannan in *C. albicans* cell wall obviously enhanced TNF-α, IL-6 and IL-12 production in DC (25). Hence, the structure of cell wall mannan

may play a crucial role in the differential DC responses and T cell differentiation.

4.4 *Saccharomyces cerevisiae* single gene deletion and its benefit

4.4.1. Saccharomyces cerevisiae

Saccharomyces cerevisiae is a fungal species with cell wall containing mannans, glucans, and chitins (10, 11). It is a candidate for development of new adjuvant because *S. cerevisiae* is non-pathogenic. Furthermore, genes involved in mannan glycosylation are well characterized, and strains with deletions of these genes are readily available in a deletion library.(11, 26, 27) In addition, previous studies showed that whole heat-killed recombinant *S. cerevisiae* expressing HIVAX-Gag markedly enhanced DC maturation and IL-12 production, and subsequently augmented antigen-specific CTL responses (28). Furthermore, recombinant *S. cerevisiae* containing E.G7 protein notably increased CTL numbers resulting in the reduction of lymphoma progression (29).

The glycosylation process of N-linked mannan is regulated by many genes (30). It has been shown that *OCH1* gene plays a role in the initiation of the first α-1,6 mannosylation, and *MNN9* and *VAN1* genes are involved in the elongation of α-1,6 mannose backbone (31). *MNN2*, *MNN5 and MNN1* genes have been revealed to control the glycosylation of the first, the second α-1,2 mannose, and the terminal α-1,3 mannose residues, respectively (26, 30). In addition, previous reports have shown that *MNN4* and *MNN6* genes encode enzymes transferring mannosylphosphate to both O-linked and N-linked mannan (Fig. 1)(30, 32).

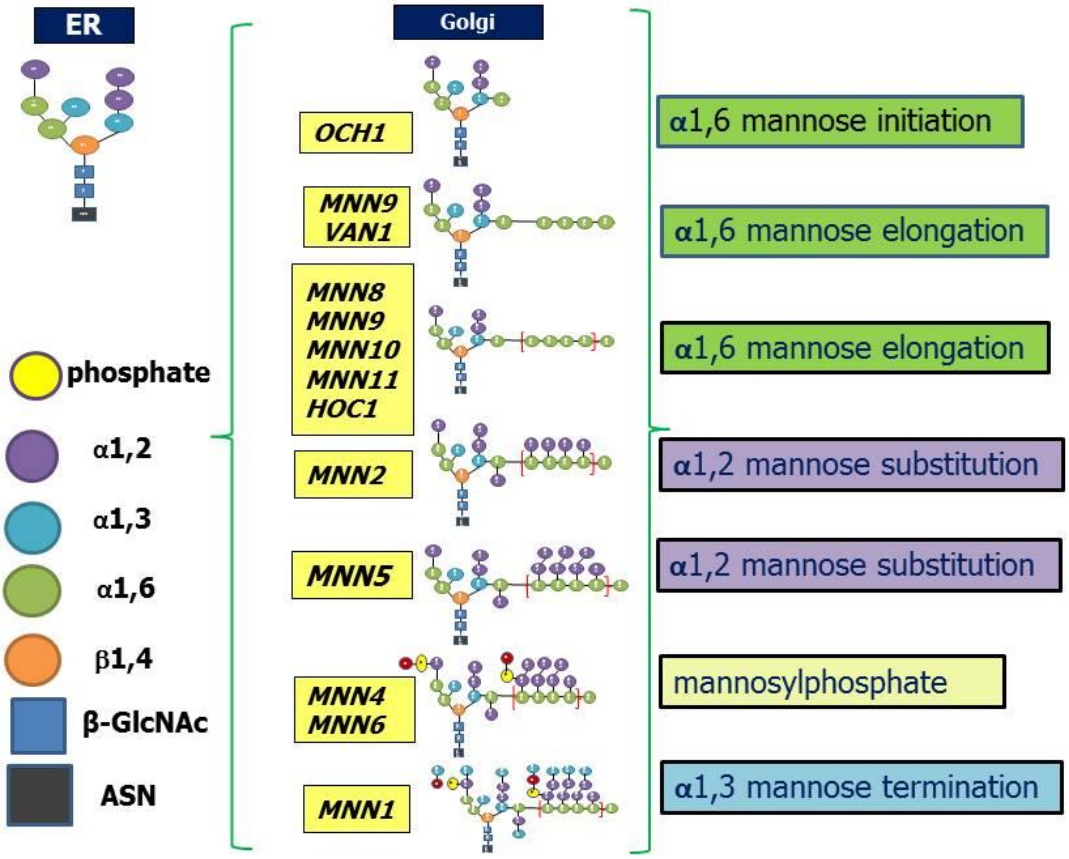


Figure 1 Glycosylation of N-linked mannan infungal cell wall (30).

If we select the single gene deletion from the library in each step we assumed that the structure of the mutant would be like in **(Fig. 2)**

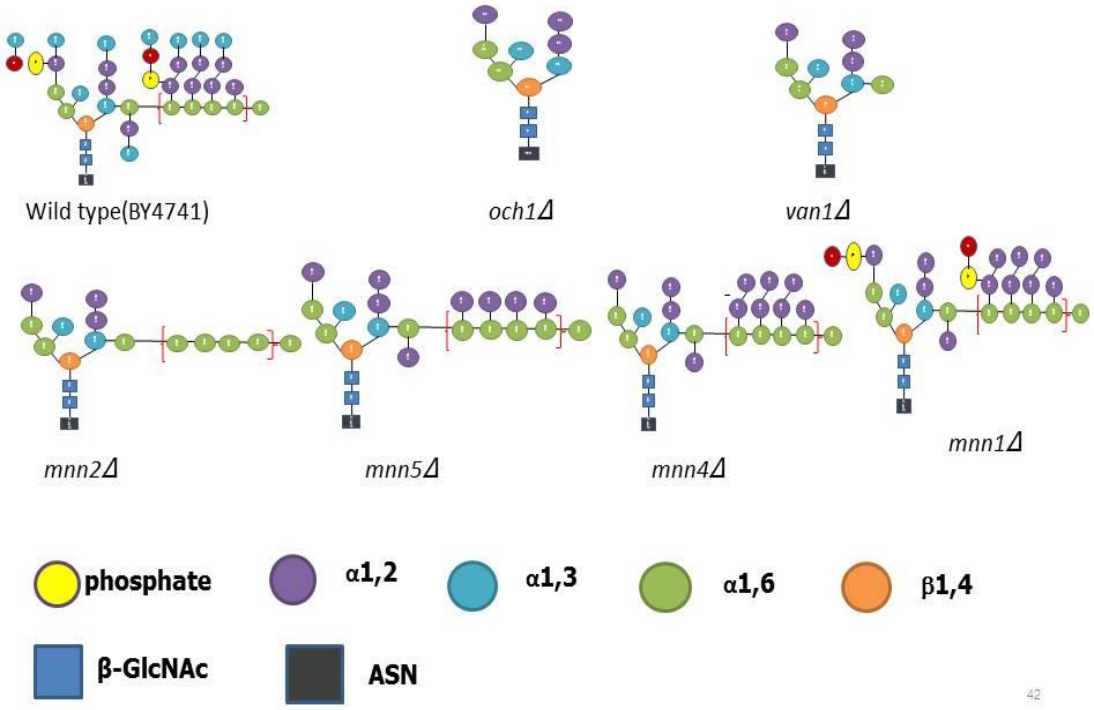


Figure 2 N-linked mannan cell wall structure of single gene deletion mutants.

We hypothesize that single deletions of *S. cerevisiae* genes controlling the production of N-linked mannans in the cell wall will affect their structures, and lead to differential responses of DCs. Our result will provide crucial information for the development of natural carbohydrate adjuvants, and will be able to apply for the public health prevention and clinical therapeutic treatment.

CHAPTER IV
MATERIALS AND METHODS

PART I Yeast culture and preparation

1. *Saccharomyces cerevisiae* strains

Saccharomyces cerevisiae BY4741 WT(wild type), *och1Δ, mnn1Δ, mnn2Δ, mnn4Δ, mnn5Δ, and van1Δ* mutants were obtained from the Mat**a** haploid yeast deletion library (Invitrogen, USA), and stored in 15% glycerol at -80^{o}C.

2. Yeast culture

Yeast strains were cultured in Yeast Peptone Dextrose (YPD) media, containing yeast extract (HiMedia), peptone (HiMedia), and glucose (Univar), at 30^{o}C with 200 rpm shaking for 15 hours. Then, the yeast suspension was diluted to OD_{600} of 0.1 and was continued incubating until OD_{600} reach 0.6 (24).

3. Growth curve determination

Yeasts were cultured from OD_{600} of 0.1 for 24 hours, and the growth curve were determined by measuring OD_{600} at 2, 4, 6, 8, 16 and 24 hours.

4. Heat-killed yeast preparation

Yeast cells were collected from the culture, washed 3 times in sterile PBS, and resuspended in PBS at the concentration of $1x10^8$ cells/ml. The cells were incubated at 65^oC for 2 hours in the preliminary experiments and 100^oC for 10 minutes in all subsequent experiments.

PART II Bone marrow-derived dendritic cell culture and stimulation

1. Balb/c mice

The 5-week old female Balb/c mice were purchased from The National Laboratory Animal Center, Mahidol University (NLAC-MU). All procedures were reviewed and approved by the Animal Care and Use Committee of Faculty of Medicine, Chulalongkorn University (Ethics certificate number 7/58).

2. Generation of BM-DCs

Bone marrow cells were collected from femurs of mice and cultured in RPMI medium (GIBCO®, Invitrogen)

supplemented with 10% fetal bovine serum (GIBCO®, Invitrogen), 100 U/ml Penicillin and 0.1 mg/ml Streptomycin (GIBCO®, Invitrogen), 10 ng/ml GM-CSF (PeproTech) and 10 ng/ml IL-4 (PeproTech). The cells were seeded in 24-well plate at the concentration of $1x10^6$ cells/ ml. BM cells were cultured at 37^oC in 5% CO_2 for 7 days, and the culture media were changed every 2 days.

3. BM-DC stimulation

In preliminary experiment, BM-DCs were stimulated with heat-killed WT and mutants at various DC:Yeast ratios for 2 and 4 hours for gene expression analysis, and 24 hours for flow cytometric analysis.

In all subsequent experiments, BM-DCs were stimulated with heat-killed WT and mutants at DC:Yeast ratio of 1:5 for 2 and 4 hours for gene expression analysis and 24 hours for flow cytometric analysis.

PART III Genes expression analysis

1. RNA extraction

The stimulated BM-DCs were harvested and total RNA was extracted by using TRIzol® Reagent (Invitrogen, USA) and chloroform, and precipitated by isopropanol and ethanol. RNA was dissolved in DEPC water, and the concentration was measured by Nanodrop (Thermo Scientific, USA) and stored at -80^{o}C.

2. cDNA synthesis

The mRNA was converted to cDNA by SuperScript® III First-Strand Synthesis reverse transcriptase kits (Invitrogen) according to manufacturer's instruction, and stored at -80^{o}C.

3. Quantitative PCR analysis

The mRNA levels were examined by Real-Time PCR (Light cycler 480, Roche). The PCR mixture contained 2 μl of water, 0.5 μl of 10 pmol/μl primers, 5 μl of Master mix (Roche, USA), and 2 μl of cDNA. The PCR was amplified for 40 cycles

with the conditions shown in Table 3. The sequences of the primers are as previously described (33) and shown in Table 4.

Table 3 Real-time PCR Cycle

Step	Temperature	Time
Pre-incubation	95°C	5 minutes
Denature	95°C	10 seconds
Annealing	60°C	10 seconds
Extension	72°C	30 seconds

Table 4 Real-time PCR primer sequences (33).

Primers	Sequences
1. *Il1a*	5’- TCCAGGGCAGAGAGGGAGT -3’ 5’- GGAACTTTGGCCATCTTGATTT-3’
2. *Il1b*	5’- GTGGCTGTGGAGAAGCTGTG -3’ 5’- GAAGGTCCACGGGAAAGACAC -3’
3. *Il14*	5’- GCAACGAAGAACACCACAGA -3’ 5’- AAATATGCGAAGCACCTTGG -3’
4. *Il16*	5’- CCAGAAACCGCTATGAAGTTCC -3’ 5’- TTGTCACCAGCATCAGTCCC -3’
5. *Il110*	5’- GAAGCTGAAGACCCTCAGGA -3’ 5’- TTTTCACAGGGGAGAAATCG -3’
6. *Il12a*	5’- CAGAAACCTCCTGTGGGAGA -3’ 5’- GGAGCTCAGATAGCCCATCA -3’
7. *Il12b*	5’- ATCCAGCGCAAGAAAGAAAA -3’ 5’- GGAACGCACCTTTCTGGTTA -3’
8. *Il123a*	5’- GCACCTGCTTGACTCTGACA -3’ 5’- CATGGGGCTATCAGGGAGTA -3’

9. *Ifng*	5’- ATCTGGAGGAACTGGCAAAA -3’ 5’- TCTGGCTCTGCAGGATTTTC -3’
10. *Tgfb1*	5’- CCTGAGTGGCTGTCTTTTGACG -3’ 5’- AGTGAGCGCTGAATCGAAAGC -3’
11. *Gapdh*	5’- ATCACTGCCACCCAGAAGAC -3’ 5’- ATGAGGTCCACCACCCTGTT -3’

4. mRNA expression calculation

The relative mRNA expression was calculated by the formula: $2^{-(\Delta CPtarget\ gene\ -\ \Delta CPgapdh)}$, and the fold changes were calculated by comparing the relative mRNA expression of stimulated BM-DCs with those of negative controls.

PART V. Flow cytometric analysis.

1. Cell staining

BM-DCs were stimulated with yeasts for 24 hours. The cells were harvested and stained for the maturation markers using specific antibodies as follow: APC-conjugated anti-mouse CD11c (eBiscience), FITC-conjugated anti-mouse CD80 (eBioscience), PE-conjugated anti-mouse CD86 (eBioscience),

and PerCP Cy5.5-conjugated anti-mouse I-A/I-E mAbs (Biolegend).

2. Flow cytometric analysis

The stained samples were analyzed by flow cytometry (BD FACSCalibur™ system) and BD CellQuest™ software.

PART VI. Statistical analysis

The number of samples was five per group. Statistical analysis was performed using Mann-Whitney U Test, and p value of ≤ 0.05 was considered statistically significant.

CHAPTER V
RESULTS

PART I. Saccharomyces cerevisiae growth curve

The yeasts WT and mutants were cultured overnight and the OD_{600} was measured at 2, 4, 6, 8, 16 and 24 hours (Fig. 3). The log phase of all yeasts started at 4 hours and ended at 16 hour, and the mid log phase was 10-12 hours. The growth rate of *och1Δ* mutant was slowest, however, its log phase was still in the same period as the others.

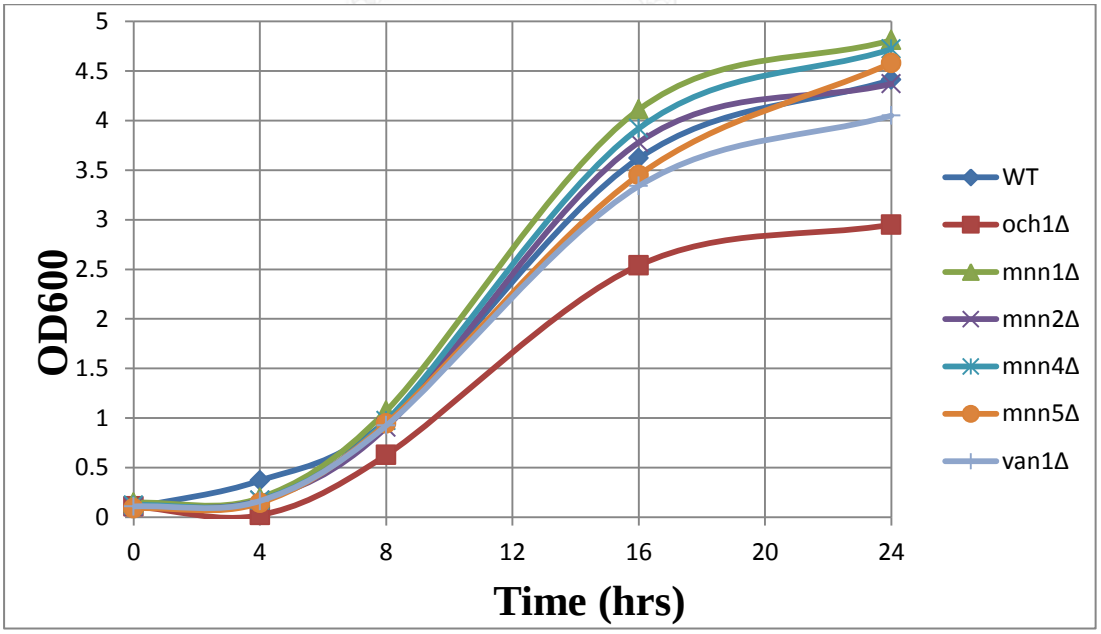
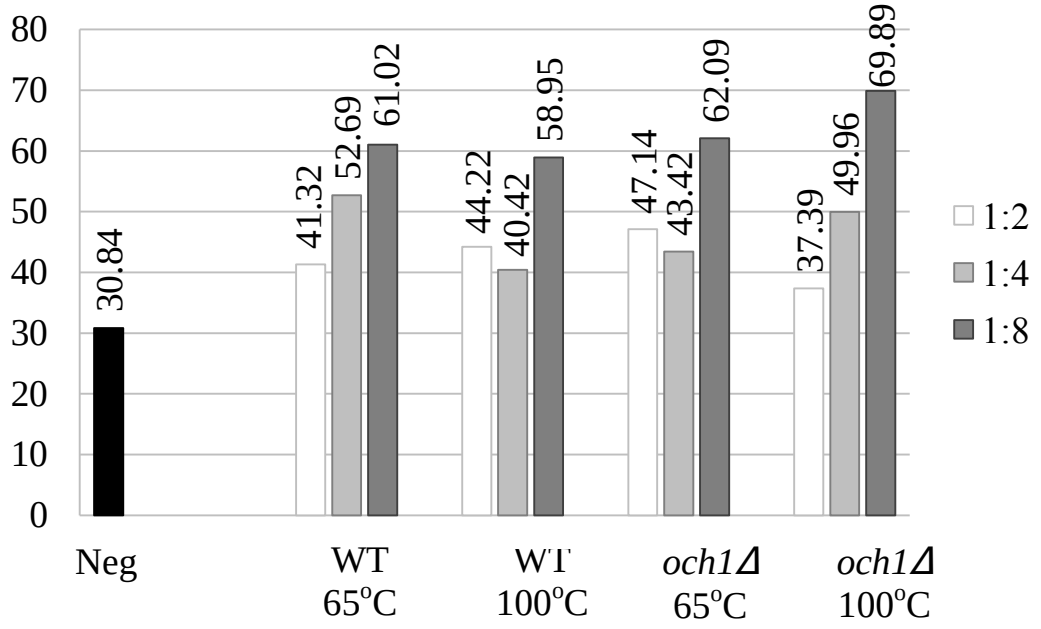


Figure 3 Growth curve of *S. cerevisiae* WT and mutants.

PART II. Selection of the condition for BM-DC stimulation

To investigate the effect of yeast cell wall to DC responses, yeast must be inactivated to eliminate the undesired signal from secreted products. High temperature was applied to inactivate yeasts, but the heat may also affect their immunogenicity. In addition, the ratio of DC:yeasts may affect DC activation. To determine the suitable temperature for yeast inactivation and proper DC:yeasts ratio, BM-DCs were stimulated with 65^{o}C (24) and 100^{o}C (34) heated-killed WT and *och1Δ*, at the ratio of DC:yeasts of 1:2, 1:4 and 1:8. Then, the levels of DC maturation marker CD86 were determined by flow cytometry (Fig 4). The different temperatures used to kill yeast cells did not influence BM-DC maturation. The results showed that BM-DC maturation was induced in a dose-dependent manner. From the result, thus, heat-killed condition of 100^{o}C for

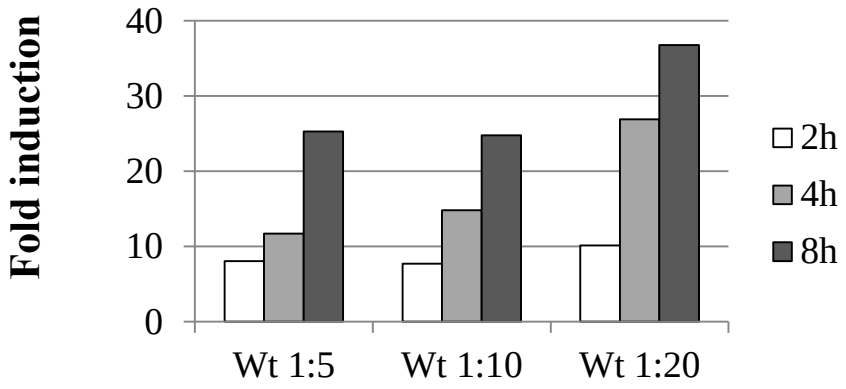


various ratios of heat-killed yeast. *S. cerevisiae* WT and *och1Δ* were incubated at 65°C for 2 hours or 100°C for 10 minutes, and then were added to BM-DC culture at DC:Yeast ratios of 1:2, 1:4 and 1:8. BM-DCs were stimulated for 24 hours, and CD86 expression was analyzed by flow cytometry. The indicated numbers are mean fluorescence intensity of CD86 surface molecules. Neg : Negative control or unstimulated BM-DCs.

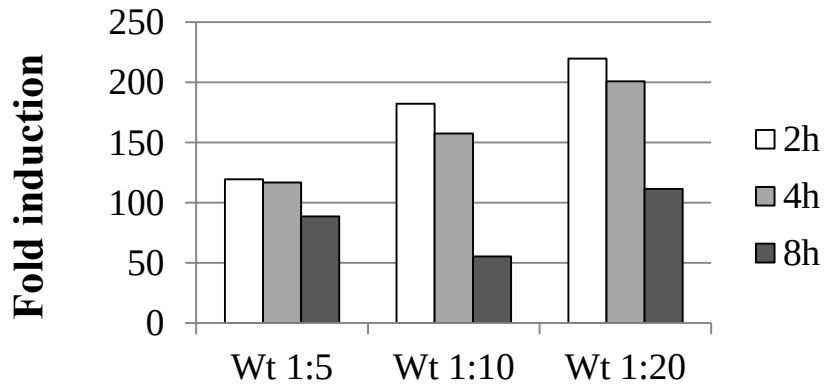
Although, the DC:Yeast ratio of 1:8 (Fig. 4) showed the highest CD86 expression on BM-DCs, it was still necessary to find the proper amount of yeasts for mRNA analysis of BM-DC stimulation. Therefore, in the next experiment, we aimed to find the suitable ratio and stimulation time by observing the mRNA expression of *Il12a*, encoding IL-12 subunit p35; *Ifng*, encoding IFN-γ; and *Il23a*, encoding IL-23 subunit p19, following the previous report that WT *S. cerevisiae* enhanced

IL-12 and IFN-γ production in monocyte-derived DCs and *och1Δ* mutant drove Th17 differentiation (24). BM-DCs were stimulated with 100^{o}C heat-killed WT *S. cerevisiae* at the ratio of 1:5, 1:10 and 1: 20 for 2, 4 and 8 hours, and mRNA expression levels of *Il12a*, *Ifng* and *Il23a* were determined (Fig. 5). There was no difference in mRNA expression of *Il12a*, *Ifng* and *Il23a* genes when BM-DCs were stimulated with heat-killed yeasts at all ratios. However, the viability of BM-DCs in the cultures was decreased when stimulated with 1:10 and 1:20 (data not shown). The *Il12a* expression in BM-DCs was increased with the stimulation time (Fig. 5A), while *Ifng* and *Il23a* expressions were highest at 4 hours and 2 hours, respectively (Fig. 5B and 5C). Thus, the DC:Yeast ratio of 1:5 and the stimulation time of 2 and 4 hours were selected for BM-DC stimulation in the analysis of mRNA expression.

(A) *Il12a*



(B) *Ifng*



(C)

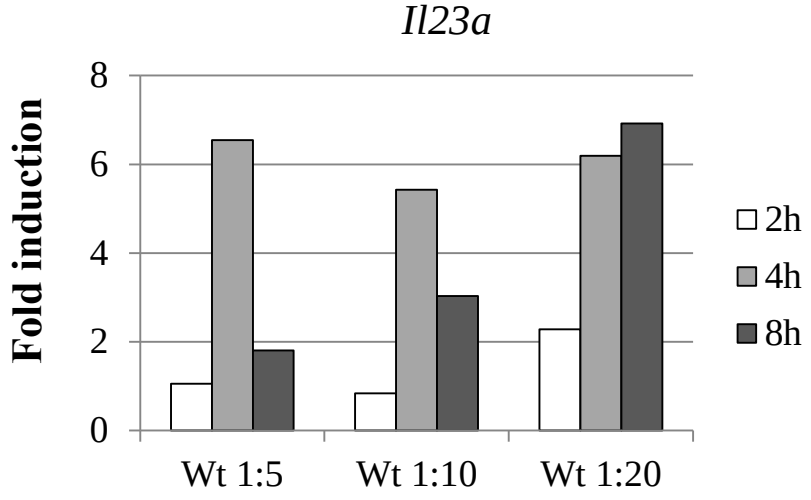


Figure 5 The mRNA expression of *Il12a, Ifng* and *Il23a* genes in BM-DCs stimulated with heat-killed WT at various ratios. *S. cerevisiae* was inactivated by 100°C heat and incubated with BM-DCs at DC:Yeast ratio of 1:5, 1:10 and 1:20 for 2, 4, and 8 hours. The relative mRNA expression of (A) *Il12a* (B) *Ifng* and (C) *Il23a* was shown as fold induction over unstimulated controls.

PART III. Determination of mRNA expression of the target genes involving in Th induction

The generation of CD4 T cell subsets is mainly dependent on the type of cytokines in the microenvironment during T cell activation (Fig. 6).

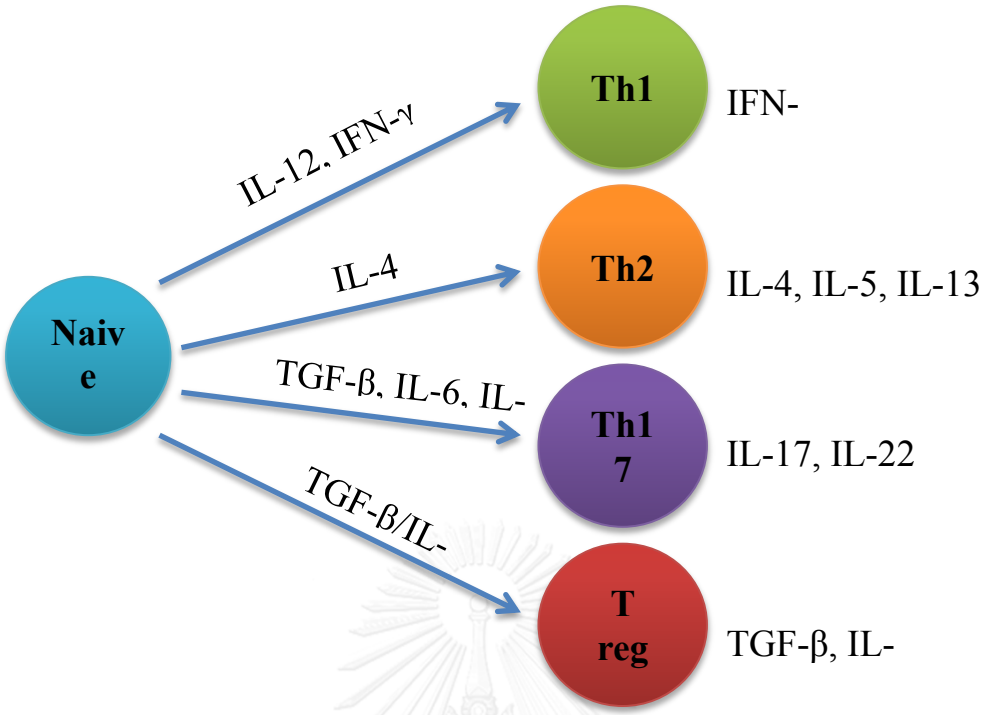
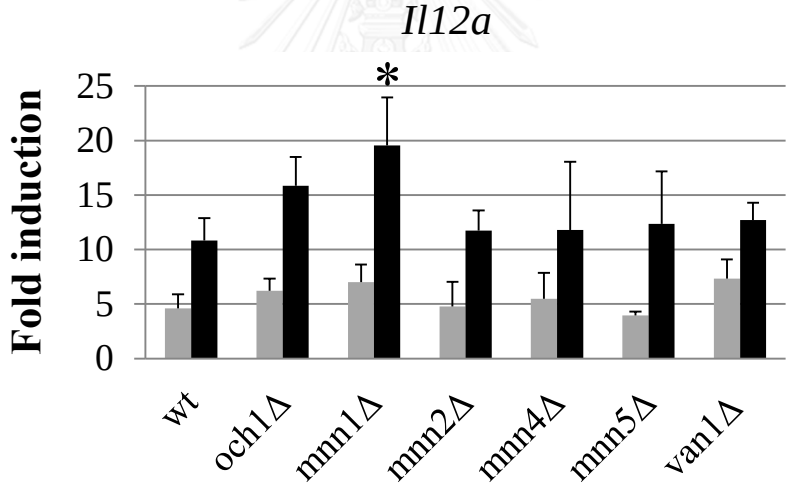


Figure 6 T helper differentiation. When naïve CD4 T cells are activated in the presence of different groups of Th-inducing cytokines, CD4 T cells are induced to differentiate into distinct subsets. IL-12 and IFN-γ direct Th1 differentiation, while IL-4 is essential for Th2 differentiation. In addition, TGF-β, IL-6 and IL-21 are crucial for Th17 differentiation, and TGF-β or IL-10 plays a role in regulatory T cells (Treg) induction.

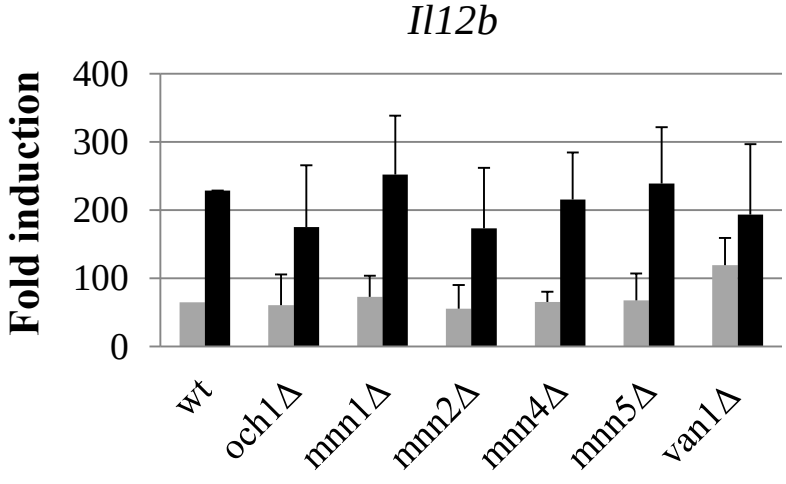
To investigate mRNA expression of the genes involving in Th1 induction (Fig. 7), BM-DCs were stimulated with heat-killed WT and mutant yeasts for 2 and 4 hours. BM-DCs stimulated with *mnn1Δ* mutant showed a notable increase in *Il12a* (Fig. 7A), encoding IL-12p35, which is the subunit of IL-12, and *Ifng* expression at 4 hours (Fig. 7B) when compared to

BM-DCs stimulated with WT. However, *mnn1Δ* stimulation did not alter *Il12b*, which encodes IL-12p40, the subunit of IL-12 and IL-23. BM-DCs stimulated with *mnn2Δ* mutant also significantly enhanced *Ifng* expression at 4 hours (Fig. 7C). The data suggested that the lack of terminal α-1,3 mannan (in *mnn1Δ*) and the second branch of α-1,2 mannan (in *mnn2Δ*) in *S. cerevisiae* led to the augment of Th1-inducing cykines in DCs.

(A)



(B)



(C) *Ifng*

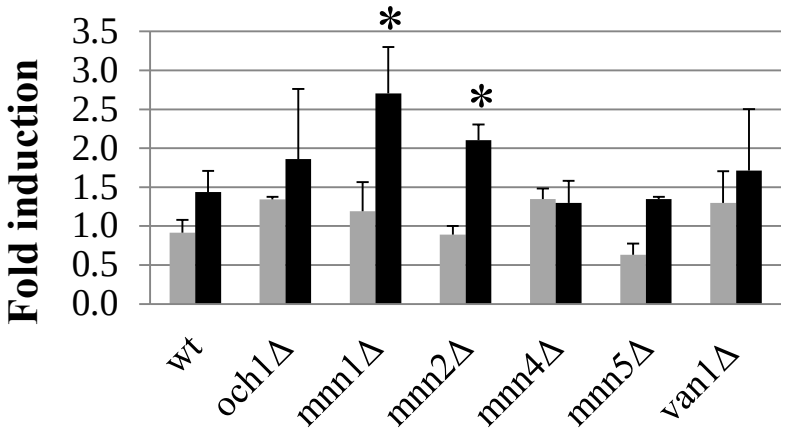
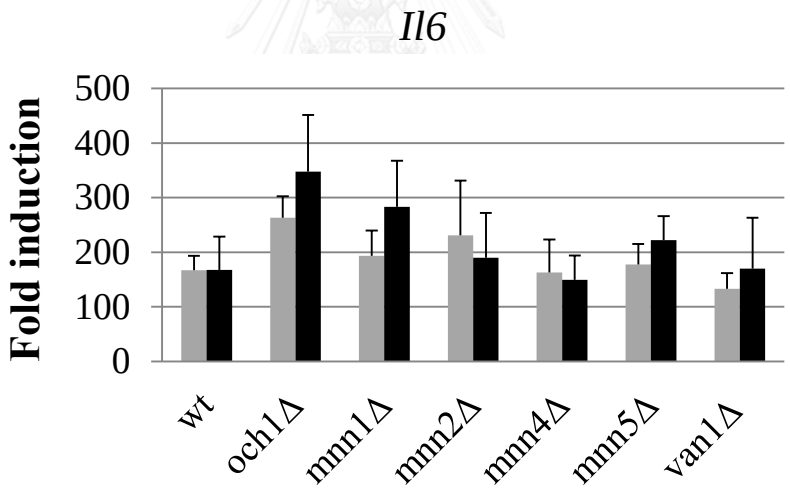


Figure 7 The mRNA expression of Th1-inducing cytokine genes. BM-DCs were stimulated with heat-killed WT and mutants at DC:Yeast ratio of 1:5 for 2 (gray bar) and 4 (black bar) hours. The relative mRNA expression of (A) *Il12a* (B) *Il12b* and (C) *Ifng* was shown as the fold induction. * P value ≤ 0.05 compared to WT at 4 hours; n = 5.

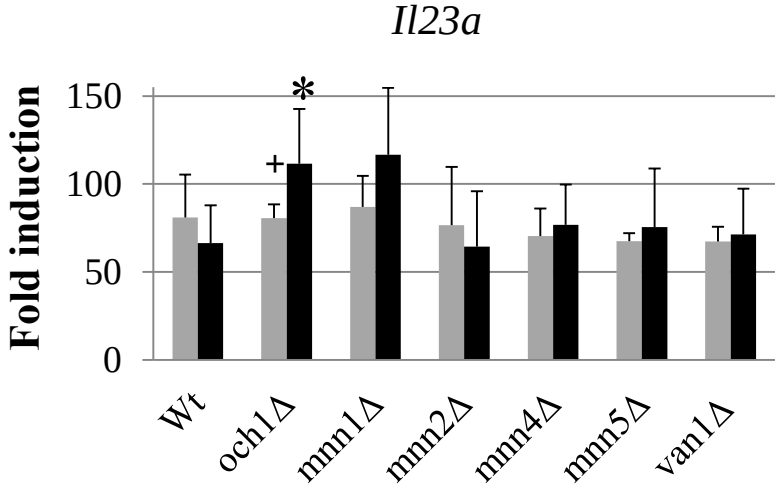
In general IL-6, IL-21, and small amount of TGF-β, are the key cytokines for Th17 induction (35). It has been reported that IL-1 and IL-23 play crucial roles in finalizing Th17 differentiation program and maintaining Th17 functions (35). BM-DCs can produce TGF-β, IL-1, IL-6 and IL-23 upon stimulation, however, we could not detect IL-21 (data not shown). Thus, IL-1, IL-6, and IL-23 were selected as Th17

inducing cytokines to be examined in this work (Fig. 8). The data demonstrated that *och1Δ* mutant did not affect the level of *Il6* mRNA (Fig. 8A), while it enhanced the induction of *Il23a* (Fig. 8B) encoding IL-23p19 subunit, *Il1a* (Fig. 8C) and *Il1b* (Fig. 8D) at 2 and 4 hours. The results suggested that N-linked mannan may play a role in the control of Th17 induction by DCs.

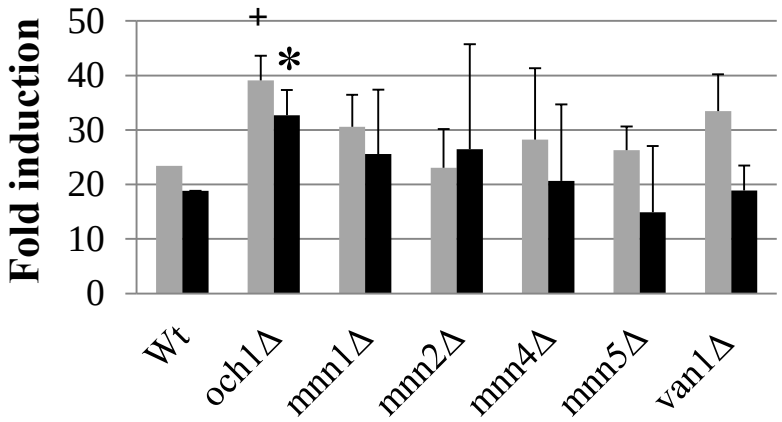
(A)



(B)



(C) *Il1a*



(D) *Il1b*

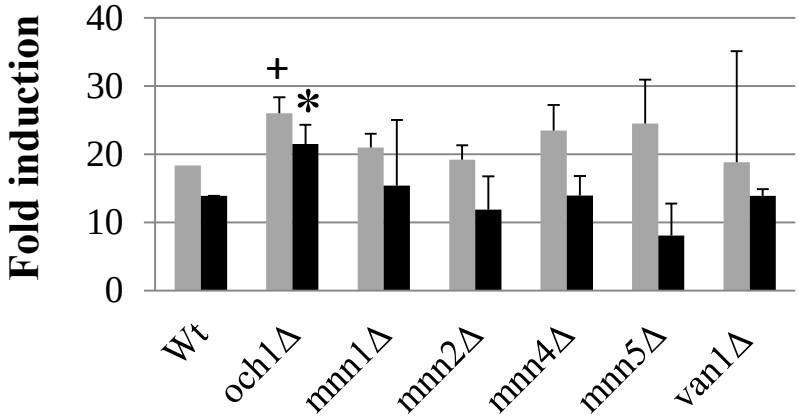


Figure 8 The mRNA expression of Th17-inducing cytokine genes. BM-DCs were stimulated with heat-killed WT and mutnats at DC:Yeast ratio of 1:5 for 2 (gray bar) and 4 (black bar) hours. The relative mRNA expression of (A) *Il16* (B) *Il23a* (C) *Il1a* and (D) *Il1b* was shown as the fold induction. $^{+}$ P value ≤ 0.05 compared to WT at 2 hours; * P value ≤ 0.05 compared to WT at 4 hours; n = 5.

Next, we investigated whether the different mannan structures affect the expression of *Il4*, a Th2-inducing cytokine, in BM-DCs (Fig. 9). BM-DCs stimulated with *och1Δ*, *mnn1Δ,* and *mnn2Δ* displayed significant reduction in *Il4* expression when compared to WT-stimulated BM-DCs. According to the above data, *mnn1Δ* and *mnn2Δ* stimulated the gene expression of Th1-inducing cytokines (Fig. 7), and *och1Δ* stimulated Th17-inducing cytokines (Fig. 8), in BM-DCs.

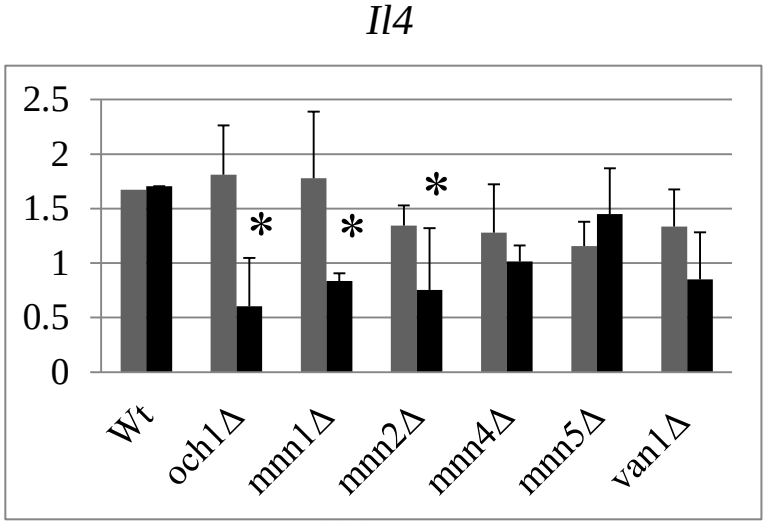
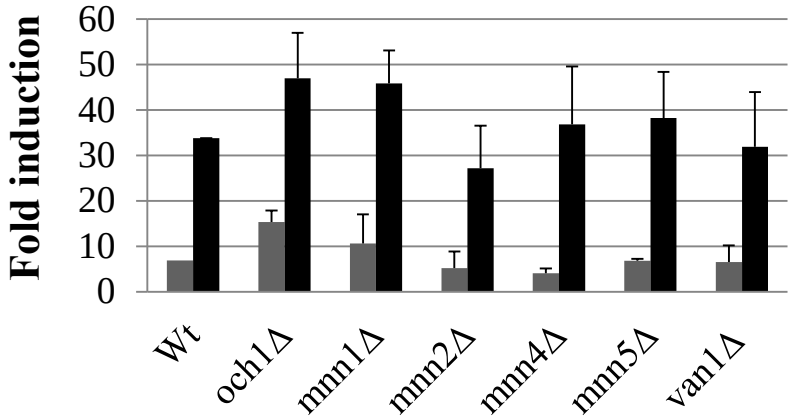


Figure 9 The mRNA expression of Th2-inducing cytokine genes. BM-DCs were stimulated with heat-killed WT and mutnats at DC:Yeast ratio of 1:5 for 2 (gray bar) and 4 (black bar) hours. The relative mRNA expression of *Il4* was shown as the fold induction. * P value ≤ 0.05 compared to WT at 4 hours; n = 5.

Finally, we determined the capability of *S. cerevisiae* WT and mutants in the mRNA expression of Treg-inducing cytokines (Fig. 10). There was no difference in *Il10* (Fig 10A) and *Tgfβ* (Fig. 10B) among all *S. cerevisiae* strains. From the results, it is possible that mannan may not be involved in Treg induction.

(A) *Il10*



(B) *Tgfb1*

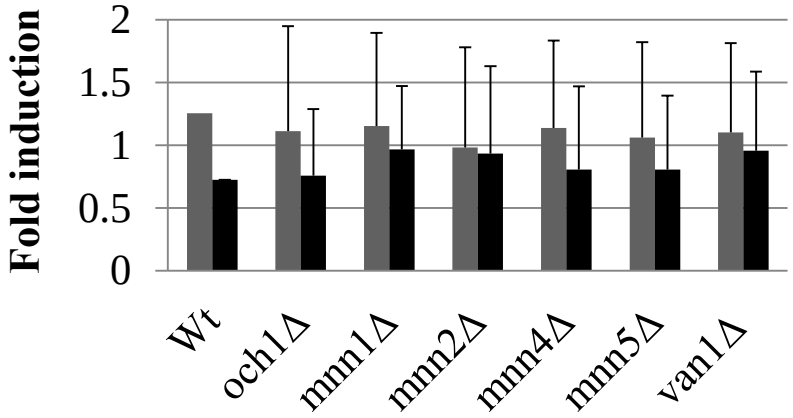


Figure 10 The mRNA expression of Treg-inducing cytokine genes. BM-DCs were stimulated with heat-killed WT and mutnats at DC:Yeast ratio of 1:5 for 2 (gray bar) and 4 (black bar) hours. The relative mRNA expression of *Il4* was shown as the fold induction. n = 5.

CHAPTER VI
DISCUSSIONS

In this study, we investigated the influence of various fungal cell wall mannan structures on BM-DC responses. Our results demonstrated that the absence of terminal α-1,3 mannan in *mnn1Δ* (Fig. 1 and 2) markedly induced the expression of *Il12a* (Fig. 7A) and *Ifng* (Fig. 7C). In contrast, the lack of α-1,2, and terminal α-1,3 mannan in *mnn2Δ* (Fig. 1 and 2) specifically induced the expression of *Ifng* (Fig. 7C). It is possible that α-1,2 mannan binds to a receptor transducing the signal to target *Il12a*. The interaction of mannan and dectin-2 receptor has been shown to target *Il12b* (36), however, there is no evidence of the specific receptor for *Il12a* induction.

In contrast to the increased expression of *Ifng*, BM-DCs stimulated with *och1Δ*, *mnn1Δ,* and *mnn2Δ* displayed significant reduction in *Il4* expression comparing to WT. It has been demonstrated that the suppression of *Il4* expression in T cells was epigenetically controlled by *Ifng* (37). Therefore, it is

possible that the reduced *Il4* expression in *och1Δ*, *mnn1Δ,* or *mnn2Δ* stimulated BM-DCs possibly resulted from epigenetic control.

Deletion of *OCH1* gene in *S. cerecisiae* has been shown to involve in the shift of Th1 to Th17 in vitro (24). Consistently, BM-DCs stimulated with *och1Δ* showed a significant increase in *Il23a* (Fig. 8B), *Il1a* (Fig. 8C) and *Il1b* (Fig. 8D) expression. Ample evidences demonstrated that signal transductions via the engagement of β-glucan and dectin-1 receptor led to Th17 induction (38-41). Mannan is located in the outermost layer of the yeast cell wall and it covers the glucan layer. Thus, it is possible that lack of N-linked mannan in *och1Δ* mutant led to exposure of glucan, which then binds to dectin-1 on BM-DCs, and consequently enhanced Th17-inducing cytokines.

Similar to *OCH1*, *VAN1* involves in the first step of mannan synthesis. However, mRNA expression of cytokine profile in BM-DCs stimulated with *van1Δ* mutant and *och1Δ* mutant differed. *VAN1* is a gene in *MNN9* family (42). It

encodes Van1p, a component of mannan polymerase I, which form a complex with Mnn9p that encoded by *MNN9*. *VAN1* and *MNN9* elongate α-1,6-mannosyl linkage on the backbone, and there is no further α-1,6-linked residues added in *van1Δ* and *mnn9Δ* mutant (42, 43). However, it was found that instead of α-1,6-linked residues, some α-1,2-linked residues were added in *van1Δ* and *mnn9Δ* mutant (43, 44). Thus, the differed responses of BM-DCs to *van1Δ* may be possible from the recognition of α-1,2-mannosyl residues that are absent in *och1Δ*.

A previous study in *C. albicans* showed that the O-linked and phosphomannan in the cell wall did not influence the production of proinflammatory cytokines in DCs (45). Consistently, our data demonstrated that the phosphomannan structure in *S. cerevisiae* cell wall was not involved in BM-DC stimulation. *MNN5* encodes α-1,2-mannosyltransferase that is responsible for the addition of second alpha-1,2-linked mannose of the branches on the mannan backbone of oligosaccharides (46). Our results demonstrated that lack of second alpha-1,2-

linked residues in *mnn5Δ* did not affect BM-DC responses, whereas lack of first α-1,2-linked residues in *mnn2Δ* leaded to the induction of IFN-γ mRNA expression. Although there is no enough supporting evidence, it is possible that terminal α-1,3-linked mannose may be transferred to the first α-1,2-linked residues directly. Therefore, *mnn5Δ* stimulated BM-DCs showed the similar gene expression profile to BM-DCs stimulated with *Wt S. cerevisiae*.

Our data demonstrated the responses of DCs *in vitro* in mRNA level, however, the production of cytokines should be further observed. In addition, *in vitro* BM-DCs responses to purified mannan from *Wt* and *mutant* cell wall must be evaluated, and *in vivo* immunization experiment will be necessary to determine the direction and magnitude of actual adaptive immune responses.

CHAPTER VII
CONCLUSIONS

In the past decades, several lines of evidence suggested that the lack of some α- or β-mannan structures caused a deviation of immune responses (24, 45, 47). It is possible that DCs recognize various mannan structures via different PRRs, resulting in distinct signal transduction pathways, and consequently leading to differences in immune responses. According to our data, genetic engineering of mannan structure in *S. cerevisiae* influenced DC responses, which possibly affect T cell differentiation. This work will be advantage for the further development of carbohydrate vaccine adjuvants.

REFERENCES

1. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. Nat Med. 2013;19(12):1597-608.

2. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805-20.

3. Awate S, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. Front Immunol. 2013;4:114.

4. Didierlaurent AM, Morel S, Lockman L, Giannini SL, Bisteau M, Carlsen H, et al. AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol. 2009;183(10):6186-97.

5. Rappuoli R, Mandl CW, Black S, De Gregorio E. Vaccines for the twenty-first century society. Nat Rev Immunol. 2011;11(12):865-72.

6. Rimaniol AC, Gras G, Verdier F, Capel F, Grigoriev VB, Porcheray F, et al. Aluminum hydroxide adjuvant induces

macrophage differentiation towards a specialized antigen-presenting cell type. Vaccine. 2004;22(23-24):3127-35.

7. Stern AM, Markel H. The history of vaccines and immunization: familiar patterns, new challenges. Health Aff (Millwood). 2005;24(3):611-21.

8. Astronomo RD, Burton DR. Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov. 2010;9(4):308-24.

9. Petrovsky N, Cooper PD. Carbohydrate-based immune adjuvants. Expert Rev Vaccines. 2011;10(4):523-37.

10. Free SJ. Fungal cell wall organization and biosynthesis. Adv Genet. 2013;81:33-82.

11. Lesage G, Bussey H. Cell wall assembly in Saccharomyces cerevisiae. Microbiol Mol Biol Rev. 2006;70(2):317-43.

12. Brown GD. Innate antifungal immunity: the key role of phagocytes. Annual review of immunology. 2011;29:1-21.

13. Parkin J, Cohen B. An overview of the immune system. Lancet. 2001;357(9270):1777-89.

14. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12(4):265-77.

15. Moyle PM, Toth I. Modern subunit vaccines: development, components, and research opportunities. ChemMedChem. 2013;8(3):360-76.

16. Tam JP. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc Natl Acad Sci U S A. 1988;85(15):5409-13.

17. Cui Z. DNA vaccine. Adv Genet. 2005;54:257-89.

18. Cox JC, Coulter AR. Adjuvants--a classification and review of their modes of action. Vaccine. 1997;15(3):248-56.

19. Guy B. The perfect mix: recent progress in adjuvant research. Nat Rev Microbiol. 2007;5(7):505-17.

20. Rey-Ladino J, Ross AG, Cripps AW, McManus DP, Quinn R. Natural products and the search for novel vaccine adjuvants. Vaccine. 2011;29(38):6464-71.

21. Gupta RK, Siber GR. Adjuvants for human vaccines--current status, problems and future prospects. Vaccine. 1995;13(14):1263-76.

22. Romani L. Immunity to fungal infections. Nat Rev Immunol. 2004;4(1):1-23.

23. Luis A Perez-Garcia DFD-J, Adolfo Lopez-Esparza and Hector M Mora-Montes. Role of Cell Wall Polysaccharides during Recognition of Candida albicans

by the Innate Immune System. Glycobiology. 2012;1(1).

24. Rizzetto L, Kuka M, De Filippo C, Cambi A, Netea MG, Beltrame L, et al. Differential IL-17 production and mannan recognition contribute to fungal pathogenicity and commensalism. J Immunol. 2010;184(8):4258-68.

25. Ueno K, Okawara A, Yamagoe S, Naka T, Umeyama T, Utena-Abe Y, et al. The mannan of Candida albicans lacking beta-1,2-linked oligomannosides increases the production of inflammatory cytokines by dendritic cells. Med Mycol. 2013;51(4):385-95.

26. Corbacho I, Olivero I, Hernandez LM. A genome-wide screen for Saccharomyces cerevisiae nonessential genes involved in mannosyl phosphate transfer to mannoprotein-linked oligosaccharides. Fungal Genet Biol. 2005;42(9):773-90.

27. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, et al. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science. 1999;285(5429):901-6.

28. Barron MA, Blyveis N, Pan SC, Wilson CC. Human dendritic cell interactions with whole recombinant yeast: implications for HIV-1 vaccine development. J Clin Immunol. 2006;26(3):251-64.

29. Stubbs AC, Martin KS, Coeshott C, Skaates SV, Kuritzkes DR, Bellgrau D, et al. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. Nat Med. 2001;7(5):625-9.

30. Dean N. Asparagine-linked glycosylation in the yeast Golgi. Biochim Biophys Acta. 1999;1426(2):309-22.

31. Striebeck A, Robinson DA, Schuttelkopf AW, van Aalten DM. Yeast Mnn9 is both a priming glycosyltransferase and an allosteric activator of mannan biosynthesis. Open Biol. 2013;3(9):130022.

32. Conde R, Cueva R, Larriba G. Rsc14-controlled expression of MNN6, MNN4 and MNN1 regulates mannosylphosphorylation of Saccharomyces cerevisiae cell wall mannoproteins. FEMS Yeast Res. 2007;7(8):1248-55.

33. Kim C, Sano Y, Todorova K, Carlson BA, Arpa L, Celada A, et al. The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. Nat Immunol. 2008;9(9):1019-27.

34. Franzusoff A, Duke RC, King TH, Lu Y, Rodell TC. Yeasts encoding tumour antigens in cancer immunotherapy. Expert Opin Biol Ther. 2005;5(4):565-75.

35. Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol. 2008;8(5):337-48.

36. Saijo S, Ikeda S, Yamabe K, Kakuta S, Ishigame H, Akitsu A, et al. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. Immunity. 2010;32(5):681-91.

37. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol. 2009;9(2):91-105.

38. Kashem SW, Igyarto BZ, Gerami-Nejad M, Kumamoto Y, Mohammed J, Jarrett E, et al. Candida albicans morphology and dendritic cell subsets determine T helper cell differentiation. Immunity. 2015;42(2):356-66.

39. Mintz-Cole RA, Gibson AM, Bass SA, Budelsky AL, Reponen T, Hershey GK. Dectin-1 and IL-17A suppress murine asthma induced by Aspergillus versicolor but not Cladosporium

cladosporioides due to differences in beta-glucan surface exposure. J Immunol. 2012;189(7):3609-17.

40. Zenaro E, Donini M, Dusi S. Induction of Th1/Th17 immune response by Mycobacterium tuberculosis: role of dectin-1, Mannose Receptor, and DC-SIGN. J Leukoc Biol. 2009;86(6):1393-401.

41. Chamilos G, Ganguly D, Lande R, Gregorio J, Meller S, Goldman WE, et al. Generation of IL-23 producing dendritic cells (DCs) by airborne fungi regulates fungal pathogenicity via the induction of T(H)-17 responses. PLoS One. 2010;5(9):e12955.

42. Southard SB, Specht CA, Mishra C, Chen-Weiner J, Robbins PW. Molecular analysis of the Candida albicans homolog of Saccharomyces cerevisiae MNN9, required for glycosylation of cell wall mannoproteins. Journal of bacteriology. 1999;181(24):7439-48.

43. Jungmann J, Munro S. Multi-protein complexes in the cis Golgi of Saccharomyces cerevisiae with alpha-1,6-

mannosyltransferase activity. The EMBO journal. 1998;17(2):423-34.

44. Tsai PK, Frevert J, Ballou CE. Carbohydrate structure of Saccharomyces cerevisiae mnn9 mannoprotein. The Journal of biological chemistry. 1984;259(6):3805-11.

45. Cambi A, Netea MG, Mora-Montes HM, Gow NA, Hato SV, Lowman DW, et al. Dendritic cell interaction with Candida albicans critically depends on N-linked mannan. The Journal of biological chemistry. 2008;283(29):20590-9.

46. Rayner JC, Munro S. Identification of the MNN2 and MNN5 mannosyltransferases required for forming and extending the mannose branches of the outer chain mannans of Saccharomyces cerevisiae. The Journal of biological chemistry. 1998;273(41):26836-43.

47. Ueno K, Okawara A, Yamagoe S, Naka T, Umeyama T, Utena-Abe Y, et al. The mannan of Candida albicans lacking beta-1,2-linked oligomannosides increases the production of

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APPENDIX

VITA

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