THE DIFFERENTIAL DENDRITIC CELL PROPERTIES IN RESPONSE TO *CANDIDA ALBICANS* AND NON-*ALBICAN CANDIDA* β-GLUCAN

Miss Truc Thi Huong Dinh

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Medical Microbiology
Medical Microbiology,Interdisciplinary Program
GRADUATE SCHOOL
Chulalongkorn University
Academic Year 2020

คุณสมบัติของเดนไดรติกเซลล์ที่แตกต่างกันเมื่อตอบสนองต่อเบต้ากลูแคนของเชื้อราแคนดิดาอัลบิแคนส์ และ แคนดิดาที่ไม่ใช่อัลบิแคนส์

น.ส.ทรุค ธิ ฮวง ดินห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาจุลชีววิทยาทางการแพทย์ สหสาขาวิชาจุลชีววิทยาทางการแพทย์
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	THE DIFFERENTIAL DENDRITIC CELL PROPERTIES IN RESPONSE TO *CANDIDA ALBICANS* AND NON-*ALBICAN CANDIDA* β-GLUCAN
By	Miss Truc Thi Huong Dinh
Field of Study	Medical Microbiology
Thesis Advisor	Assistant Professor PATCHAREE RITPRAJAK, D.D.S., Ph.D.
Thesis Co Advisor	Professor TANAPAT PALAGA, Ph.D.
	Assistant Professor ASADA LEELAHAVANICHKUL, M.D., Ph.D.

Accepted by the GRADUATE SCHOOL, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the GRADUATE SCHOOL
(Associate Professor THUMNOON NHUJAK, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman
(Professor NATTIYA HIRANKARN, M.D., Ph.D.)

..... Thesis Advisor
(Assistant Professor PATCHAREE RITPRAJAK, D.D.S., Ph.D.)

..... Thesis Co-Advisor
(Professor TANAPAT PALAGA, Ph.D.)

..... Thesis Co-Advisor
(Assistant Professor ASADA LEELAHAVANICHKUL, M.D., Ph.D.)

..... Examiner
(Associate Professor ARIYA CHINDAMPORN, Ph.D.)

..... Examiner
(Sita Virakul, Ph.D.)

..... External Examiner
(Associate Professor Pornpimon Angkasekwinai, Ph.D.)

ทรุค ธิ ฮวง ดินห์ :

คุณสมบัติของเดนไดรติกเซลล์ที่แตกต่างกันเมื่อตอบสนองต่อเบต้ากลูแคนของเชื้อราแคนดิดาอัลบิแคนส์ และแคนดิดาที่ไม่ใช่อัลบิแคนส์. (THE DIFFERENTIAL DENDRITIC CELL PROPERTIES IN RESPONSE TO *CANDIDA ALBICANS* AND NON-*ALBICAN CANDIDA* β-GLUCAN) อ.ที่ปรึกษาหลัก : Assist. Prof.พัชรี ฤทธิ์ประจักษ์D.D.S., Ph.D., อ.ที่ปรึกษาร่วม : Prof.ธนาภัทร ปาลกะPh.D,Assist. Prof.อัษฎาศ์ ลีพหวนิชกุลM.D., Ph.D.

ในปัจจุบันนี้อัตราการดื้อยาต้านเชื้อรา และการติดเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ (non-*albicans* candidiasis) ของผู้ป่วยมีจำนวนเพิ่มมากขึ้น และอัตราการเสียชีวิตของผู้ป่วยในกลุ่มนี้มีเพิ่มสูงขึ้น ในขณะที่องค์ความรู้เกี่ยวกับกลไกการเกิดโรคยังมีอยู่อย่างจำกัด ระบบภูมิคุ้มกันทั้ง innate immunity และ adaptive immunity มีหน้าที่สำคัญในการป้องกันร่างกายจากการติดเชื้อรา Dendritic cell เป็นเซลล์ภูมิคุ้มกันที่สำคัญในการทำงานร่วมกันของ innate immunity และกระตุ้น adaptive immunity เพื่อการตอบสนองที่จำเพาะมากขึ้นในการต่อสู้กับการติดเชื้อแคนดิดา นอกจากนี้องค์ความรู้เกี่ยวกับบทบาทและหน้าที่ของ dendritic cell กับกระบวนการติดเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ และการศึกษาหน้าที่ของ dendritic cell ต่อการตอบสนองต่อโมเลกุลของผนังเซลล์ของเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ เช่น beta-glucan ยังมีอยู่อย่างจำกัด ดังนั้น งานวิจัยนี้จึงทำการศึกษาหน้าที่ของ Dendritic cell ต่อการตอบสนองของ β-glucan จากเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ จากการศึกษาพบว่า β-glucan จากเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ต่างชนิดกัน ทำให้ Dendritic cell มีการตอบสนองที่แตกต่างกัน เช่น maturation และการหลั่ง cytokine อาจจะเนื่องมาจากรูปร่างและโครงสร้างที่แตกต่างกันของ β-glucan จากชนิดของเชื้อที่แตกต่างกัน เช่น β-glucan จาก *C. krusei* สามารถกระตุ้น Dendritic cell ทำให้เกิดการ maturation และการหลั่ง cytokine ที่เกี่ยวข้องกับกระบวนการอักเสบและต้านการอักเสบ ได้มากสุดเมื่อเทียบกับแคนดิดาชนิดอื่นๆ ในกลุ่ม ในขณะที่ *C. tropicalis* และ *C.albican* กระตุ้น Dendritic cell ได้เหมือนกันในระดับที่ต่ำกว่า และการตอบสนองของ Dendritic cell ต่อ β-glucan ทั้งสองชนิดผ่านกลไก dectin1-dependent pathway และ dectin1-independent pathway ตามลำดับ ซึ่งทำให้สามารถจำแนกให้เห็นความแตกต่างจากการถูกกระตุ้นด้วย β-glucan ของทั้งสอง species ขณะที่ β-glucan จาก *C. krusei* เพิ่มการหลั่งของ IL10 ใน Dendritic cells ซึ่ง IL10 มีอาจจะความสำคัญต่อกระบวนกวนการเพิ่มจำนวนของ regulatory T cells ในสิ่งมีชีวิตที่เกิดจากการติดเชื้อ *C. krusei* ดังนั้นการตอบสนองของระบบภูมิคุ้มกันต่อ β-glucan จาก *C. krusei* อาจจะเป็นกลไกในการป้องกันการติดเชื้อของร่างกายไปถึงกลไกการหลบหลีกของเชื้อ *C. krusei* ดังนั้น การเปลี่ยนแปลงหน้าที่ของ Dendritic cell ต่อการตอบสนองของ β-glucan อาจจะมีบทบาทสำคัญต่อการดำเนินไปของโรคและกลไกทางระบบภูมิคุ้มกันต่อการติดเชื้อแคนดิดาในกลุ่มที่ไม่ใช่อัลบิแคนส์ แต่การงานวิจัยนี้ยังคงต้องการทำการศึกษาเพิ่มเติมต่อไป

สาขาวิชา	จุลชีววิทยาทางการแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5987840420 : MAJOR MEDICAL MICROBIOLOGY
KEYWORD:

Truc Thi Huong Dinh : THE DIFFERENTIAL DENDRITIC CELL PROPERTIES IN RESPONSE TO *CANDIDA ALBICANS* AND NON-*ALBICAN CANDIDA* β-GLUCAN. Advisor: Assist.Prof. PATCHAREE RITPRAJAK, D.D.S., Ph.D. Co-advisor: Prof. TANAPAT PALAGA, Ph.D.,Assist. Prof. ASADA LEELAHAVANICHKUL, M.D., Ph.D.

Increased mortality and antifungal drug resistance of non-*albicans Candidiasis* patients have become an alarming in health problems nowadays, whereas profound knowledge of their pathogenesis mechanisms is still limited. The immune responses of innate and adaptive immunity play a crucial role in protecting from *Candida* infections. Dendritic cells (DCs) are the most notable because of their roles in bridging an innate immunity and specific adaptive immunity to eliminate fungal invasion. It is little known about DC functions in non-*albicans Candida* (NACs) infections. Besides, there is less study on evaluating the DC properties upon the interaction between DCs and NACs-derived ß-glucans. Therefore, this work compared DC properties in response to ß-glucans isolated from currently common NACs. As a result, DC maturation and cytokine production altered at various degrees in *Candida* species-dependent manner. This observation possibly resulted from the structural and morphological differences of ß-glucans of distinct *Candida* species. Notably, *C. krusei* ß-glucan could increase the most robust DC activation of enhanced maturation, pro-inflammatory and anti-inflammatory cytokine secretion, whereas ß-glucan of *C. tropicalis* and *C. albicans* had similar lower levels of those effects. Moreover, ß-glucan of the former species could influence DCs in both dectin-1-dependent and -independent, distinguished from two latter species. Interestingly, ß-glucan of *C. krusei* enhanced IL-10 production of DCs, which might expand the high IL-10-producing FoxP3- regulatory T (Treg) cells in *vivo C. krusei* infection. However, this hypothesis needs further proof. We propose that increased immunomodulation due to *C. krusei* ß-glucan might prevent the host's protective immune activities and promote immune evasion of *C. krusei*. In summary, we have shown that altering DC functions of NACs-derived ß-glucans might play a role in NACs infections' pathogenesis.

Key words: Glucan, dendritic cells, non-*albicans Candida* species (NACs)

Field of Study:	Medical Microbiology	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....
		Co-advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

To achieve these results as now, at first, I would like to express the deepest gratitude to my dear advisor, Assist. Prof. Dr. Patcharee Ritprajak for all her supports, instructions, and kindness. With her characteristics of enthusiasm, straightforwardness, and responsibility, she inspired me the right and efficient way to do science research which I had no experience with before. She gave me a gold chance at my age to set up my Ph.D. study here that I always appreciate and engrave in my mind from the beginning. She always encouraged and guided me to realize the weak and strong points, to train myself gradually in science research. For my extra-time period without a scholarship, she supported me financially and spiritually to continue my study here. During my Ph.D. life, aside from transferring the knowledge, she taught me so much more than immunology. For all these reasons, I profoundly express gratitude to my dear advisor.

I would like to thank Prof. Nattiya Hirankarn, Assoc. Prof. Tanapat Palaga, Assist. Prof. Asada Leelahavanichkul, Assoc. Prof. Ariaya Chindamporn, Sita Virakul, Ph.D. and Assoc. Prof. Pornpimon Angkasekwinai in the committee of my thesis defense for spending time with giving me valuable suggestions for completing my works the best.

I would like to especially thank Assist. Prof. Panuwat Padungros and Ms. Pranpariya Ponpakdee (Department of Chemistry, Faculty of Science, CU) for supporting NMR results of articles; Assist. Prof. Dr. Asada Leelahavanichkul and Ms. Wilasinee Saisorn (Department of Microbiology, Faculty of Medicine, CU) for supporting in vivo experiment; Ms. Lawan Boonprakong (Oral Biology Research Center, Faculty of Dentistry, CU) for supporting SEM results; Ms. Phawida Tummamunkong in our laboratory, for her enthusiastic companion during doing experiments of articles. I would like to extend my acknowledge to Professor David L. Williams (from Department of Surgery, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA) for kindly providing my advisor the protocols of setting up beta-glucan extraction here. Without these special supports, I thought I could not complete my study as now.

I greatly thank the ASEAN scholarship of Chulalongkorn University for sponsoring three years of my study program. I also thank the research funding of Ratchadapisek Sompoch Endowment Fund, Chulalongkorn University for supporting my research. Next, I sincerely appreciate the supports of the committee, lecturers, and staff of the Medical

Microbiology program, Medicine faculty, Chulalongkorn University. The committee waived the tuition fee for me during the extended time without scholarship support.

I sincerely thank the support of the Oral Biology Research Center and staff, Dentistry faculty, Chulalongkorn University for facilitating the equipment to perform my experiments there. Of note, every staff of this center is very kind, friendly, and enthusiastic in helping international students.

I especially gratitude Assoc. Prof. Dr. Nguyen Trung Kien- the principal and Assoc. Prof. Dr. Tran Ngoc Dung-the former head of Physiopathology and Immunology Department in Can Tho University of Medicine and Pharmacy, and Assoc. Prof. Dr. Tran Thi Minh Diem-the former head of Physiopathology and Immunology Department in Hue University of Medicine and Pharmacy. They encouraged and wrote recommend letters for my opportunity at the beginning. Besides, I also thank other colleagues in my department for facilitating the time and spirit for me to complete the study.

One of the most important things, to reach the success of my study, I have never forgotten the great support both spiritually and materially from every member of my great family and friends. I endlessly thank my parents and husband for looking after my daughter and son instead of me for over 4 years with endless love. My relatives, the best Vietnamese and Thai friends have been encouraging and sharing cheerful smiles for me to overcome the challenges of studying far away from home.

Looking back values of my Ph.D. studying here, aside from the obtained knowledge, that time showed the most remembered memories which I was lucky to be experienced. When the ahead path becomes rougher and harder, I remind myself with keeping on an optimistic attitude and positive efforts to enjoy a Ph.D. life. Ultimately, I profoundly thank you, this great opportunity very much!

Truc Thi Huong Dinh

TABLE OF CONTENTS

Page

ABSTRACT (THAI) iii

ABSTRACT (ENGLISH) iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vii

LIST OF TABLES 1

LIST OF FIGURES 2

LIST OF ABBREVIATIONS 4

CHAPTER I 7

INTRODUCTION 7

Importance and rational 7

Research questions 12

Hypothesis 12

Objectives 12

Benefits of study 12

The conceptual framework 13

CHAPTER II 14

LITERATURE REVIEWS 14

1. *Candida* species 14

1.1. Overview of *Candida albicans* and *Candida* non-*albicans* species 14

1.2. The medical challenges to invasive infections of *Candida albicans* and NACs15

1.3. The diversity of virulence in *Candida* pathogenicity 18

1.3.1. Similar and dissimilar virulence features of common *Candida* spp. 18

1.3.2. The association of cell wall components with the virulent ability of *Candida* spp. 24

2. Dendritic cells with their roles in immunity against *Candida* infections 26

2.1. Characteristics and functions of dendritic cells 26

2.2. The roles of dendritic cells in host immunity against *Candida* spp. 31

3. ß-glucan of *Candida albicans* and non-*albicans* species 33

3.1 Structure and properties of *Candida* ß-glucan 33

3.2. Receptors of ß-glucan 35

3.2.1. Dectin-1 receptor 35

3.2.2. Completementary receptor 3 (CR3) 38

3.2.3. The concurrent activation of many receptors during dectin-1- ß-glucan interaction 39

CHAPTER III 41

MATERIALS AND METHODS 41

1. Animals and ethics statement 41

2. *Candida* yeast strains and culture 41

3. Cell wall ß-glucan extraction 42

4. Scanning Electron Microscopy 45

5. Generation of bone marrow-derived dendritic cells (BMDCs) 45

6. BMDC stimulation with *Candida* ß-glucan 46

7. Dectin-1 blockade 47

8. Spleen-tyrosine kinase (Syk) inhibition 47

9. Flow cytometry analysis 47

10. *In vitro* DC and T cell co-culture assay 48

11. *In vivo* subcutaneous immunization of ß-glucan and *ex vivo* re-stimulation assay 49

12. *In vivo* systemic *C. krusei* infection and *ex vivo* re-stimulation assay 49

14. Cell viability assay 51

15. Cytokine quantification with enzyme-linked immunosorbent assay (ELISA) 51

16. Statistical analysis 52

CHAPTER IV 54

RESULTS AND DISCUSSION 54

Part I: Preliminary results 55

Objective 1: To screen the difference of DC activation via maturation and cytokine secretion in response to cell-wall ß-glucan of *Candida* non-*albicans* spp. and *C. albicans*. 55

Microparticulate ß-glucan of *Candida* species promoted the maturation and cytokine secretion of BMDCs at different levels 55

Discussion 58

Part II: Results 63

Objective 2: To determine the influence mechanisms of NACs cell wall ß-glucan on DCs and the adaptive responses respectively 63

Morphology and structure analysis of *Candida* ß-glucans 63

Effects of *Candida* ß-glucans on DC viability and CD11c expression 66

C. krusei ß-glucans had differential impacts on DC maturation 69

Massive pro-inflammatory and anti-inflammatory cytokine secretion of BMDCs upon stimulation of *C. krusei* ß-glucan 74

C. krusei ß-glucan enhanced IL-10-producing T cells, but not FoxP3^{+} regulatory T cells in DC-T cell co-culture assay 78

In vivo C. krusei ß-glucan immunization expanded IL-10-producing T cell population in antigen-specific manner 81

High expansion of IL-10-producing T cell population in systemic *C. krusei* infected-mice 83

C. krusei ß-glucan-induced DC maturation and cytokine production partially depends on dectin-1 receptor 86

Differential DC activation via Syk-dependent signaling pathway of *Candida* ß-glucan 94

Different expression of surficial dectin-1 receptor on *Candida* ß-glucan-stimulated BMDCs 100

Discussion 102

CHAPTER V 113

CONCLUSION AND PERSPECTIVE 113

APPENDIX 114

REFERENCES 116

VITA 138

LIST OF TABLES

Page

Table 2.1 Some properties of common *Candida* spp. 23

Table 2.2 Cell wall components of common yeast *Candida* spp.....26

Table 4.1 Blockade of DC maturation and cytokine production by dectin-1 antagonist 91

LIST OF FIGURES

Page

Figure 2.1 The proportion of NACs and *C. albicans* in some countries 16

Figure 2.2 DCs dictate the direction of adaptive immune responses 29

Figure 2.3 Several molecules relate to the fate of DC phenotypes 30

Figure 2.4 The basic structure of yeast ß-glucan 35

Figure 2.5 Intracellular signaling cascades of dectin-1 receptor 38

Figure 3.1 Process of *Candida* yeast microparticulate ß-glucan extraction 45

Figure 3.2 Timeline of systemic *C. krusei* infection model 51

Figure 4.1 Representative dot plots and histograms of BMDC maturation markers in flow cytometry analysis 55

Figure 4.2 CD11c expression of *Candida* ß-glucan-stimulated BMDCs at 24 h 56

Figure 4.3 Altered maturation markers among *Candida* ß-glucan-stimulated BMDCs 57

Figure 4.4 Secreted cytokine profile of BMDCs stimulated with *Candida* ß-glucans .. 60

Figure 4.5 Morphology and size of *Candida* ß-glucans 63

Figure 4.6 DC maturation after stimulation with *Candida* ß-glucans pre-treated with polymyxin B 65

Figure 4.7 Cytokine expression after stimulation with *Candida* ß-glucans pre-treated with polymyxin B 66

Figure 4.8 Effects of *Candida* ß-glucans on DC viability 67

Figure 4.9 Changes of CD11c marker among BMDCs stimulated with *Candida* ß-glucans 68

Figure 4.10 Effects of *Candida* ß-glucans on the proportions of mature DCs.....70

Figure 4.11 Different effects of *Candida* ß-glucans on the expression of DC maturation.....71

Figure 4.12 Expression levels of surface protein PD-L1, PD-L2, CD206 on BMDCs stimulated with *Candida* ß-glucans.....73

Figure 4.13 Excessive pro-inflammatory and anti-inflammatory cytokine production in *C. krusei* ß-glucan-stimulated BMDCs at 24 h.....76

Figure 4.14 Cytokine profiles of *Candida* ß-glucan-stimulated BMDCs at 48 h.....77

Figure 4.15 Effects of *Candida* ß-glucan-stimulated BMDCs on T cell-produced cytokines.....78

Figure 4.16 Similar effects of *Candida* ß-glucans-stimulated BMDCs on Treg cells....80

Figure 4.17 High IL-10-producing T cells in in vivo *C. krusei* ß-glucan immunization..82

Figure 4.18 Systemic *C. krusei* infection possibly promoted IL-10-producing T cells.85

Figure 4.19 Impact of dectin-1 blockade on DC proportion and CD11c expression...87

Figure 4.20 Inhibition of DC maturation by dectin-1 blockade.....90

Figure 4.21 Contribution of dectin-1 blockade to DC cytokine production at 24 h....92

Figure 4.22 Effects of dectin-1 blockade on DC cytokine production at 48 h.....93

Figure 4.23 Inhibition of DC maturation by Syk inhibitor 97

Figure 4.24 Effects of Syk inhibitor on DC cytokine production at 24 h 98

Figure 4.25 Effects of Syk inhibition on DC cytokine production at 48 h 99

Figure 4.26 Differential dectin-1 expression on *Candida* ß-glucan-stimulated BMDCs... 101

LIST OF ABBREVIATIONS

Als	Agglutinin-like sequence
Hwp1	Hyphal wall protein 1
Hyr1	Hyphal regulated cell wall protein 1
HAGs	Hyphae-associated genes
ALDH	Aldehyde dehydrogenase
APCs	Antigen-presenting cells
Bcl10	B cell leukemia 10 protein
BMDCs	Bone marrow dendritic cells
CARD9	Caspase recruitment domain-containing protein 9
CCR	C-C Chemokine receptor
CD	Cluster of differentiation
CLEC7A	C-type lectin domain family 7 member A
CLRs	C-type lectin receptor
CR3	Complement receptor 3
CTL	Cytotoxic T lymphocytes
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DCs	Dendritic cells
Dectin-1Rc	Dectin-1 receptor
DPBS	Dulbecco's Phosphate Buffered Saline
ELISA	Enzyme-linked immunosorbent assay
EphA2	Ephrin type A receptor 2
ERK	Extracellular signal-regulated kinase
FoxP3	Forehead box protein P3
GM-CSF	Granulocyte-macrophage-cloning stimulating factor

HI-FBS	Heat-inactivated fetal bovine serum
ICU	Intensive care unit
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon gamma
IL	Interleukin
IL-1RA	Interleukin 1 receptor antagonist
ITAM	Immunoreceptor tyrosine-based activator motifs
MAC-1	Macrophage integrin 1
Malt1	Mucosa-associated lymphoid tissue lymphoma transcription protein 1
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
mp-ß-glucan	Microparticulate beta-glucan
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NACs	Non-*albicans Candida* species
$NADPH^+$	Nicotinamide adenine dinucleotide phosphate H^+
NFAT	Nuclear factor of activated T-cells
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	Nucleotide-binding domain and leucine-rich repeat
NLRP3	NLR family pyrin domain containing 3
NMR	Nuclear magnetic resonance
NODs	Nucleotide-binding oligomerization domain receptors
OVA	Oval albumin
PAMPs	Pathogen-associated molecule patterns
PD-1/ PD-Ls	Program death 1 / Program death - ligands
PKC	Protein kinase C
PLCγ	Phospholipase C gamma

PRRs	Pathogen-recognizing receptors
Raf1	Rapidly Accelerated Fibrosarcoma 1
Ras	Rat sarcoma
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute medium
SEM	Scanning electronic microscopy
Syk	Spleen tyrosine kinase
TCR	T cell receptors
TGF-β	Transforming growth factor beta
Th cells	Helper T cells
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
Treg cells	Regulatory T cells
YPD medium	Yeast - Peptone - D-glucose Medium
WGD	Whole genome duplication

CHAPTER I

INTRODUCTION

Importance and rational

Candidiasis, the well-known opportunistic *Candida* fungi infection, is caused by the most common species such as *Candida albicans* and non-*albicans Candida* spp. (NACs) (Lamoth et al., 2018; Walsh et al., 2019). These *Candida* species frequently infect high-risk people who are suffering from primary or acquired immunocompromised states. They could be neonates or the elderly bearing underlying diseases, for example, HIV/AIDS, cancers, hematological malignancies or organ transplantations, and abdominal surgeries (Lamoth et al., 2018; Walsh et al., 2019). There is a variety of clinical symptoms of candidiasis which varies from mild to severe state or from mucocutaneous sites to life-threatening conditions. This diversity depends on the infected *Candida* species and how potent the host anti-fungal immunity has. In previous decades, *C. albicans* was responsible for the first cause of almost candidiasis cases, but until now, an increased rate of NACs has been recorded via clinical data reports throughout the world with morbidity and mortality as high as these of *C. albicans* (Hachem et al., 2008; Leroy et al., 2009; Lamoth et al., 2018). *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei, Candida glabrata* and *Candida dubliniensis* are five of the dominant NACs responsible for human invasive candidiasis globally (Turner & Butler, 2014; Melanie Polke, 2015; Lamoth et al., 2018; Walsh et al., 2019). This is likely alarming for medicine because epidemiological and phenotype alterations of *Candida* species in candidiasis also reflect a wide non-response to current antifungal drugs of this fungi. The antifungal drug-resistance has been recorded more along with infections of these non-*albicans Candida* spp. that give rise to many obstacles in candidiasis treatment (Pappas et al., 2018). Especially, *C. tropicalis, C. krusei* and *C. glabrata*, these species have high intrinsic antifungal

drug-resistance such as flucytosine, amphotericin B, echinocandins and fluconazole, even multidrug resistance (Pappas et al., 2018; P. Y. Chen et al., 2019; Jamiu et al., 2020). Hence, these challenges continue to require further investigations.

Several activities of immunity have highlighted an indispensable magnitude to combat the fungi in invasive candidiasis patients. The host immunity is depicted in general via a typical *C. albicans* infection with a harmony of innate immunity and adaptive immunity (Mihai G. Netea, 2015; Pappas et al., 2018), yet little has known about those processes in NACs-related infections. In the case of *C. albicans*, Th1 and Th17 cells are previously considered as the specific immune cells resistant to *C. albicans* infection (Mihai G. Netea, 2015). Each type of *Candida* spp. has different virulence and pathogenesis likely leading to the different infectivity of each species. Among the virulence factors, the morphological alteration for adaptation and secreted aspartyl proteases (Saps) have been considered as the major virulence of *Candida* genus, but it could not infer from *C. albicans* to other species (Neil AR Gow, 2002; Banerjee et al., 2019). Although *C. dupliniensis* and *C. parapsilosis* also contain a filamentous form, they possess lower infectivity and a less virulence (Arendrup et al., 2002; Ortega-Riveros et al., 2017). It is known that some species can survive and escape from the phagolysosome of phagocytes depending on the forming of filamentous morphotype (García-Rodas et al., 2011); however, some species such as *C. krusei* and *C. glabrata* could survive with resistance to the killing of these cells and could cause high infectivity with less ability of forming filamentous form (García-Rodas et al., 2011; Seider et al., 2011). It is possible that these species of *Candida* genus may exploit these cells as vectors to avoid the eradication of immunity and may facilitate a chronic infection (Kasper et al., 2015). Notably, it was demonstrated that filamentation of *C. parapsilosis* and *C. tropicalis* shows a negative correlation with their virulence since filamentous formation leads to downregulation of some other virulence genes and reduce their pathogenicity (Banerjee et al., 2019). These

reasons hint that the interaction of *Candida* fungi with immune cells is complex and can be manipulated by other factors prior to a filamentous transition (Gilbert et al., 2015). Notably, cell wall compositions are highly potential factors, as the first interface of *Candida* spp. contacts with the host cells directly. Possibly, the cell wall alteration of each species leads to trigger the outcomes of host immune cell responses correspondingly (Arana et al., 2009). Therefore, the magnitude of cell wall components likely influences *Candida* pathogenicity as important as other virulence factors.

The reciprocal relationship between host immune cells and *Candida* spp. initially comes from the direct physical contact of the fungal cell wall components and host cell PRRs (Arana et al., 2009; Mihai G. Netea, 2015). Mannan, glucan, and chitin account for the most amount of the cell wall structure, and the residue is protein components (Arana et al., 2009; Gow & Hube, 2012). Regarding cell wall glucan of *Candida* genus, it is mostly structured by ß-glucan which interweaves ß-(1,3)-glycosidic linkages of backbone and ß-(1,6)-glycosidic linkages of branches. Diverse properties of fungal ß-glucan, which has been described in several previous studies, could result in abundant biological impacts such as anticancer responses, immunomodulation, and so on (Xiao et al., 2020). Of which, the antifungal immunity activated by this component plays an essential role in host beneficial protection. The settle of inflammatory Th1 and Th17 cells is probably efficient to eliminate fungal infection (Mihai G. Netea, 2015; Goyal et al., 2018). Furthermore, the ß-glucan release from *Candida* cell wall possibly associates with the severity of human invasive candidiasis (Sims et al., 2012; Giacobbe et al., 2015). Thus, circulating ß-glucans may interact with immune cells directly and induce either protective immunity or pathologic inflammatory responses.

Through the discoveries of ß-glucan receptors including dectin-1 and other receptors, the effects of ß-glucan gradually become more obvious. Dectin-1 receptor

is the most critical PRR of CLRs family on host immune cells that recognize ß-glucan structures (Brown et al., 2002; Saijo & Iwakura, 2011). Expression of this receptor is predominant in many innate immune cells (Taylor et al., 2002) and could induce distinct functions depending on cell type such as dendritic cells (Leibundgut-Landmann et al., 2008; Goodridge et al., 2009), macrophages (Brown et al., 2002; Steele et al., 2005) and neutrophils (Hopke et al., 2016). This specific receptor operates its signaling mostly via activities of spleen tyrosine kinase (Syk) adaptor and numerous downstream factors and transcriptional factors, such as NF-kB, NFAT, and NLRP3-inflammasome typically (Brown & Gordon, 2001; Tang et al., 2018). Moreover, a platform of gathering ß-glucan receptors is possibly formed on the immune cell membrane when immune cells encounter fungal surficial ß-glucan (Inoue & Shinohara, 2014). This coordination consists of dectin-1 and other receptors such as CR3, TLRs and scavenger receptors to recognize ß-glucan compositions, but it remains unclear in all cases of *Candida* infection (Ross & V̌etvicka, 1993; Goodridge et al., 2011; Inoue & Shinohara, 2014; Ostrop & Lang, 2017; Camilli et al., 2018). Furthermore, dectin-1 defect could result in increased susceptibility of *Candida* infection in the murine model (Thompson et al., 2019).

Dendritic cells (DCs), one group of the innate immune cells obtains the most highlighted functions in antifungal immunity (Durai & Murphy, 2016; Feldman et al., 2019). Since they function as a bridge of activities between innate and adaptive immune responses through their professional antigen-presenting skill. In anti-fungal responses, by intrinsic abilities, DCs sense pathogen-associated molecule patterns (PAMPs) via their pathogen recognition receptors (PRRs), then they can phagocytosis *Candida* and migrate quickly to draining lymph nodes (Newman & Holly, 2001; Cambi et al., 2008; Ramirez-Ortiz & Means, 2012). At that time, an upgrade from immature to mature cells enables DCs to present fungal processed antigens via surface expression of major histocompatibility (MHC) - antigen complex which is the first required signal

to stimulate T lymphocytes (Kikuchi et al., 2002; Mihai G. Netea, 2015). The maturation status of DCs associates with their function as immunogenic or tolerogenic phenotype (Schmidt et al., 2012). Together with this, expression of many co-stimulatory markers and various cytokines secretion from DCs are required signals for naïve T cell lymphocyte differentiation and effector T helper (Th) cell proliferation afterward (Saravia et al., 2019). In turn, adaptive immune responses of Th1 and Th17 continuously promote the more powerful action of innate immune cells (Mihai G. Netea, 2015; Richardson et al., 2019). Th1-secreted interferon (IFN)-γ elicits enhanced killing abilities of neutrophils, macrophages to eradicate *Candida* fungi invasion, while Th17-related cytokines IL-17 and IL-22 enhance more epithelial cells-secreted ß-defensin to prevent localized *Candida* infection (Mihai G. Netea, 2015). All the above properties may promote DC to be the central cells of the immune system. DCs could importantly contribute to determine alterations of entire immune responses which may differ among *Candida* spp. infections.

However, it is noticed that the different sources of ß-glucan could induce different immune responses (Dalia Akramienė, 2007; Godfrey Chi-Fung Chan et al., 2009). The diversity of cell wall mannan has been found in distinct *Candida* species promoted differential DC responses (Nguyen et al., 2018; Thu Ngoc Yen Nguyen, 2018). These notions become a direction arrow for interested doubts, whether different species of *Candida* genus could comprise structurally different ß-glucans and how they distinctly influence in a bias of host protective immunity and NACs infection outcomes. Moreover, at present, there is still little observation of the direct influence of NACs ß-glucan on DCs and host adaptive immunity. Therefore, this study accessed this interesting information by characterizing the properties of DCs which interact with microparticulate ß-glucans from some common NACs and focused on mechanisms of this relationship.

Research questions

Whether cell wall ß-glucans plays a critical role in the pathogenicity of NACs that impacts fungi-specific immunity differently through the central role of DCs.

-) Does ß-glucan of NACs and *C. albicans* have a differential effect on dendritic cell maturation and cytokine production?

-) Does ß-glucan of NACs impact on dendritic cells through dectin-1 receptor pathway similar in ß-glucan of *C. albicans*?

-) Are the distinct T cell responses driven by NACs ß-glucan-stimulated DCs?

Hypothesis

1) The maturation and cytokine production of DCs in response to *Candida* non-*albicans* ß-glucan differ from those responses to *C. albicans* ß-glucan.

2) ß-glucans of NACs impact on DCs through dectin-1-dependent and dectin-1-independent pathway in comparison with ß-glucan of *C. albicans*.

3) DCs stimulated by NACs-derived ß-glucans determine the fate of the T cell responses which differ from *C. albicans* ß-glucan-stimulated DCs.

Objectives

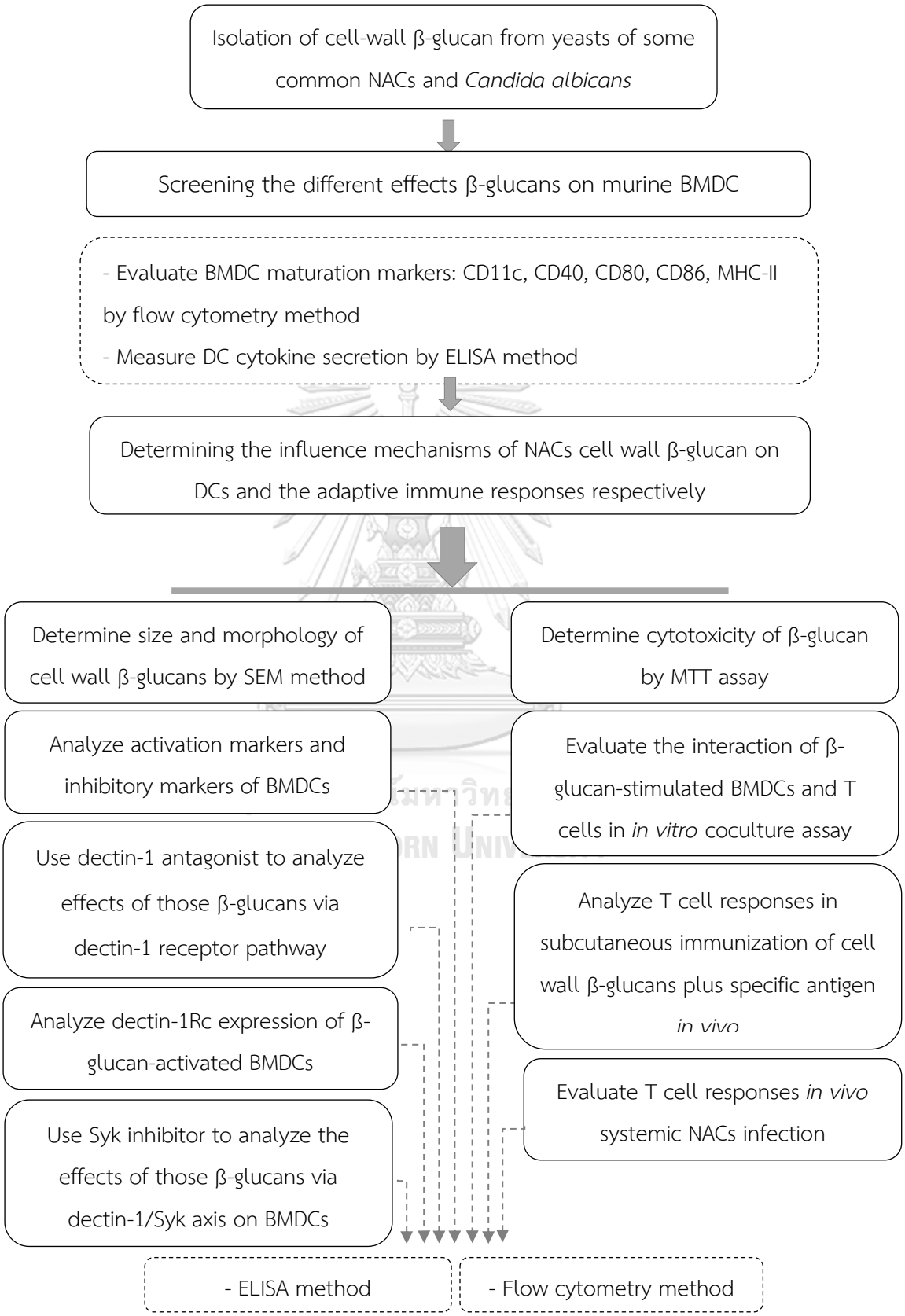
1) To screen the difference of DC activation via maturation and cytokine secretion in response to cell-wall ß-glucan of some *Candida* non-*albicans* spp. and *C. albicans*.

2) To determine the influence mechanisms of NACs cell wall ß-glucan on DCs and the adaptive responses respectively.

Benefits of study

From this observation, a better understanding of the interaction between NACs ß-glucans and DCs would shed light partly on the pathogenesis of NACs infections. Furthermore, these data become an important fundamental knowledge to apply for the treatment against *Candida* genus.

The conceptual framework



CHAPTER II

LITERATURE REVIEWS

1. *Candida* species

1.1. Overview of *Candida albicans* and *Candida* non-*albicans* species

Candida species are generally known as commensal and harmless organisms on healthy people. They could present in many positions throughout the body such as in the skin, oral oropharynx, vagina, and gastrointestinal tract about 30-70% without causing any symptom (Melanie Polke, 2015). Among *Candida* genus, *Candida albicans* presents as the most popular species and is investigated scientifically much more than others. About 200 species of *Candida* genus are classified into two main groups *Candida albicans* and non-*albicans Candida* spp. (NACs). About 15 isolates of 200 species can colonize and infect humans (Melanie Polke, 2015).

However, *Candida* species could invade inside tissues opportunistically under advantageous conditions when the body is deficient in immunological controlling or broken down the integrity of epithelial barriers. These fungi could attack at superficial locations with mild symptoms to systemic invasion with severe tissue damages, even life-threatening situations. Some *Candida* infections have been reported in patients including oral candidiasis, vaginitis, candidemia, candidiasis peritonitis, biliary candidiasis, hepatosplenic candidiasis, pancreatic candidiasis, gastrointestinal candidiasis, renal candidiasis, endocarditis, and meningoencephalitis consequences of candidemia (Gupta et al., 2015; Azim et al., 2017; Miranda-Cadena et al., 2018; Walsh et al., 2019).

To an extent of clinical reports, *Candida* fungi have been evaluated to be one of the most common causes of opportunistic infections and rise life-threatening of patients who have primary or secondary immune deficiencies. The increased number of secondary immunocompromised states often associates with underlying medical diseases such as cancers hematologic malignancy, massive chemotherapy,

organ transplantations, abdominal surgeries, diabetes, HIV/AIDS, low-birth-weight neonates, and the elderly (Wu et al., 2017; Lamoth et al., 2018; Öncü et al., 2019; Walsh et al., 2019). In addition to immune impairment, the long-term using of antibiotic treatment, invasive medical devices such as parenteral nutrition, mechanical ventilation, catheters, or long staying in an intensive care unit (ICU) are of high risks of nosocomial candidiasis (Lamoth et al., 2018; Öncü et al., 2019). A positive correlation between the source of *Candida* infection and mortality is considered because increased mortality of candidiasis infants is parallel with a diverse combination of many sources of *Candida* infections in the blood, urine and cerebrospinal fluid (Walsh et al., 2019).

1.2. The medical challenges to invasive infections of *Candida albicans* and NACs

In recent years, the epidemiological changes of *Candida* infection have been alarming as many massive challenges to global public health. Comparing to the past, *Candida* non-*albicans* spp. have tended to be more dominant than *C. albicans* which has been known as a common pathogen previously (Azim et al., 2017; Lamoth et al., 2018). As shown in Figure 2.1, the remarkable increase of NACs infections was higher when compared to *C. albicans* with the percentage ranging up to 80% in some countries, for example in USA, Brazil, Peru, and India (Lamoth et al., 2018). Another study of invasive candidiasis in Turkey hospitalized children also found that the proportion of NACs infection accounted for 70% of total candidiasis (Öncü et al., 2019).

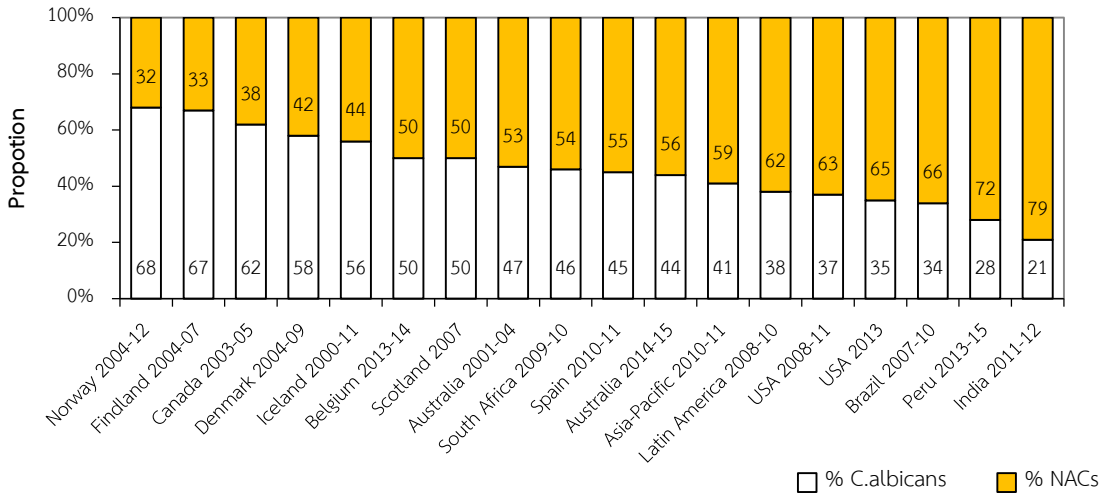


Figure 2.1 The proportion of NACs and *C. albicans* in some countries (Lamoth et al., 2018)

Among *Candida* non-*albicans* spp., five species – *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei,* and *C. dubliniensis* are the most frequent pathogens that have been detected in candidiasis cases via many reports recently (J. C. O. Sardi, 2013; Melanie Polke, 2015; Whibley & Gaffen, 2015; G. Y. Kim et al., 2016; Azim et al., 2017; Lamoth et al., 2018; Öncü et al., 2019). The infected frequency of each above species among candidiasis alters depending on underlying diseases, geometric areas throughout the world, research designs (Pfaller et al., 2008; Lamoth et al., 2018). *C. tropicalis* accounted for the highest proportion of NACs-related candidiasis in cancer patients (Wu et al., 2017) or ICU patients (Gupta et al., 2015). The most common species represent in many conditions are *C. glabrata*, because it has presented in oral candidiasis (Miranda-Cadena et al., 2018), diabetes patients (Khatib et al., 2016), ICU patients (Glöckner & Cornely, 2015) and also has the highest exposure in hands and lab-coats of health-care assistants and hospital environments being accountable to transfect this species to immunosuppressed patients (Savastano et al., 2016). Together with *C. glabrata*, *C. krusei* is the major cause accountable for a high candidiasis incidence in hematological malignancy patients (Hachem et al., 2008). By

contrast, *C. parapsilosis* is the most dominant species of candidiasis in hospitalized neonates and children (Öncü et al., 2019) and *C. dubliniensis* mainly causes diverse candidiasis on HIV/AIDS patients (Coleman et al., 1997; Sullivan & Coleman, 1998).

Moreover, the anti-fungal drug resistance also increases in association with these common NACs infections and contributes to the increased mortality of candidiasis patients seriously (Lamoth et al., 2018). Currently, anti-fungal drugs including azoles, echinocandins, allylamines, and nucleosides are using to treat *Candida* infections. Also, the proportion of anti-fungal drug resistance varies on *Candida* spp. In which, *C. krusei* is the first species with the least susceptibility to anti-fungal drugs, the second is *C. glabrata* and then *C. tropicalis* is the third (Jamiu et al., 2020). Azole drugs are the first-class used to treat candidiasis, however, anti-azoles resistance has been found widely in *C. krusei, C. tropicalis, C. glabrata,* and others (Deorukhkar et al., 2014; Savastano et al., 2016; Y. Wang et al., 2016; Wu et al., 2017; Lamoth et al., 2018; Miranda-Cadena et al., 2018). Furthermore, these *Candida* species currently tends to possess a more dangerous multidrug-resistant ability because the resistance of these species to fluconazole, amphotericin B, echinocandins and flucytosine has been recorded typically in *C. glabrata* and *C. krusei* infection (Pfaller et al., 2008; Lamoth et al., 2018; Jamiu et al., 2020).

The mortality and prognosis of NACs infections have been evaluated as dangerous as *C. albicans* infections and fluctuate on specific patient populations and countries (Lamoth et al., 2018; Jamiu et al., 2020). For example, *C. glabrata* infection has been accentuated on causing high mortality (approximately 50%) as the same as *C. albicans* infection does in diabetes patients and abdominal surgeries (Khatib et al., 2016; Savastano et al., 2016). *C. tropicalis* is the main pathogen responsible for around 45% of 28 day-mortality of candidiasis-infected cancer patients (Wu et al., 2017). *C. parapsilosis* causes about 24% of candidiasis-related death within the first 30 hospitalized days in neonates and children (Öncü et al., 2019). Also, it has been

found that the high mortality of patients tends toward on cases of *Candida* infection which plays an independent factor of survival prognosis (Montravers et al., 2006; Wu et al., 2017). Nowadays, no target vaccine is applied for preventing *Candida* infection. Altogether, candidiasis is still considered as a burden of mortality and aggravates severe consequences of risky patients.

1.3. The diversity of virulence in *Candida* pathogenicity

1.3.1. Similar and dissimilar virulence features of common *Candida* spp.

Some significant factors have been considered as the virulence of *Candida* spp. consist of filamentous transformation, hydrolase secretion, adherence capacity, fitness traits and biofilm formation (Ramage et al., 2005; Tsang et al., 2007; Silva et al., 2012; Mayer et al., 2013). Each species of *Candida* genus probably possesses some similar and private properties (table 2.1) that influence differently their virulence and pathogenicity.

Candida albicans is a diploid of CTG clade phylogeny that appears to be equipped with sharp weapons of pathogenicity according to natural evolution because these effective strategies enable this species to survive and widespread more than other species of *Candida* genus. As well-known, *C. albicans* can transform rapidly from yeast to filamentous form (pseudo-hyphae and hyphae) plus with fitness attributes, which could invade active epithelial barrier when host immunity is impaired (Odds, 1994). The yeast-hyphae transition occurs when this species cope with disadvantageous conditions by sensing high temperature, high CO_2, changes of pH or nutrient changes (Mayer et al., 2013; Nadeem et al., 2013). This transition is mediated through turning on the activation of several hyphae-associated genes (HAGs) plus multifunction-relating target proteins and a high metabolic adaptation to environment factors (S. Brunke & B. Hube, 2013; Mayer et al., 2013; Melanie Polke, 2015). Also, many cell wall Als, Hwp1, Hyr1 proteins are produced as adhesins responsible for high adhesion on cell or non-cell surfaces also influence the

pathogenicity of *C. albicans* through the degree of colonization (Mayer et al., 2013). Besides, it supports this species to adapt quickly to environmental stresses, to escape from host cells and to settle infection sites widely on many risky patient populations. Yet, the magnitude of morphotype changes to *C. albicans* is still not coherent (Neil AR Gow, 2002). On the one hand, during invading deep tissues of the host, this species could salvage a nutritious serum source of the blood stream and tissues to reproduce and disseminate rapidly throughout the body (Mayer et al., 2013; Melanie Polke, 2015). On the other hand, *C. albicans* produces several Saps and phospholipase to destroy the host tissue barrier (Tsang et al., 2007; Melanie Polke, 2015). These also contribute to more virulence and the strongest pathogenicity of this species (Odds, 1994).

Among non-*albicans Candida* species, *Candida dubliniensis* possesses the closest CTG clade phylogeny with *C. albicans* and this species also shares such similar properties that it may be difficult to distinguish from *C. albicans* by only morphology observation (Papon et al., 2013; Merseguel et al., 2015; Whibley & Gaffen, 2015). *C. dubliniensis* can form filamentous morphology and chlamydospores (Coleman et al., 1997; Jabra-Rizk et al., 1999). This species also expresses some virulence-associated proteins such as Saps and Hpw1 (O'Connor et al., 2010). The main difference of two species is genetic which probably determines some alterations of growth and lower virulence of *C. dubliniensis* in comparison with *C. albicans* (Jackson et al., 2009; Moran et al., 2012). For instance, some critical virulence-related genes are not expressed in *C. dubliniensis* when compared with *C. albicans*, such as *ALS3*, *HYR1*, some genes of *SAP* family and so on, thereby, virulence may be reduced in *C. dubliniensis* (Moran et al., 2012). Besides, they could be distinguished by ß-glucosidase activity which is lacking in *C. dubliniensis*, while it presents in *C. albicans* (Sullivan & Coleman, 1998). Unlike *C. albicans*, the filamentous transition of *C. dubliniensis* does not occur during neutrophil culture

medium *in vitro* (Svobodová et al., 2012). In line with this, the filamentous formation of *C. dubliniensis* varies on the presence of nutrient components such as peptone and glucose in the medium due to influence on the expression of UME6 transcription factor (O'Connor et al., 2010).

Regarding to phylogeny, *Candida tropicalis* is of the CTG clade. This species also resembles *C. albicans* in possessing all-powerful virulence factors (Papon et al., 2013; Deorukhkar et al., 2014). Some of *C. tropicalis* isolates could form hyphae on polystyrene material surface with high virulence of invasion, and these species remarkably express genes of top virulence factors such as *ALST2-3* and *SAPT3* (Yu et al., 2016). Nevertheless, few properties of *C. tropicalis* also differ from those of *C. albicans*. For example, the filamentation ability of *C. tropicalis* is not supported in a rich nutritious medium, while *C. albicans* favors the filamentous transition in that medium (Lackey et al., 2013). Besides, this ability also depends on each isolate of *C. tropicalis*. Aside from containing four Saps proteases, *C. tropicalis* reveals the activity of lipase, phospholipase and hemolysin as crucial virulence factors (Silva et al., 2012). However, this notion is still not consistent in some studies (Deorukhkar et al., 2014; Yu et al., 2016).

In similar to the above species, *Candida parapsilosis* is also classified into CTG clade of *Candida* phylogeny (Papon et al., 2013). However, the morphological transition of *C. parapsilosis* merely limits on yeast to pseudo-hyphae form under the association of various regulator genes (Tóth et al., 2019). Besides, this species possesses a few adhesins of Als, Hwp and Hyr protein family that involve in its capacity of colonization on both cellular and material surface (Silva et al., 2012; Tóth et al., 2019). Additionally, the secretion of hydrolase is also not excluded in virulence factors of this species. It has proven that many isolates of *C. parapsilosis* secrete adhesins, three kinds of Saps proteases, lipase and phospholipase enzyme that correlate tightly with strong virulence (Silva et al., 2012; Tóth et al., 2019). Typically,

protein Sapp1 and Sapp2 have been recently shown their crucial role in attenuating the functions or damaging innate immune cells (Singh et al., 2019). Also, higher expression of phospholipase and protease activity is shown in *C. parasilosis* isolates adhering toward material surfaces (Costa et al., 2010).

Although *Candida krusei* is not a CTG clade or WGD clade of phylogeny, this species probably possesses some similarities to other species among *Candida* genus (Papon et al., 2013). *C. krusei* is a diploid, dimorphic and can induce filamentous form as the primary virulence in some circumstances. For example, the transformation of yeast to hyphae has been found on *C. krusei* infection at oral epithelial tissues of immunosuppressed mice (Samaranayake et al., 1998). The pseudo-hyphae form of *C. krusei* also occurs inside phagocytes after phagocytosis (García-Rodas et al., 2011). However, *C. krusei* requires a longer time to this transition and less aggressive than *C. albicans*, so pseudo-hyphae form may not the primary invasive virulence of the former (Samaranayake et al., 1998; Jamiu et al., 2020). Besides, *C. krusei* differs from *C. albicans* inability of adhesion on epithelial cells because the former adheres to epithelial cells lower but higher on the acrylic surface of materials than the latter does (Samaranayake et al., 1994). Consistently, *C. krusei* also contains a low activity of Saps or phospholipase on an epithelial surface which contrasts with those activities of isolates that adhere to material surface (Costa et al., 2010). Furthermore, *C. krusei* has been described to reveal a high primary resistance to many antifungal drugs (Jamiu et al., 2020).

Regarding to phylogeny, unlike any species, *C. glabrata* is a haploid of WGD clade which is the closest to a non-pathogenic species *Saccharomyces cerevisiae* (Papon et al., 2013; Timmermans et al., 2018). Thereby, *C. glabrata* has known to possess different pathogenicity verse *C. albicans* and other species, because it contains less virulent factors (Fidel et al., 1999; Sascha Brunke & Bernhard Hube, 2013; Galocha et al., 2019; Kumar et al., 2019). Together with the smallest size of

yeast form, it has known that *C. glabrata* can produce pseudo-hyphae but fail to induce hyphae form (Fidel et al., 1999; Csank & Haynes, 2000). Consistently, *C. glabrata* mainly grows under yeast form (Silva et al., 2012) and lacks of morphological change that is sensitive to environmental factors, for example, high temperature when comparing to *C. albicans* (Csank & Haynes, 2000). Virulence-related SAPs are also absent in *C. glabrata* as well as no phospholipase activity is seen in this species (Galocha et al., 2019; Kumar et al., 2019; Pais et al., 2019). However, instead of possessing *ALS* adhesin-producing gene family like in *C. albicans*, the adhesion of *C. glabrata* is typically encoded by 18 genes of *EPA* gen family which shows similar functions to *ALS* gene, especially gene *EPA1* and *EPA 6* (De Groot et al., 2008; Silva et al., 2012; Timmermans et al., 2018). Some studies demonstrated that adhesins Epa1 protein of *C. glabrata* relates to adhesion ability on cell surfaces, while Epa6 influences its ability on polystyrene materials *in vitro* (El-Kirat-Chatel et al., 2015; Vale-Silva et al., 2016; Tian et al., 2020). This feature is promoted via abundant kinds of adhesins secreted by *C. glabrata*, whereby the adhesion ability could play an important role in *C. glabrata* virulence (Timmermans et al., 2018). Furthermore, together with *C. krusei*, *C. glabrata* possesses a high intrinsic anti-fungal multi-drug resistance which enhances their pathogenicity more than other species (Kumar et al., 2019; Pais et al., 2019).

Generally, the expression of virulence factors varies on each *Candida* species defines the different capacity of pathogenicity and infectivity. Most of species has filamentation as the essential strategies to inhibit functions of immune cells and escape the eradication of immunity, except *C. glabrata* (García-Rodas et al., 2011; Lewis et al., 2012; Tóth et al., 2014). Adhesion property of *Candida* spp. is for initial colonization as well as for pointing out their virulence, because four NAC species (*C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata*) with tendency of adhering more dominantly on medical material surfaces possess higher virulent (Costa et al., 2010;

Yu et al., 2016). Also, this ability relates to biofilm formation of these species on different human-made biomaterials and resistance to anti-fungal immunity (Ramage et al., 2005; Timmermans et al., 2018). On the other hand, the infectivity difference of each species is also illustrated subjectively in a previous *in vivo* study, in which these species are grouped into three levels of virulence based on burdens of *Candida* infections, the mortality and lessons of internal organs. Particularly, *C. albicans* and *C. tropicalis* are of the most virulent group, *C. glabrata* is of the intermediate virulent group, and *C. parapsilosis* and *C. krusei* are of the lowest virulent group (Arendrup et al., 2002). However, in *Caenorhabditis elegans* model to test virulent factors, *C. krusei* could cause the mortality rate as high as *C. albicans* and higher than other NACs (Ortega-Riveros et al., 2017). Accordingly, other study observed that *C. albicans* and *C. tropicalis* succumbed mice within 10-15 days after gut mucosa infection and within 2-5 days after systemic infection, whereas high doses of *C. krusei*, *C. glabrata* and *C. parapsilosis* could kill mice within 10-15 days after systemic infection (Hirayama et al., 2020).

Table 2. 1 Some properties of common *Candida* spp. (Papon et al., 2013; Whibley & Gaffen, 2015; Ortega-Riveros et al., 2017; Gómez-Gaviria & Mora-Montes, 2020)

Candida spp.	Yeast size (μm)	Phylogeny	Morphology			Numbers of virulent Saps
		Clade	Yeast	Pseudo-hyphae	Hyphae	
C. albicans	4-10	CTG	+	+	+	10
C. dubliniensis	4-10	CTG	+	+	+	8
C. tropicalis	5-11	CTG	+	+	+	4
C. parapsilosis	3-9	CTG	+	+	-	3
C. krusei	3-10	Other	+	+	+/-	+/-
C. glabrata	2-4	WGD	+	+/-	-	-

1.3.2. The association of cell wall components with the virulent ability of *Candida* spp.

The cell wall of *Candida* genus is quite tight and dynamic structure because a cell membrane-embedding network of three major carbohydrate compositions plus proteins could be rearranged coherently under some environmental changes (Ruiz-Herrera et al., 2006; Latge, 2010; Free, 2013). These components are polymers of carbohydrate units including mannans from mannose units, glucans from glucose units and chitin from N-acetyl-D-glucosamine units (Free, 2013). The different proportion of each component is shown (in Table 2.1) and varies depending on each strain of *Candida* genus. The flexible structure of cell wall also gives many benefits for *Candida* spp. Particularly, by a transition of morphologies (yeast to pseudo-hyphae and to hyphae), this fungi is easy to handle external stressful alterations such as temperature, pH, humidity and nutrient sources to retain their viability (Gow & Hube, 2012).

In addition, cell wall components are considered as the key factors of fungal pathogenicity and physiology of fungal infection process through the first interplay with host cells directly (Ruiz-Herrera et al., 2006; Latge, 2010). Particularly, cell wall components could involve in the adhesion abilities of *Candida* species on the surface. Cell wall structure could promote the ability of fungal colonization in epithelial cells of tissues through an interaction of revealed ß-glucan on fungi and its receptors on host cells (Sem & 2016). Also, these components help to shape the morphotypes and the hardness of cell wall of *Candida* spp. A complex of glucan and chitin supports fungi to actively penetrate deeper or form a biofilm to prevent fungi from an immune attack (Arana et al., 2009; Mihai G. Netea, 2015).

The influence on reducing immune responses could be referred to as the virulence capacity of cell wall carbohydrates. For cell wall ß-glucan, *C. albicans* ß-

glucan could restrain IL-6 production of endotoxin-stimulated human peripheral mononuclear cells (PBMCs) and monocytes. Besides, the functional cytokines of T cells in the collected PBMC population were suppressed under stimulating *C. albicans* ß-glucan through decreased IL-2 and IFN-γ production in this study (Nakagawa et al., 2003). In addition, the release of cell wall components into the blood was found in candidiasis patients (Mokaddas et al., 2011), which may cause a suppression of antifungal immunity leading to severe infections (Arana et al., 2009). CR3 with dectin-1 receptor regulate the effects of *C. albicans* ß-glucan on the natural balance of IL-1ß and IL-1 receptor antagonist (IL-1RA) specifically and cell deaths (Ganesan et al., 2014; Smeekens et al., 2015). Serum ß-glucan concentration of candidemia patients negatively correlates with the prognosis degree of infection outcomes (Giacobbe et al., 2015). The higher level of ß-glucan is in serum, the more mortality is likely in these candidiasis patients (Sims et al., 2012). For cell wall mannan, in recent studies, mannan composition locates at the outermost of *Candida* cell wall could trigger cell death together with the activation of innate immune cells and even turn the fate of adaptive immune cells. In which, mannan of *C. krusei* triggered the apoptosis-related DC death via TLR2 and MyD88 signaling pathway in concomitant with inhibiting Th1 and enhancing Th17 response (Nguyen et al., 2018; Thu Ngoc Yen Nguyen, 2018). Strikingly, another study found that *C. glabrata* increases its virulence and anti-fungal resistance during its growth through remodeling cell wall components (Bouklas et al., 2017). For cell wall chitin, it was found that it displayed to dampen cytokine responses of innate cells (Mora-Montes et al., 2011). From these findings, it implies that cell wall components could associate closely with the severity of candidiasis, as well as these components probably decide the virulence of each *Candida* species.

Table 2. 2 Cell wall components of common yeast *Candida* spp.

Candida spp. Strain	Cell wall components				References
	Mannan (%)	Glucan (%)	Chitin (%)	Proteins	
C. albicans	38–40	58–60	2	-	(Ruiz-Herrera et al., 2006)
	26.6	64.0	4.2	3.5 (%)	(De Groot et al., 2008)
	36.1	61.7	2.0	146.4 (µg)	(Navarro-Arias et al., 2019)
	35.31	62.97	1.72	-	(Thompson et al., 2019)
C. dubliniensis	-	-	-	-	
C. tropicalis	36.2	62.9	2.3	129.7 (µg)	(Navarro-Arias et al., 2019)
	37.47	59.29	3.24	-	(Thompson et al., 2019)
C. parapsilosis	58.88	40.75	0.37	-	(Thompson et al., 2019)
C. krusei	23.9	67.8	8.2	76.4 (µg)	(Navarro-Arias et al., 2019)
C. glabrata	43.8	54.0	1.2	6.4 (%)	(De Groot et al., 2008)
	28.09	69.51	2.40	-	(Thompson et al., 2019)

2. Dendritic cells with their roles in immunity against *Candida* infections

2.1. Characteristics and functions of dendritic cells

Dendritic cells were discovered by Ralph Steinman in decades of the 70s and 80s of century XX and then known widely for their master functions in the immune system. In 2011, the Nobel prize was gifted for his important explorations (Mildner & Jung, 2014). Up to date, DCs has known about their properties and functions as well as their classification in mouse and human (Solano-Gálvez et al., 2018). They originally derive from bone marrow cells and distribute in any tissue lymphoid or non-lymphoid throughout the body. Depending on the original lineage, position, specialized markers (surface and transcriptional regulators) and functions,

differentiated DCs are classified into many major subpopulations such as myeloid conventional DCs (cDCs) with two subtypes of cDC1s and cDC2s, plasmacytoid DCs (pDCs), inflammatory monocyte-derived DCs (mo-DCs) and other distinct subsets such as cutaneous (Solano-Gálvez et al., 2018; Chrisikos et al., 2019; Eisenbarth, 2019; Ritprajak et al., 2019).

In general, like their name with nerve cell-like stellate dendrites, DCs play a role as sentinels in immunity for rapidly recognizing an invasion of certain pathogens. With high mobility, pathogenic information-bearing DCs migrate to local peripheral lymph nodes to communicate with naive T lymphocytes there and triggering powerful immune responses combating targets (Alvarez et al., 2008). Upon the ability of antigen presentation and activation of adaptive immune cells, DCs are specialized as the professional antigen-presenting cells (APCs) (Solano-Gálvez et al., 2018).

For sensing pathogens, many PRRs expressed on DC surface or in intracellular space could bind with their ligands as PAMPs from pathogens quite specific. Some PRRs are toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain receptors (NODs) and retinoid-inducible gene 1-like receptors (RIG-I) (Wüthrich et al., 2012). On the one hand, DCs engulf pathogens through many processes as macro-pinocytosis, phagocytosis, or endocytosis. Later, they digest pathogens and process to combine antigens to MHC class I or II molecules which are identifications for DC-T cell communication. On the other hand, an upgrade from non-activated to activated state or from immature to mature state enables DCs to express many functional molecule markers. For instance, the chemokine receptor CCR7 molecule is an important signal required for DCs exactly locate where to migrate and gather with T cells in lymph nodes (Alvarez et al., 2008; Mildner & Jung, 2014).

For activating T cells, DC-T cell communication requires many molecules expressed by DC as important signals for this immune synapse (Mildner & Jung, 2014;

Chrisikos et al., 2019). In detail, MHC molecules help for displaying pathogenic antigens from DCs and bind with T cell receptors (TCR) that is the first signal. MHC class II is required for DC contact with T $CD4^+$ cells, whereas MHC class I is required for T $CD8^+$ cells. To promote activation of T lymphocytes, a requirement of costimulatory markers (such as CD80, CD86) expressed by DCs is the second important signal. DC-secreted cytokines as the third key signal combine with other two signals inducing the specific transition of transcriptomic factors of T cells to optimally orient environment for T cell differentiation and proliferation longer time targeting to pathogens (Leung et al., 2010; Saravia et al., 2019). T helper (Th) 1 cells are known to require promotion of cytokine IL-12, Th2 cells need IL-4, IL-5, Th17 cells require a presence of IL-23, IL-6, and regulatory T (Treg) cells need IL-10. However, Th17 cells also share some signaling of TGF-ß with Treg cells, so these groups of cells could be separated by IL-7 involvement of Th17 differentiation and IL-2 requirement for Treg development (Zhou et al., 2009; Leung et al., 2010; Saravia et al., 2019). To maintain the effective immune responses, DCs also promote the memory T cells with life-long for advancing secondary pathogen-targeted defense (Anderson et al., 2008). In addition to inducing T cell activation against pathogens, DCs affect the tolerance responses by adjusting the activity of autoreactive T cells to self-antigens under a normal state for keeping a balance of immune activities throughout the body (Probst et al., 2014). Through mechanisms of inducing peripheral tolerance, autoimmune diseases could be prevented from wrong reactions of immunity (Iberg et al., 2017; Chrisikos et al., 2019). Hence, DCs play a pivotal role in managing both tolerance immune responses and protective immune responses diversely depending on DC subtype, context, and location.

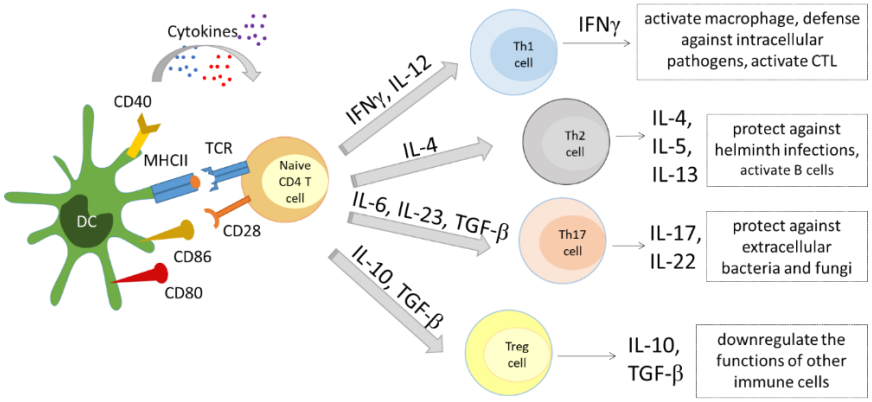


Figure 2.2 DCs dictate the direction of adaptive immune responses (Zhou et al., 2009; Leung et al., 2010; Saravia et al., 2019)

According to those DC functions of immunity, these cells could be separated phenotypically and functionally into two DCs groups: immunogenic and tolerogenic DCs (Figure 2.3) (Ritprajak et al., 2019). Under certain conditions, several molecules of maturation, cytokine production, anti-inflammatory mediators, chemokines, chemokine receptors, and metabolic control could be up- or down-regulated involving in the biological status of DCs in many studies (Ritprajak et al., 2019). These events enable DCs to skew toward one of phenotype directions and bring about changes in the T cell responses appropriately. Immunogenic DCs can elicit the immune responses to eradicate certain invasive pathogens through enhancing Th1 or/and Th17 responses, while tolerogenic DCs tend to modulate or suppress immune responses through Treg responses aiming to balance immune reactions or maintain an immune tolerance throughout the body (Roussey et al., 2016; Ritprajak et al., 2019).

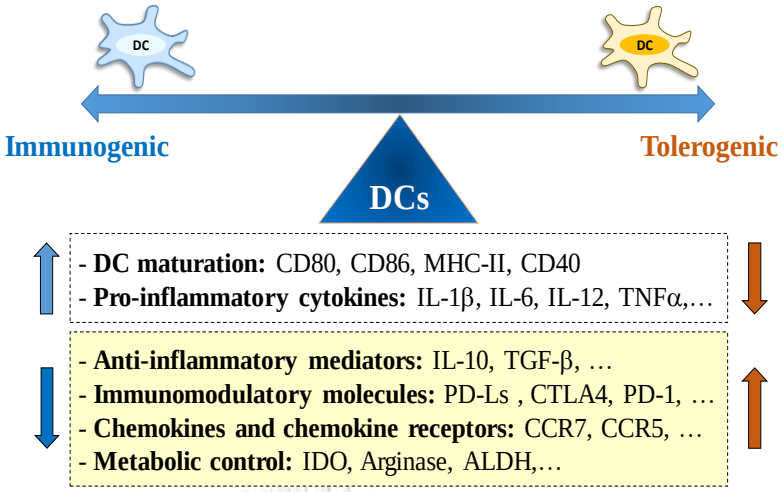


Figure 2.3 Several molecules relate to the fate of DC phenotypes (Serrano et al., 2018; Ritprajak et al., 2019)

In parallel to the above classification, DCs are also named as immature, semi-mature and mature cells, which correlates to the different activation states of DCs in their proliferation and pathological contexts (Lutz, 2012; Schmidt et al., 2012). It is described that immature DCs which have a low expression of activation markers (such as CD80, CD86, MHC-II), high release of anti-inflammatory mediators (such as IL-10), and low secretion of pro-inflammatory cytokines (such as IL-6, TNF-α, IL-12), tend to display the tolerogenic features. On the contrary, mature DCs that show the immunogenic properties, tend to have high expression of maturation, low levels of anti-inflammatory mediators and high amount of pro-inflammatory cytokines. For semimature DCs, these cells exhibit the tolerogenic features, but differentially integrate phenotypical signatures between immature and mature DCs. Possibly, they have a low or high expression of maturation markers, high or low amounts of anti-inflammatory mediators, high or low amount of pro-inflammatory cytokines depending on certain contexts (Schmidt et al., 2012; Dudek et al., 2013).

2.2. The roles of dendritic cells in host immunity against *Candida* spp.

Being one of the key phagocytes in immunity, DCs is crucial for the establishment of effective immune responses against fungal infections in general and *Candida* infections in particular (Mihai G. Netea, 2015; Qin et al., 2016; Roussey et al., 2016; Feldman et al., 2019). Several studies explored the interaction of DCs and *C. albicans* infection in various aspects. Previously, it is known that DCs can respond differently to yeast form and hyphae form of *C. albicans* morphology. Thereby, IL-12 secretion and the protective Th1 responses were triggered by DCs in response to *C. albicans* yeasts, while these activities were inhibited when DCs responded to *C. albicans* hyphae (d'Ostiani et al., 2000). Owning a phagocytosis potency, DCs could uptake *C. albicans* yeasts, produce efficiently killing capacity of this species and activate T cell proliferation (Newman & Holly, 2001). Interestingly, some studies found that DCs responses were affected by cell wall components and these provided fundamental evidence of a DC- *Candida* interaction, because DCs possess many kinds of PRRs to recognize PAMPs of *Candida* spp. Particularly, DCs became more mature and secreted high amount of IL-12 due to ß-glucan on *C. albicans* cell wall (Kikuchi et al., 2002). Through ß-glucan binding capacity of dectin-1 receptor, DCs operated intracellular signaling pathways in response to *C. albicans* (Rogers et al., 2005; Goodridge et al., 2007; Gow et al., 2007). DCs increased phagocytosis of *C. albicans* through recognition of mannan which is mediated by CLRs (Cambi et al., 2008). In addition, the susceptibility of *C. albicans* infection was increased in dectin-1 deficiency mice (Thompson et al., 2019). The more important thing, DCs dictate the *Candida*-specific adaptive immunity including Th1 and Th17 responses can protect from *C. albicans* infection (Wüthrich et al., 2012). It was found that *C. albicans* ß-glucan-exposed DCs promoted Th1 and Th17 proliferation *in vivo* through activating dectin-1 signaling pathway (LeibundGut-Landmann et al., 2007). Besides, DCs could recognize the mannan structure of *C. albicans* through dectin-2 receptor and

secreted IL-1ß, IL-23 which promoted the protective Th17 proliferation (Saijo et al., 2010). Moreover, another study indicated that Th1 responses were promoted by *C. albicans* yeast-exposed DCs, while hyphae-exposed DCs preferred to elicit Th17 response (Rizzetto et al., 2010). Other study showed both mannan and glucan of *C. albicans* could trigger DCs produced prostaglandin E2 leading to upregulate Th17-priming cytokines such as IL-1ß, IL-6 and IL-23 (Smeekens et al., 2010).

Recently, the magnitude of DCs in *Candida* infection has drawn attention more because DCs differently respond depending on each species. Different kinds of yeasts mediated various expression levels of CD80, CD86, CD40, CD83, and cytokine TNF-α, IL-6, IL-1ß, IL-8, and IL-10 of DCs (Bazan et al., 2018). Some NACs could mediate differently DC responses which differed from *C. albicans*. Different amounts of pro-inflammatory cytokines were released from DCs in responses to various *Candida* spp. such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. albicans* (Thompson et al., 2019). In addition, another study found DC maturation and cytokine productions vary on different types of mannan structures of *C. albicans* and NACs. Of which, mannan of NACs such as *C. krusei* and *C. parapsilosis* tended to upregulate those DC properties higher than *C. albicans* (Nguyen et al., 2018; Thu Ngoc Yen Nguyen, 2018). Furthermore, the different outcomes of immunity combating fungi also depend on various DC subtypes (Salazar & Brown, 2018). In *C. albicans* cutaneous infection, yeast-exposed Langerhans triggered the adaptive immune response toward Th17, while they shifted toward Th1 when responded to hyphae form (Kashem et al., 2015). From different DC-*Candida* interactions, DCs change their properties discretely, therefore, the outcomes of adaptive immunity possibly differ in relevance to those *Candida* spp. (Thompson et al., 2019). Besides, survival rate and burden infections of *Candida* spp.– infected mice exhibited distinctly, in which *C. albicans* and *C. tropicalis* could cause higher mortality than

other species (Hirayama et al., 2020). Although DCs play a crucial role in anti-*Candida* immunity, the direct interaction of DCs and NACs is still poorly understood.

3. ß-glucan of *Candida albicans* and non-*albicans* species

3.1 Structure and properties of *Candida* ß-glucan

Glucan, one of the major polysaccharides constructs the inner layer of the fungal cell wall and accounts the most weight of the cell wall mass (Arana et al., 2009; Free, 2013). Glucan contains various structures of α-or ß-linkages with glycosidic bonds between glucose units, depending on the sources of glucan such as α-(1,3)- or α-(1,4)-linked glucan, ß-(1,2)-; ß-(1,3)-; ß-(1,4)-; ß-(1,6)-linked glucan in linear glucan or (2,3)-; (3,6)-linkages in cyclic hyphae glucan. The number of these linkages has various effects on the conformation and solubility of glucan structure (Dalia Akramienė, 2007; Free, 2013; Lowman et al., 2014). Also, it is found that almost glucans of *Candida* genus are structured by ß-(1,3)-linkages and ß-(1,6)-linkages of glucose monomers (Figure 2.4) (Godfrey Chi-Fung Chan et al., 2009; Korolenko et al., 2019). Structures of ß-glucan profoundly affect their bioactivities on host cells, since a diversity of ß-glucan characteristics has been described in many previous studies (Han et al., 2020).

Notably, the complexity of length, degree of branching, and branching structures of ß-glucans may vary among *Candida* species and fungal morphotype (Godfrey Chi-Fung Chan et al., 2009; Lowman et al., 2014; Mihai G. Netea, 2015; Xiao et al., 2020). For *C. albicans*, yeast cell wall glucan structure possesses a linear backbone of ß-(1,3)-glucan along with ß-(1,6)-glucan branches, but ß-glucan of filamentous form possesses cyclic structure and more branches (Lowman et al., 2014). For example of NACs, *C. glabrata* ß-glucan also contains a linear backbone of ß-(1,3)-glucan with ß-(1,6)-linked branch chains as same as in *C. albicans* yeast glucan, but the average length of glucan backbone, the position of branches

extruded from the backbone and the number of branched chains are different from *C. albicans*. Furthermore, the ß-(1,6)-glucan branches of *C. glabrata* with 4 to 5 units of glucose are curled and longer than this of *C. albicans*. While branches of *C. albicans* ß-glucan start at the terminal end of the backbone, branches of *C. glabrata* glucan extrude from intra-chain at every 21 repeated units of the backbone (Lowman et al., 2011; Lowman et al., 2014). Currently, it still has less information about how the structural difference of ß-glucan of other NACs.

Additionally, the diverse effects of ß-glucan on host cells vary depending on their size, molecular weight, and water solubility, since these properties could determine the conformation of ß-glucan in a solution and impact on the affinity of ß-glucan and its receptors (Sletmoen & Stokke, 2008; Godfrey Chi-Fung Chan et al., 2009; Batbayar et al., 2012; Han et al., 2020). Different levels of IL-1ß and reactive oxygen species (ROS) are produced by DCs in different sizes of ß-glucans (Elder et al., 2017). The proportion of ß-glucan exposure varies on strains of *Candida* species (Graus et al., 2018), and this suggests that various *Candida* spp. possibly express the different areas of ß-glucan exposure on *Candida* cell wall leading to the different effects of host immune cells against these fungi. The soluble or particulate extracted glucan can vary their properties and biological effects during interacting with host immune cells (Sletmoen & Stokke, 2008; Batbayar et al., 2012). Soluble ß-glucan of *C. albicans* could inhibit the pro-inflammatory cytokine (IL-6) release of endotoxin-activated monocytes and monocytes exposed with this ß-glucan could suppress the functional cytokine secretion (IL-2 and IFN-γ) of T lymphocytes (Nakagawa et al., 2003). Besides, soluble glucans induced lower effects on macrophages and none effect on DCs, while particulate glucans could mediate high effects on these cells in anti-tumor responses (Goodridge et al., 2011; Qi et al., 2011). In line with this, yeast-derived micro-particulate ß-glucan with the suitable size was easier to be taken up

by phagocytes and impacted on functionally cellular activities such as the maturation of phagosome, autophagy and ROS production (Fatima et al., 2017).

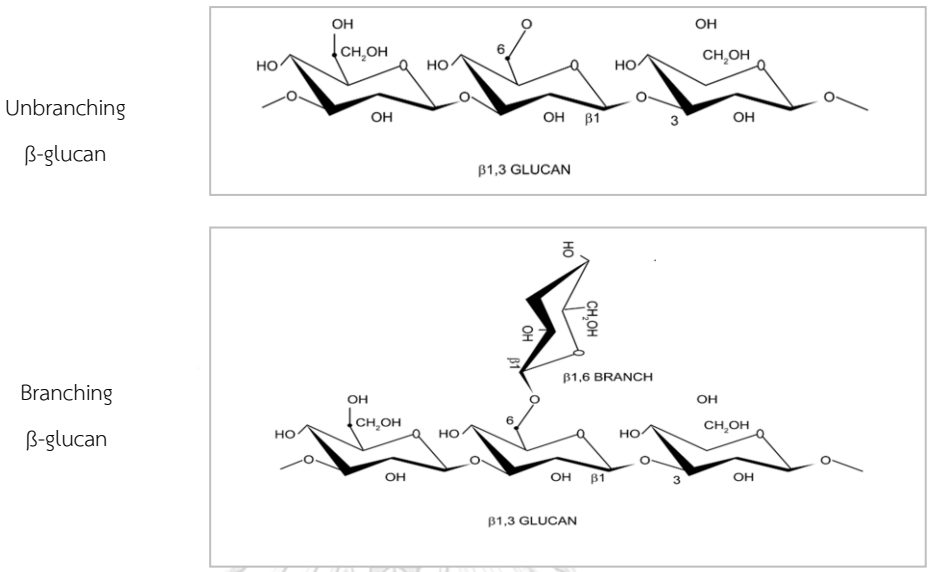


Figure 2.4 The basic structure of yeast ß-glucan **(**Godfrey Chi-Fung Chan et al., 2009**)**

3.2. Receptors of ß-glucan

The sensation of fungal ß-glucan is performed by many PRRs on host cells. Historically, the first found receptor is complement receptor 3 (CR3), then dectin-1 receptor, TLRs, other C-type lectin receptors, scavenger receptors, and EphA2 receptor (Godfrey Chi-Fung Chan et al., 2009; Xiao et al., 2020). Of which, dectin-1 and CR3 are paid the most attention in immune effects of ß-glucan. The difference of above ß-glucan properties could lead to the various interaction between ß-glucan and their receptors. Also, the effects of ß-glucan could vary depending on cell types during interacting with each other.

3.2.1. Dectin-1 receptor

Dectin-1 receptor or CLEC7A is the main protein for host immune cells detecting fungal ß-(1,3)-glucan specifically (Brown & Gordon, 2001; Taylor et al., 2007; Adams et al., 2008; Latge, 2010). Dectin-1 is proven that only recognize polymers of

ß-glucans, not monomers of glucose or other carbohydrates (Brown & Gordon, 2001). This receptor belongs to a type II transmembrane protein of C-type lectin receptor (CLRs) family located on various myeloid cells (Plato et al., 2013). An expression of dectin-1 is found to be higher on macrophages than this on DCs (Brown et al., 2002; Taylor et al., 2002). Interestingly, dectin-1 has a different affinity to ß-glucans. This receptor could bind specifically with only ß-(1,3)-glucan containing at least 7 glucose units with short chains of ß-(1,6)-glucan branches or ß-(1, 4)-glucan branches, while only ß-(1,6)-glucan chain or α-(1, 3)-glucans do not interact with this receptor (Adams et al., 2008; Latge, 2010).

Regarding to structure, dectin-1 receptor is constructed by three portions including a single C-type lectin extracellular domain, a transmembrane portion as a stalk, and an intracellular portion as a polypeptide of 40 amino acids (Goodridge et al., 2009). In functionally, the C-type lectin domain is for ß-glucan engagement and a single YxxI/L motif of the intracellular portion called the hemi immunoreceptor tyrosine-based activator motifs (hemi-ITAM) structure is responsible for initiating to transduce the signal from ligand engagement (Goodridge et al., 2009; Tang et al., 2018). For triggering hemi-ITAM structure, a dimerization of two single-dectin-1 receptors binding to ß-glucan at the same time is started to activate the signal transduction through ITAM structure into many downstream parts of Syk (canonical pathway) (Rogers et al., 2005) and non-Syk signaling such as Ras/Raf1 pathway (Gross et al., 2006; Gringhuis et al., 2009; Plato et al., 2013; Salazar & Brown, 2018). Ultimately, the functions of this pathway are shown via the activation of transcription factors such as NF-kB, NF-AT, ERK, and enzyme pro-caspase 1 and pro-caspase 8 leading to result in phagocytosis, cell maturation, cytokine and chemokine production (Figure 2.5) (Goodridge et al., 2007; Goodridge et al., 2009; Sonja I Gringhuis, 2012; Tang et al., 2018). Thereby, priming for adaptive immune responses is also triggered via typical T cell differentiation to Th1 and Th17 (Goyal et al., 2018),

Th2 (Joo et al., 2015), Th9 (Wang et al., 2018) and even CD8 T cells (Leibundgut-Landmann et al., 2008).

Several discoveries of dectin-1 receptor have paved a better understanding of fungal pathogenesis and anti-fungal defense, especially in *C. albicans* infection (Gow et al., 2007; Taylor et al., 2007; Gringhuis et al., 2009; Reid et al., 2009). Recently, dectin-1 is found as the essential factor of the antifungal immune response against NACs in some recent studies. They found that the cytokine production and phagocytic ability of human innate cells differed among NACs due to different dectin-1 dependent manner (Navarro-Arias et al., 2019). Through dectin-1 receptor, dendritic cells (DCs) and macrophages differently responded to the same *Candida* species in cytokine production (Thompson et al., 2019). Under exposure to *C. tropicalis*, innate immune cells released TNF-α, IL-6 and some downstream molecules of dectin-1 signaling pathway (Duan et al., 2018). Another study has found that dectin-1 pathway could be important for resistance to *C. krusei* infection and susceptibility of *C. krusei* infection increased in dectin-1 knockout mice (S. M. Chen et al., 2019). Similarly, it has shown that dectin-1 played a crucial role in combating *C. glabrata* infection, because the dectin-1 knockout mice are susceptible to *C. glabrata* more than wildtype mice (Chen et al., 2017). Moreover, it also described that *C. albicans* ß-glucan induced IL-1RA in dectin-1- independent manner (Smeekens et al., 2015).

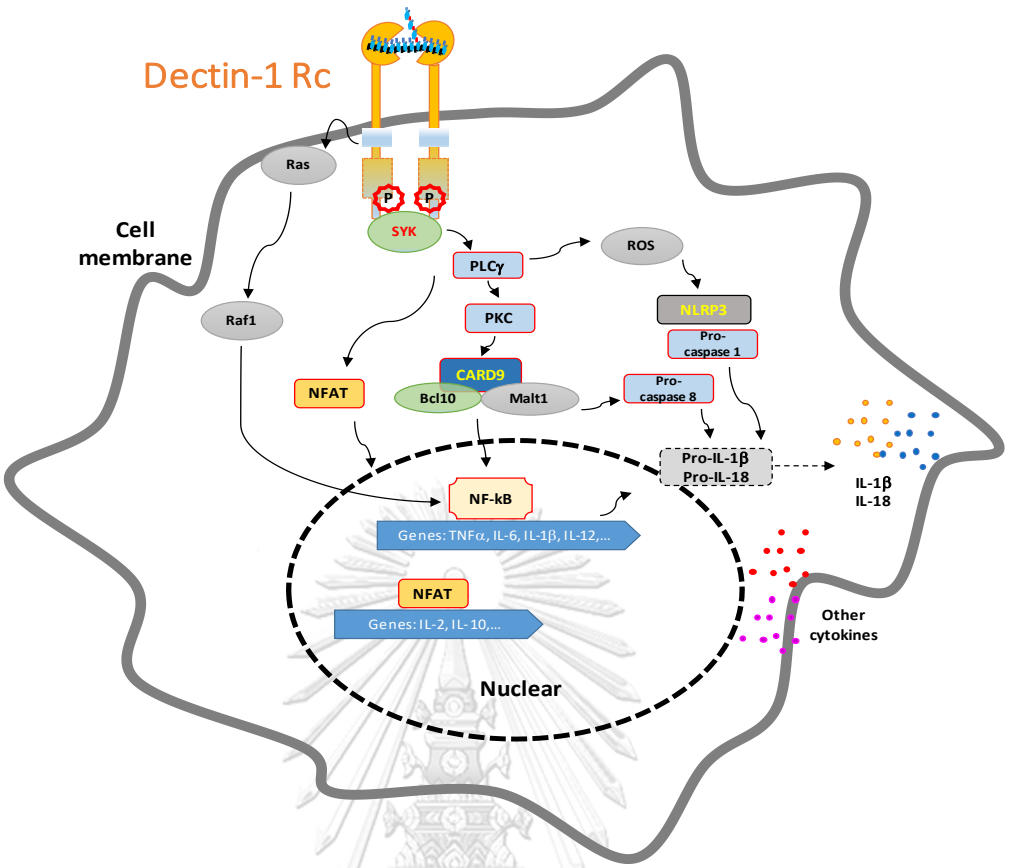


Figure 2.5 Intracellular signaling cascades of dectin-1 receptor triggered by ß-glucan (Brown & Gordon, 2001; Tang et al., 2018)

3.2.2. Completementary receptor 3 (CR3)

CR3 is a heterodimer of two transmembrane glycoproteins CD11b (αM) and CD18 (ß2) known as MAC-1 of β_2 integrin family expressed highly on DCs and other immune cells (Ehlers, 2000) (Schittenhelm et al., 2017; Goyal et al., 2018). CR3 has early known binding to fungal ß-glucan in previous studies (Ross & Větvicka, 1993). Particularly, the lectin sites of CD11b subunit specifically engages with ß-glucan on zymosan (Thornton et al., 1996). In the case of CR3 deficiency, innate immune cells reduced the uptake capacity of ß-glucan (Xia et al., 1999). As known, the ß-glucan engagement of CR3 has mentioned widely as a crucial effect of ß-glucan and innate immunity in anti-tumor approaches (Xiao et al., 2020). Aside from binding complement factor C3b, CR3 can bind with ß-(1,6)-glucan on *Candida* cell wall and

enhance cellular responses including ROS production or phagocytosis (Rubin-Bejerano et al., 2007; Tang et al., 2018).

The activities of CR3 depend on the origin of signals from inside-out or outside-in that could activate the subunits of CD11b and CD18 integrin molecule and impact the cell responses differently (Schittenhelm et al., 2017). Normally, under the steady-state of cells, these subunits exist on non-activated and low-affinity forms through a dynamic hinge between two subunits of each molecule. An activation of CR3 relates to mediate adhesion of cell migration and support to locate for cells in a certain position in tissues; however, it has found that this receptor also involves in immune regulation of both stimulation and suppression depending on cell type (Schittenhelm et al., 2017). For example, activation of CR3 of DCs intimately associated with DC functions and cell viability has been shown in previous studies, thereby it also influences innate and adaptive immune responses (Ehlers, 2000; Varga et al., 2006; Ehirchiou et al., 2007). In the case of activated CR3, DCs enhanced CD86 and MHC-II expression, but reduced Th1-cytokine induction and suppressed Th1 cell proliferation (Behrens et al., 2007; Podgrabinska et al., 2009). Besides that, it was described that DCs enhance maturation and migratory function when blocking CR3 (Morrison et al., 2014; Lukácsi et al., 2020).

3.2.3. The concurrent activation of many receptors during dectin-1- β-glucan interaction

Although dectin-1 reveals as the main receptor of β-glucan recognition, it has been described to cooperate with other receptors during the interplay between innate immune cells and β-glucan (Latge, 2010; Xiao et al., 2020). Depending on the type of glucans, the combination of dectin-1 and TLRs changes the immune response of phagocytes. Thereby, TLR2 and TLR4 could inhibit IL-6, IL-8 and TNF-α resulted from the efficiency of dectin-1 signaling of macrophages in response to particulate β-glucan, while this combination only inhibited IL-6 and IL-8 production

when these cells exposed to soluble ß-glucan (Kanjan et al., 2017). Recently, one of CLRs, CD23 has been reported that it relates to the ß-glucan recognition in anti-*Candida* responses of phagocytes. CD23 is upregulated through NF-AT activation in dectin-1 signaling pathway leading to enhance nitric oxide production of innate immune cells (Zhao et al., 2017). Another study found that a cooperation of CR3 and dectin-1 receptor enhanced the inflammasome activity, IL-1ß and cell deaths of DCs exposed to *C. albicans* ß-glucan (Ganesan et al., 2014). A few receptors of scavenger receptor family also involve in recognizing particulate ß-glucan, and they may cooperate with dectin-1 receptor with an unclear mechanism (Salazar & Brown, 2018). The coexistence of many ß-glucan receptors possibly generates diversity and complexity of antifungal immune responses in a species-dependent manner.

CHAPTER III

MATERIALS AND METHODS

1. Animals and ethics statement

Healthy female C57BL/6 mice from five to six weeks old were purchased from Nomura Siam International Company Ltd., Bangkok, Thailand. All animal procedures were performed following the guidelines and approved by the Chulalongkorn University Institutional Animal Care and Use Committee (IACUC) (Animal protocol number 19-33-010 and 031/2561).

2. *Candida* yeast strains and culture

Candida albicans strain SC5314, *Candida glabrata* strain ATCC2001, *Candida krusei* strain ATCC6258, *Candida tropicalis* strain ATCC750, *Candida parapsilosis* strain ATCC90018, and *Candida dubliniensis* strain NCPF3490 were used in this work as the reference strains are used for quality control and antifungal drug susceptibility testing.

This study used YPD medium consisting of 1% of Yeast (HiMedia Laboratories, Mumbai, India) – 2% of Peptone (HiMedia) – 2% of Dextrose (D-Glucose, QRëC, New Zealand) as the standard medium for yeast culture (Sherman, 2002). The suitable media was sterile by autoclaved at 121^{oC} within 20 min before using for growing *Candida* yeasts. Each strain was inoculated on petri dishes containing YPD medium plus 2% of agar powder (HiMedia) at 30^{oC} for 36 hours (for *C. albicans*, *C. glabrata*, *C. parasilosis* and *C. dubliniensis*) and for 12 hours (for *C. krusei* and *C. tropicalis*) in incubator (WIS-30, DAIHAN Scientific - Korea). Next, colonies of each type were picked up and resuspended in YPD broth, then counted the number by chamber Neubauer Hema-cytometer (Marienfeld, Germany) and observed under bright field of

microscope (Oly-CH2, Olympus, USA) to ensure their yeast morphology. For preparing subculture, 5.2 x 10^6 yeast cells of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. dubliniensis* and 7.8 x 10^6 yeast cells of *C. krusei* and *C. tropicalis* were transferred into 15 mL of YPD broth in 50mL-glass flasks and placed in an shaking incubator (DAIHAN Scientific) at 30^{oC} plus shaking 180 rpm for 6-8 hour to get log-phase of yeasts. Optical density (OD) index of yeast solution was measured by a spectrophotometry at wavelength 600nm (Evolution e500, Korea). Later, transferring the volume of yeast cells with started OD_{600} = 0.1 into 1.2 L of YPD broth in 2L-glass flasks were continued to incubate at 30^{oC} plus shaking 150 rpm for 10-13 hours in incubator (INFORS HT, Switzerland) to expand a large number of yeast mass for β-glucan extraction. This condition of temperature, time of culture and density of yeasts showed mostly *Candida* yeast (100%) (the recorded images in appendix) and yeast form of each strain was checked by bright filed microscope before extraction of β-glucan (Olympus BX50, Tokyo, Japan).

3. Cell wall β-glucan extraction

The protocols for *Candida* microparticulate β-glucan extraction, depyrogenation, and sterilization were kindly given by Prof. David L. Williams (Department of Surgery, James J. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA) (David L. Williams S., 1991; Lowman et al., 2003; Lowman et al., 2011; Lowman et al., 2014). The protocol consists of two main parts that ensure over 95% of purity and quality of microparticulate β-glucan (mp-β-glucan) is described as following (Figure 3.1).

a) First part is the preparation and extraction of mp-β-glucan. Log-phage yeast cells were collected by centrifuging with 8000 rpm at 4^{oC} for 10 min (Centrifuge Avanti® Beckman Coulter, USA). Then, follow the below steps

Step 1: Stir 40 g of fresh yeast cells into 400 mL of NaOH 0.75N (AppliChem, Germany) in a big glass beaker and cover with foil membrane. Boil and stir this suspension at 100^{oC} for 15 min. Cool it down and centrifuge with 5000 rpm at 4^{oC} for 5 min to get the pellet. Repeat this step more 2 times. This step is mostly to get rid of mannan, lipid, and protein out of the cell wall.

Step 2: Resuspend the pellet in 400 mL of H_3PO_4 2N (QRëC), also boil and stir at 100^{oC} for 15 min. Cool it down and centrifuge to get the pellet. Repeat this step more 2 times.

Step 3: Resuspend the pellet in 100 mL of 1% acidic ethanol that consists of 10 ml H_3PO_4 to 990 ml of absolute ethanol (QRëC). Then, boil and stir at 100^{oC} for 15 min. Cool it down and centrifuge to get the pellet. Repeat this step more 2 times.

Step 4: Wash the pellets in 200mL of free-endotoxin water (Otsuka, Bangkok, Thailand), boil and stir at 100^{oC} for 15 min. Cool it down and centrifuge to get the pellet. Repeat this step at least more 2 times. Then, measure pH by pH indicator strip (MColorpHast^{TM}, Merck, Germany) and adjust pH to 7.0 approximately.

Step 5: Collect the pellet by centrifuging, then lyophilize it for long storage (Lyophilization systems, ING, USA). Store the lyophilized mp-ß-glucan at fridge -20^{oC} until use.

To avoid endotoxin contamination, all glass containers were prepared under endotoxin-free condition by autoclaved at 121^{oC} 40 mins and heated to dry at 200^{oC} in an oven (UN55-Memmert, Germany) for at least 9 hours.

b) Second part is an endotoxin elimination and sterilization of mp-ß-glucan

Step 1: Resuspend 0.05 g of lyophilized mp-ß-glucan in 10 mL of endotoxin-free water. Then, rotate the suspension at room temperature for 18-24 hours.

Step 2: Centrifuge and wash the pellet with endotoxin-free water for 2 times. Then adjust pH to 7.0 by pH indicator strip inside sterile hood.

Step 3: Depyrogenate mp-β-glucan suspension by adding 1 - 2 mL of NaOH 250 mM and gently shaking within 1-2 min. Then neutralize the suspension by titrating the volume of H_3PO_4 250 mM respectively.

Step 4: Centrifuge 5000 rpm for 5 min at 4^{oC} and wash mp-β-glucan pellet with endotoxin-free water (repeatedly total 5 times). Then discard all the supernatant.

Step 5: Dissolve the pellet in a desired volume 5 mL of free-endotoxin water and calculate their concentration (dry weight/volume), in this case, a desired concentration is 10 mg/mL.

Step 6: Sterile mp-β-glucan suspension by autoclaving in according to standard sterilization condition (at 121^{oC} for 20 min under 15 psi of pressure). Then, store the suspension at 4^{oC} until using for experiments.

Finally, the concentration of β-glucan solution was calculated as above steps with a final 10 mg/mL concentration.

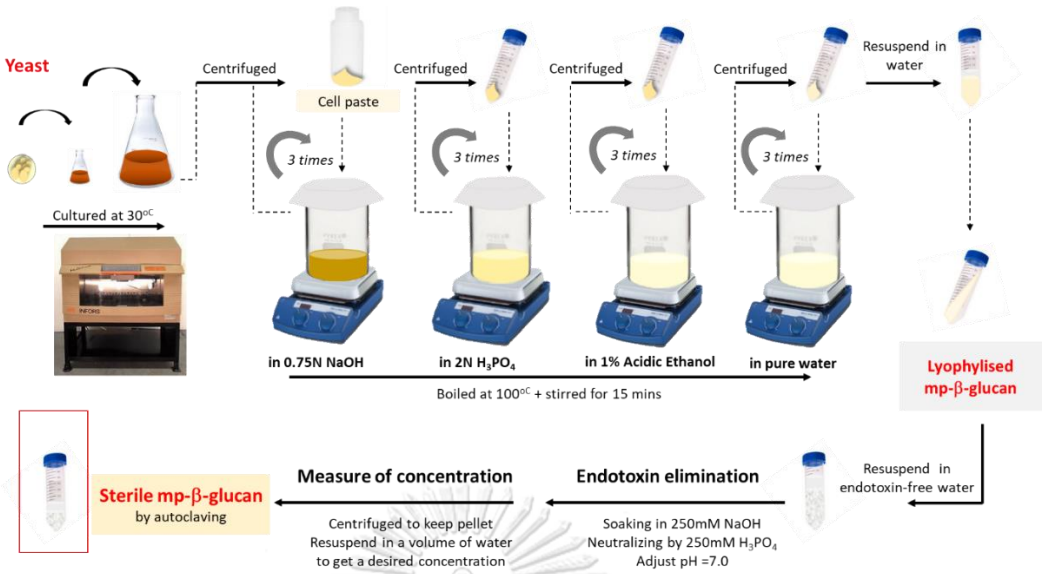


Figure 3.1 Process of *Candida* yeast microparticulate ß-glucan extraction

4. Scanning Electron Microscopy

To check the morphology and size of particulate glucan, *Candida* ß-glucan were first fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH = 7.2 for 18 h at 4^{oC}. Next, these glucans were washed with sterile deionized water and were air-dried. Finally, the ß-glucans were coated with ultra-thin gold layer (JFC-1200, JEOL, Peabody, MA, USA). The samples were captured their pictures by a scanning electron microcopy (Quanta250, FEI, Hillsboro, OR, USA) with 10.000x magnification.

5. Generation of bone marrow-derived dendritic cells (BMDCs)

DCs were generated from bone marrow cells as described in previous studies (Inaba et al., 2009; Thu Ngoc Yen Nguyen, 2018). Mice were sacrificed by dislocating the spinal cord at neck site. Then, tibia and femur bones of mice were taken out and rinsed through 70% ethanol to sterile and soaked in Roswell Park Memorial Institute (RPMI)-1640 medium (GIBCO, Thermo Fisher Scientific, NewYork, NY, USA). These bones were cut at two tips and bone marrow cells were collected in fresh media by

using 1 mL sterile syringe plus 25G needle to pump out. The pellet of cells was kept after centrifuging with 300 g at 4^{oC} in 5 mins. Next, the pellet was resuspended in RPMI 1640 media supplemented with 10% of heat-inactivated fetal bovine serum (HI-FBS), 100 U/mL of penicillin and 100 U/mg of streptomycin, and 0.2 mM L-Glutamax and passed through cell strainer 70 µm (all reagents of GIBCO). For culturing optimally, the cells at the concentration of 1 x 10^6 cells/ml were seeded in 24-well plate (NUNC™ Cell-Culture, Thermo Scientific, US) with 1 mL of completed media per well plus growth cytokines granulocyte-macrophage-cloning stimulating factor (GM-CSF), and interleukin-4 (IL-4) with final concentration 10 ng/mL (PeproTech, Rocky Hill, NJ, USA) and incubated at 37°C with 5% CO_2 incubator for 7 days (Forma Series II water jacket, Thermo Scientific, CA, USA). The half of media was removed and replenished every 2 days. The differentiated cells were ready to do a stimulation of mp-ß-glucan at day 7. All steps were done inside sterile hood (ESCO Class II BSC - AirStream®, Singapore).

6. BMDC stimulation with *Candida* ß-glucan

On day 7^{th} of culturation, BMDCs were changed a half of fresh media without cytokines. Then, cells subsequently were stimulated with 200 µL of sterile *Candida* mp-ß-glucan 5X to get final concentrations from low to high 12.5 – 25 – 50 µg/mL per well. The concentrations were selected basing on previous studies (Nakagawa et al., 2003; Thu Ngoc Yen Nguyen, 2018). At 24 and 48 hours of stimulation, the cells and supernatants were collected to analyze maturation markers and cytokine secretion.

Polymyxin B is an *Bacillus polymyxa*–derived antibiotic applied for lipopolysaccharide elimination (Cardoso et al., 2007). To test an interference of an endotoxin contamination during the extraction of mp-ß-glucan, the *Candida* ß-glucans were pretreated with 25 and 50 units/mL of polymyxin B sulfate (GIBCO) for

2 hours at room temperature. Next, the *Candida* ß-glucans were washed many times with endotoxin-free water and prepared to stimulate BMDCs for 24 and 48 hours. The cells and supernatants were collected to check the maturation markers and some cytokines.

7. Dectin-1 blockade

For dectin-1 inhibition experiments, BMDCs were pre-incubated with 25 µg/mL of dectin-1 antagonist which is water-soluble (1, 3)/(1, 6)-ß-glucan from *Saccharomyces cerevisiae* (soluble WGP, InvivoGen, USA) within 2 hours, then stimulate cells with 25 µg/mL of mp-ß-glucan. To compare dectin-1 blockade, BMDCs were stimulated with 25 µg/mL of mp-ß-glucan only. The mp-ß-glucan of *C. albicans*, *C. tropicalis* and *C. krusei* were used in this experiment. Curdlan is ß-(1,3)-glucan of bacterium *Alcaligenes faecalis* is well-known as dectin-1 agonists (InvivoGen) that are used for positive control to check effects of dectin-1 antagonist in experiments (Ganesan et al., 2014; H. S. Kim et al., 2016).

8. Spleen-tyrosine kinase (Syk) inhibition

BMDCs were pre-incubated with 0 and 1 µM of Syk inhibitor (InvivoGen) within 30 minutes prior to add 25 µg/mL of mp-ß-glucan. For comparison of Syk blockade, BMDCs were stimulated with 25 µg/mL of mp-ß-glucan only. Curdlan, mp-ß-glucan of *C. albicans*, *C. tropicalis* and *C. krusei* were used in this experiment. The cells and supernatant were harvested at 24 and 48 h post-stimulation to evaluate maturation markers and cytokine production of BMDCs.

9. Flow cytometry analysis

To evaluate BMDC maturation and inhibitory phenotype, BMDCs were stained with fluorophore-conjugated monoclonal antibodies to determine the maturation and phenotype markers including anti-CD11c (clone N418), anti-CD80 (clone 16-

10A1), anti-CD86 (clone CL-1), anti-I-A/I-E (MHC-II, clone M5/114.152), anti-CD40 (clone 3.23), anti-PD-L1 (clone MIH7), anti-PD-L2 (clone TY25), anti-CD206 (clone C086C2) and anti-dectin-1 (clone RH1). For T cell assay, LN cells and splenocytes were stained with fluorophore-labeled antibodies against CD3 (clone 145-2C11), CD4 (clone GK1.5), CD25 (clone PC61) and mouse/human FoxP3 (clone FJK-16s). All antibodies were obtained from BioLegend (San Diego-CA, USA) except for the anti-FoxP3 antibody, which was obtained from eBioscience (San Diego-CA, USA). The stained cells were assessed by a flow cytometry (CytoFLEX, Beckman Coulter, CA, USA). For flow cytometry acquisition, the live cells of all samples were acquired at 20,000 cells/samples. Accordingly, the same electronic gate was applied to all sample for flow cytometry acquisition and analyses. The analysis of percent rate and mean fluorescence intensity (MFI) of markers were performed using Kaluza software (Beckman Coulter).

10. *In vitro* DC and T cell co-culture assay

On day 7th of culturation, BMDCs were stimulated with 25 μg/mL of mp-ß-glucans for 24 h. Then the cells were collected and washed with RPMI medium twice. Parallel, T cells were prepared from spleens of intact mice by using immunomagnetic beads following the instruction of commercial kit (Pan T Cell Isolation kit II, mouse; Miltenyi Biotec, San Diego, CA, USA). The principle of this method is based on the exclusion of non-T cell populations to collect the suspension of purified T cells. The non-T cell populations which are DCs, macrophages, mononuclear leukocytes, NK cells, B cells, endothelial cells, hematopoietic stem cells and erythrocytes. They are captured by a biotin-antibodies cocktail (containing of anti-CD11c, anti-CD11b, anti-CD19, anti-CD45R, anti-CD105, anti-MHC-II, and anti-Ter-199 antibodies) and adhered to magnetic field by biotin-linked magnetic beads. Next, after collecting T cells, 1 x 10^5 cells of the BMDCs were

co-cultured with 1 x 10^6 cells of isolated T cells at DC:T cell ratio of 1 : 10 in 48-well plates. At the same time, 30 ng/mL soluble anti-CD3 antibody was added into co-cultured cells (Clement et al., 1985; Yixin Li & Roger J. Kurlander, 2010). The supernatants and cells were harvested at 72 h after co-culturation for detecting cytokines IFN-γ, IL-17, IL-2 and IL-10 and staining CD4, CD25 and FoxP3 marker respectively.

11. *In vivo* subcutaneous immunization of β-glucan and *ex vivo* re-stimulation assay

The mice were divided into 3 groups: PBS group, *C. albicans* β-glucan group and *C. krusei* β-glucan group. They were immunized twice subcutaneously in the scruff of the neck at day 0 and day 7 (Thu Ngoc Yen Nguyen, 2018). In PBS control group, mice were injected with 200 µl/injection of only 30 µg chicken oval albumin (OVA, Grade V, Sigma Aldrich) in phosphate-buffered saline (PBS). In β-glucan groups, mice were injected with 200 µl mixture of 2mg of mp-β-glucan of *C. albicans* or *C. krusei* with 30 µg OVA plus in PBS buffer. On day 14, the cervical, axillary and brachial lymph nodes (LNs) were collected after mice had sacrificed by Isofluran inhalation (Isofluran Veterinary-USP, USA). These draining LNs were digested with 300 units/mL Collagenase type IV (GIBCO) and 20 units/mL DNAse I (Sigma Aldrich) at 37°C with 200 rpm shaking for 45 min. The LN cells were washed and resuspended in RPMI 1640 medium supplemented with 10% HI-FBS, 100 U/mL penicillin and 100 U/mg streptomycin, and 0.2 mM L-Glutamax, and 55 µM 2-mercaptoethanol (2ME, GIBCO). For *ex vivo* re-stimulation assay, these cells were seeded 2 x 10^6 cells/well in 48-well plate and re-stimulated with 200 µg/mL OVA. At 72 h, the cells were collected for staining CD4, CD25, and FoxP3 marker and supernatants were measured some functional cytokines.

12. *In vivo* systemic *C. krusei* infection and *ex vivo* re-stimulation assay

The mice were divided into three groups: PBS, a low dose and high dose of *C. krusei* yeasts. Basing on *in vivo* immunosuppression model, the mice were treated with dexamethasone intraperitoneally (0.1 mg/g of body weight; Dexton-Vet, T.P. Drug laboratories (1969), Bangkok, Thailand) on day -3, -2, -1 and 5 prior to *C. krusei* infection (Figure 3.2) (Jacobsen et al., 2010). Parallel, *C. krusei* yeasts were inoculated in YPD medium, then were counted, washed and resuspend in 100 μl of PBS buffer. On day 0, three groups of mice were intravenously injected with PBS, $1x10^6$ and $5x10^6$ of *C. krusei* yeasts. On day 7 post-infection, the spleens and blood serum were collected from those mice. Spleens were minced and removed red blood cells by RBC lysis buffer (consists of 8.02 g NH_4Cl, 0.84g $NaHCO_3$ and 0.37g EDTA in 1 L sterile deionized water). Then, splenocytes were washed and transferred through cell strainers into culture medium (RPMI 1640 medium plus HI-FBS, penicillin/streptomycin, L-Glutamax and 2ME). Finally, the splenocytes were also seeded $2x10^6$ cells/well in 48 well-plate and incubated at 37^{oC} in 5% CO_2 incubator. The cells were stained with markers CD3, CD4, CD25 and FoxP3 at 48 h.

In ex vivo re-stimulation assay, splenocytes were re-boosted with immobilized anti-CD3 antibody on 24-well plate. Particularly, 10 μg/mL of purified anti-CD3 antibody in 200 μL Dulbecco's Phosphate Buffered Saline (DPBS) 1X were coated on the plate at 4^{oC} overnight. Next, to remove the extra antibodies, the plate was gently rinsed with culture medium RPMI twice. Then, the splenocytes were seeded at the concentration of $4x10^6$ cells/well in 1 mL of culture medium. After 48 h, the cells were collected for staining CD3, CD4, CD25, and FoxP3 marker and the supernatants were quantitated functional cytokines.

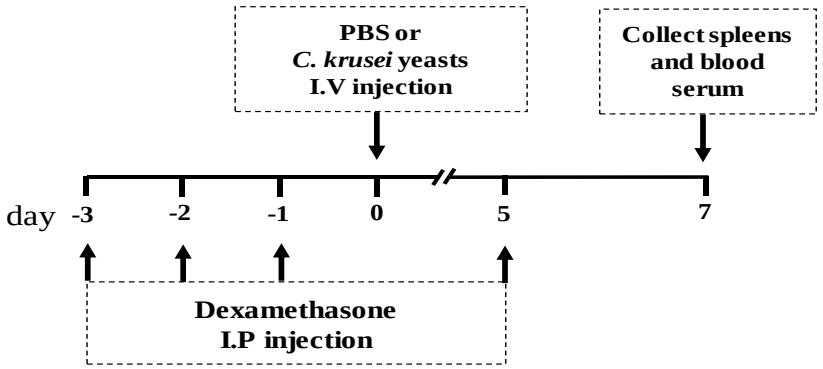


Figure 3.2 Timeline of systemic *C. krusei* infection model

14. Cell viability assay

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Life Technologies, Thermo Fisher Scientific, OR, USA) was used popularly for cell viability test as the simple and standard colorimetric method. The principle of this assay is that MTT substance penetrates inside cells and reacts with $NADPH^{+}$ released from mitochondria activities of live cells to form the intracellular formazan crystals. In details of this experiment, BMDCs were cultured in 96-well plate at the density of 1 x 10^5 cells in 200 µl cytokines-completed RPMI medium per well and taken care similarly as described above. At 24 or 48 hours of mp-ß-glucan stimulation, 20 µl of MTT 5 mg/mL were added into each well, and the continuously incubated at 37°C with 5% CO_2 in the dark for 1 hour. Subsequently, formed purple formazan crystals were dissolved by adding 180 µl of dimethyl sulfoxide (DMSO; Sigma-Aldrich Corporation, Merck, Germany). The absorbance at 570 nm (OD) was measured by Gene5.0 software of microplate reader (EPCHO2, BioTek, US). The percentage of cell viability was calculated by normalizing the absorbance of the sample to the negative control as the following formula, (OD sample-OD negative)/OD negative x 100%.

15. Cytokine quantification with enzyme-linked immunosorbent assay (ELISA)

The level of cytokines in cell supernatants is measured by sandwich ELISA method with commercial kits of TNF-α, IL-6, IL-1ß, IL-23, IL-12, IL-2, IFNγ, IL-10, IL-4 and IL-17. All ELISA kits were bought from BioLegend, CA, US, except the IL-23 ELISA kit was from eBioscience, CA, US. ELSA is the standard classical method with high sensitivity and specificity commonly used to detect the target proteins quite simply through binding activity of specific antibodies to those targets. The procedure is followed the manufacture's instruction. Briefly, first stage, coating an ELISA 96-well plate (Nunc MaxiSorp, Thermo Scientific) with monoclonal capture antibody overnight was prepared before to have enough time for antibodies catch bottom of each well. Then, washing the plate with buffer aims to remove extra antibodies and covering the plates with assay diluent solution is to block the unspecific sites at well bottom. Second stage, incubating respectively 2-fold dilution standard and supernatant samples in each well is done to the target proteins binding with capture antibodies. Next, the extra proteins were removed after washing with buffer. Third stage, the couple of antibody-target antigen is tightly bound with the second monoclonal antibody that labeled a tag of biotin substance. Biotin plays a role to connect streptavidin-horseradish peroxidase enzyme (HRP) to second antibodies which called conjugated antibodies. To detect the presence of a triple of primary antibody - target antigen – conjugated antibody in well, 3, 3'-5, 5'tetramethylbenzidine (TMB) is added and turns color of solution into blue color after reacting with HRP enzyme. Final stage, this reaction is stopped by adding acid sulfuric acid. The OD of solution in each well is determined by microplate reader (EPCHO2) at 450 nm wavelength and amount of target proteins of sample is calculated by referring sample OD to the 2-fold serial dilution standard curve.

16. Statistical analysis

All data values were displayed as mean ± SD. The statistical analysis was performed with SPSS IBM 22 software (IBM, New York, NY, USA and provided by Chulalongkorn university,) by using one-way ANOVA with post-hoc Turkey HSD for the comparison of 3-5 groups and Student's t-test for the comparison between 2 groups. The significant value was determined when $p<0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Part I:

Objective 1: To screen the difference of DC activation via maturation and cytokine secretion in response to cell-wall ß-glucan of *Candida* non-*albicans* spp. and *C. albicans*.

Part II:

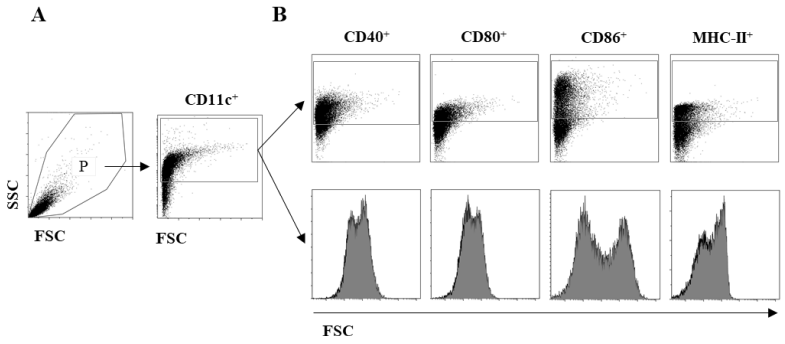
Objective 2: To determine the influence mechanisms of NACs cell wall ß-glucan on DCs and the adaptive immune responses respectively.

Part I: Preliminary results

Objective 1: To screen the difference of DC activation via maturation and cytokine secretion in response to cell-wall ß-glucan of *Candida* non-*albicans* spp. and *C. albicans*.

Microparticulate ß-glucan of *Candida* species promoted the maturation and cytokine secretion of BMDCs at different levels

ß-glucan from various sources has the different impacts during interacting with immune cells in several previous studies (Dalia Akramienė, 2007; Bashir & Choi, 2017). However, the effects from cell wall ß-glucan from many species of *Candida* genus to the immune responses and outcomes of *Candida* infections has still not clearly defined. Additionally, DCs are known to play the central role of immune responses against pathogens such as *Candida* fungi (Mihai G. Netea, 2015; Qin et al., 2016; Feldman et al., 2019). During encountering pathogens, DCs become active and more mature via a reveal of many intracellular and extracellular molecules that is vital to have a potency to set off chains of immune responses later. Hence, this study screened whether cell wall ß-glucan of the different *Candida* species induce



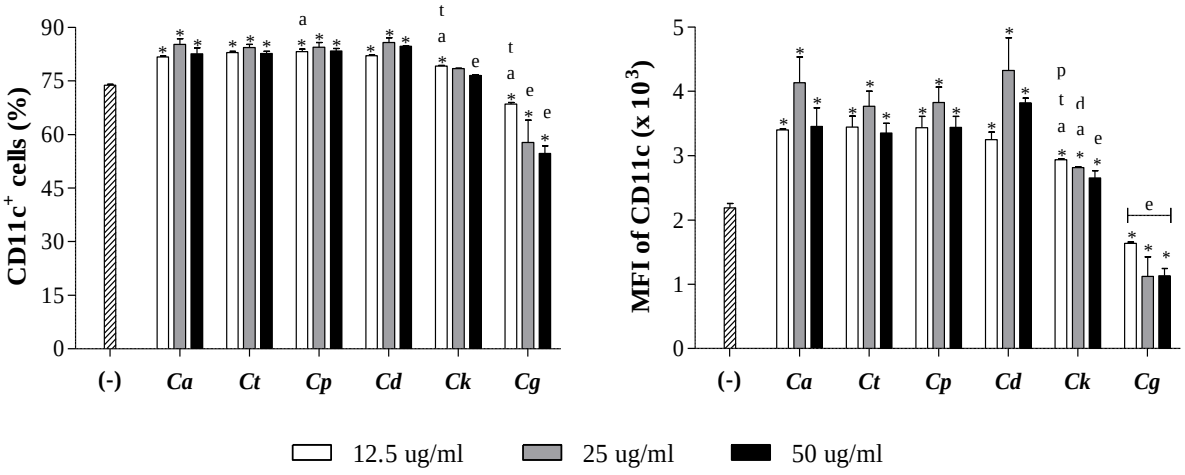
the distinct DC activation.

Figure 4.1 Representative of dot plots and histograms of BMDC maturation markers in flow cytometry analysis

(**A**) The first dot plot of live BMDCs were shown as P by using side scatter (SSC) and forward scatter (FSC), and then $CD11c^+$ BMDCs gated from P population was shown on the second of CD11c and FSC.

(**B**) Analysis of dot plots and histograms showed percentage (%) and mean fluorescence intensity (MFI) of $CD40^+$, $CD80^+$, $CD86^+$ and MHC-II^+ expression from $CD11c^+$ BMDCs.

The cultured immature BMDCs were stimulated with various doses (12.5 – 25 and 50 μg/ mL) of micro-particulate ß-glucan (mp-ß-glucan) isolated from yeasts of *Candida albicans* and five common NACs species. The alteration of DC properties was analyzed through maturation markers and cytokine production at 24 h post-stimulation by flow cytometry and ELISA method respectively. Maturation markers of BMDCs were evaluated via the expression of common molecules such as CD40, CD80, CD86 and MHC-II in $CD11c^+$ population including percentage (%) and mean fluorescence intensity (MFI) (Figure 4.1 - 4.3). Cytokine production of BMDCs was quantitated typically by ELISA method. The amount of TNF-α, IL-1ß, IL-6, IL-23, IL-12, IFN-γ and IL-10 were presented for pro-inflammatory and anti-inflammatory



cytokines (Figure 4.4). Negative control with unstimulated BMDCs was designed in this experiment.

Figure 4.2 CD11c expression of *Candida* ß-glucan-stimulated BMDCs at 24 h

At 7th day of BMDCs, cells were stimulated with 12.5, 25 and 50 μg/mL of various ß-glucans isolated form 6 *Candida* species. After 24 h of stimulation, the percentage of $CD11c^+$ DCs in the left graph and the MFI of this marker in the right graph were determined by flow cytometry. Data of *n=3*. **p*< 0.05 compared with unstimulated BMDCs; values $^a p< 0.05$, $^t p< 0.05$, $^P p<0.05$, $^d p<0.05$, $^k p<0.05$,

$^{\S}p<0.05$ and $^{e}p<0.05$ respectively compared with *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. krusei*, *C. glabrata* and all species; (-), unstimulated BMDCs, *Ca*, *C. albicans*, *Ct*, *C. tropicalis*, *Cp*, *C. parapsilosis*, *Cd*, *C. dubliniensis*, *Ck*, *C. krusei*, *Cg*, *C. glabrata*.

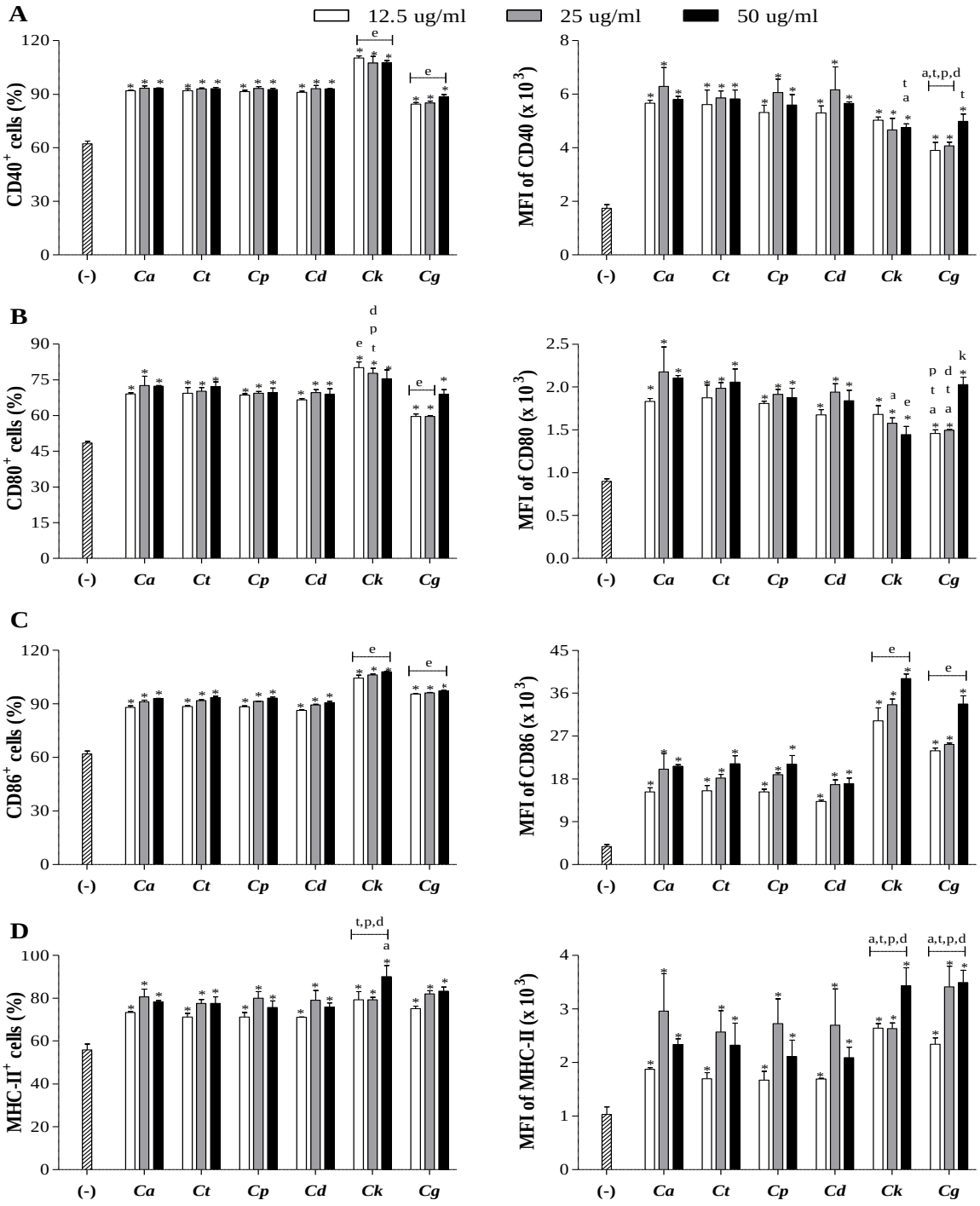


Figure 4.3 Altered maturation markers among *Candida* ß-glucan-stimulated BMDCs

At 24 h post-stimulation, expression of maturation markers in *Candida* ß-glucan-stimulated BMDCs were determined by flow cytometry. **(A)**, % and MFI of CD40 molecule; **(B)**, % and MFI of CD40 molecule; **(C)**, % and MFI of CD86 molecule; **(D)**, % and MFI of

MHC-II molecule. Data of *n=3*. *$p< 0.05$ compared with unstimulated BMDCs; values $^{a}p< 0.05$, $^{t}p< 0.05$, $^{p}p<0.05$, $^{d}p<0.05$, $^{k}p<0.05$, $^{s}p<0.05$ and $^{e}p<0.05$ respectively compared with *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. krusei*, *C. glabrata* and all species; (-), unstimulated BMDCs, *Ca*, *C. albicans*, *Ct*, *C. tropicalis*, *Cp*, *C. parapsilosis*, *Cd*, *C. dubliniensis*, *Ck*, *C. krusei*, *Cg*, *C. glabrata*.

As predicted, mp-ß-glucan of all *Candida* species significantly enhanced the expression of maturation molecules and cytokine induction of BMDCs when compared with negative control (Figure 4.3 - 4.4, respectively). Strikingly, mp-ß-glucan of *C. krusei* reduced relatively CD11c, increased higher expression of maturation markers in dose-dependent manner (Figure 4.3). Similarly, mp-ß-glucan of *C. glabrata* induced slightly high MFI level of CD80, CD86 and low MFI level of CD11c, CD40 when compared with others (Figure 4.3). In addition, BMDCs significantly increased multi-cytokine production under these two kinds of mp-ß-glucan higher than others, especially anti-inflammatory cytokine IL-10 (Figure 4.4). By contrast, mp-ß-glucan of *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis* induced relatively similar level of all maturation markers when compared with these of ß-glucan of *C. albicans* (Figure 4.3 - 4.4). Besides that, ß-glucan of these strains activated BMDCs to secrete a slight fluctuation of cytokine levels among them. From these preliminary results, cell wall ß-glucan of *Candida* spp. possibly impacted on DC functions distinctly depending on each species.

Discussion

Cell wall of *Candida* spp. has been known as a determinative factor for the reciprocal of host immunity and pathogenic fungi (Arana et al., 2009; Snarr et al., 2017). Yeast cell wall is embedded by many major components as a source of PAMPs, of which, glucan could initiate immune responses via its engagement with PRRs of the innate immune cell barrier. In term of antifungal immunity, DCs possess various PRRs associated with many crucial roles in detecting fungi to phagocytose and present fungal antigens to specific T cell response (Mihai G. Netea, 2015). Since DCs are as the centers of immune responses (van Vliet et al., 2007; Mihai G. Netea,

2015; Roussey et al., 2016; Feldman et al., 2019), these cells ultimately modulate the fate of antifungal specific T cell responses partly depending on which type of PAMPs they contact before.

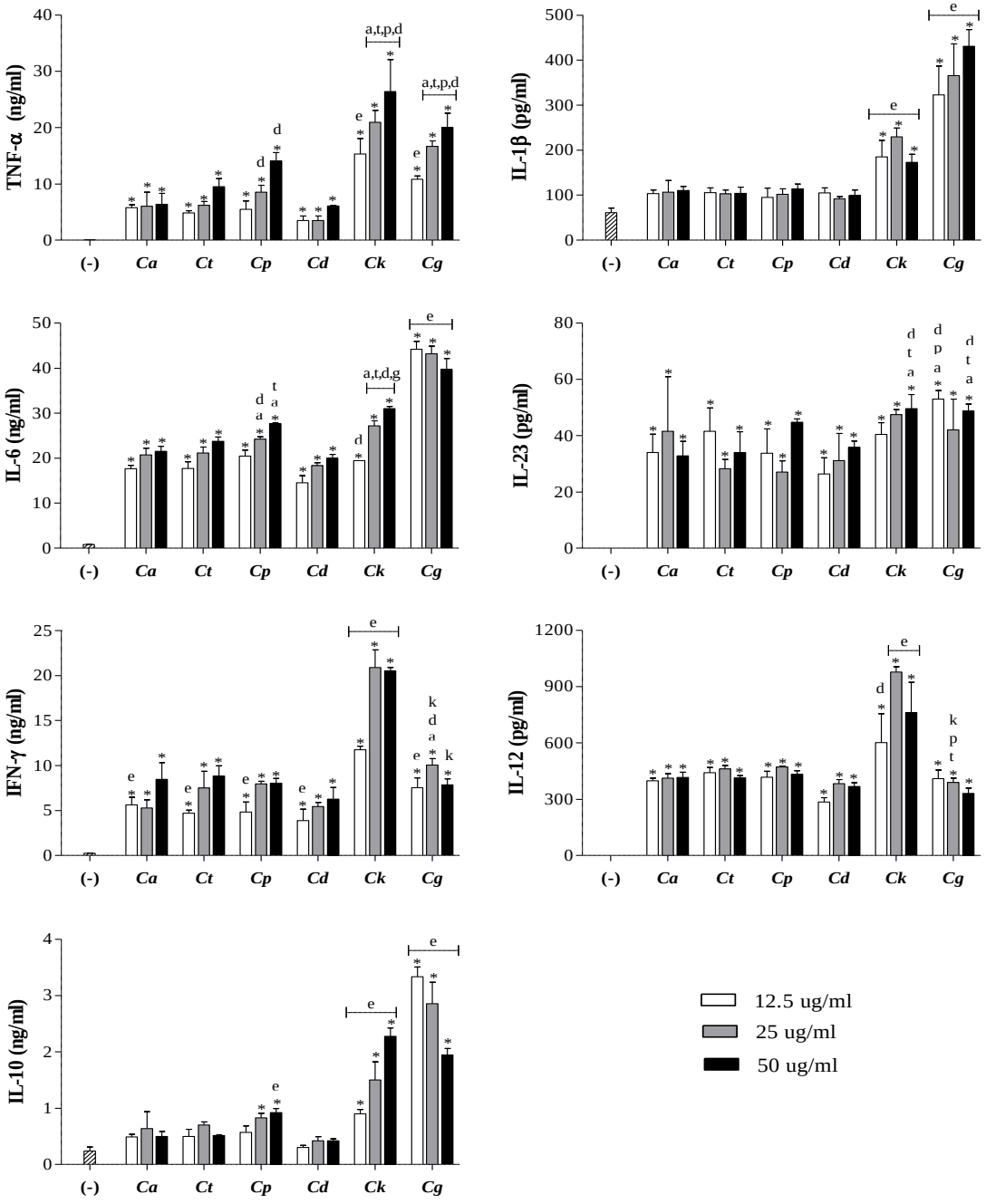


Figure 4.4 Secreted cytokine profile of BMDCs stimulated with *Candida* ß-glucans

Cytokine products of *Candida* ß-glucan-stimulated BMDCs were quantitated by ELISA method including TNF-α, IL-1ß, IL-6, IL-23, IL-12, IFN-γ and IL-10. Data of *n=3*. **p*< 0.05 compared with unstimulated BMDCs; values ^{a}p< 0.05, ^{t}p< 0.05, ^{p}p<0.05, ^{d}p<0.05, ^{k}p<0.05, ^{g}p<0.05 and ^{e}p<0.05 respectively compared with *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. krusei*, *C. glabrata* and all species; (-), unstimulated BMDCs, *Ca*, *C. albicans*, *Ct*, *C. tropicalis*, *Cp*, *C. parapsilosis*, *Cd*, *C. dubliniensis*, *Ck*, *C. krusei*, *Cg*, *C. glabrata*.

Microparticulate ß-glucan from some non-*albicans Candida* species could induce different interactions with DCs. These different effects of *Candida* ß-glucan on DC responses could be explained by some reasons. The different sizes, the different molecular structures, and the distribution of branch chains on the back bone chains of mp-ß-glucan could be the root factors (Elder et al., 2017; Han et al., 2020). In addition, the affinity of ligand and receptors could alter among these kinds of ß-glucan (Adams et al., 2008). The expression level of this component on the yeast surface may also account for its interaction with host immune cells in candidiasis (Graus et al., 2018). The differences between those ß-glucans need further investigation. From interesting results (Figure 4.3 - 4.4), it possibly implies that DCs alter their immunological properties in response to each kind of *Candida* ß-glucan leading to differently shape immune responses against those *Candida* species.

To clarify the mechanisms of these different effects of NACs mp-ß-glucans on DC responses, this study focuses on ß-glucans of *C. krusei* and *C. tropicalis* as representative NACs in comparison with ß-glucans of *C. albicans* in the next part. Some studies described interesting and enigmatic matters about these species. On the one hand, these species possess some similarities of pathogenicity rather similar *C. albicans*. They are diploid yeasts, can transform from yeast to filamentous form, as well as secrete a few similar virulent factors (Samaranayake et al., 1998; Costa et al., 2010; Papon et al., 2013; Whibley & Gaffen, 2015; Yu et al., 2016). It found that *C. krusei* was the lowest virulent species, whereas *C. tropicalis* and *C. albicans* were the highest virulent species *in vivo* murine model (Arendrup et al., 2002; Hirayama et al., 2020). On the other hand, *C. krusei* becomes an emerging multi-drug resistant pathogen with intrinsically resistant to fluconazole and other antifungal drugs (Pfaller et al., 2008; Gong et al., 2018; Jamiu et al., 2020). In addition, these species can cause invasive candidiasis with high mortality and antifungal drug resistance (Jamiu et al., 2020). Moreover, it found that *C. krusei* could survive and replication inside

innate immune cells with unclear mechanisms (García-Rodas et al., 2011). There is little known about pathogenesis strategies of these species (Gómez-Gaviria & Mora-Montes, 2020). From the preliminary results, cell wall β-glucan could be a notable candidate associated with their virulence or pathogenicity during encountering immune cells and determine subsequent outcomes of infections. Therefore, this study continues to access mechanisms of the interplay between DCs and mp-β-glucan of *C. krusei*, *C. tropicalis* and *C. albicans* in depth.

Part II: Results

Objective 2: To determine the influence mechanisms of NACs cell wall ß-glucan on DCs and the adaptive responses respectively

Morphology and structure analysis of *Candida* ß-glucans

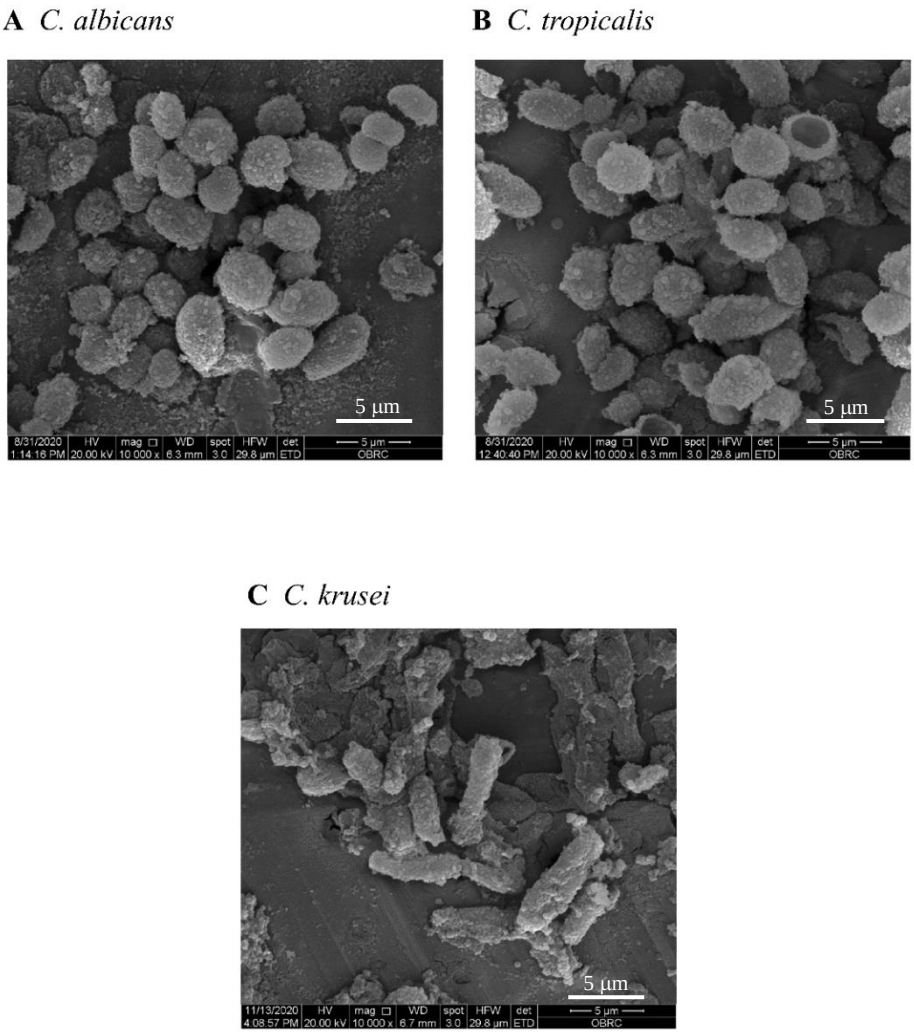


Figure 4.5 Morphology and size of *Candida* ß-glucans

ß-glucans of **(A)** *Candida albicans*, **(B)** *Candida tropicalis*, and **(C)** *Candida krusei.* were observed by SEM with 10,000x magnification

First, ß-glucan of *C. albicans*, *C. tropicalis* and *C. krusei* were morphologically characterized by scanning electron microscopy (SEM) (Figure 4.5). Interestingly, ß-glucans of *C. albicans* and *C. tropicalis* were similar in terms of size and shape (Figure

4.5A and 4.5B, respectively). Particularly, the average size of *C. albicans* ß-glucans was 4-5 μM and the average size of *C. tropicalis* ß-glucans was 4-6 μM. In addition, both ß-glucans of these two *Candida* species formed oval shape with a dense fine speckled pattern. By contrast, the morphology of *C. krusei* ß-glucans was drastically different from the other two as they formed rod shape and large in their size (average size is 7-10 μM), and surface of the ß-glucans exhibited a course speckled pattern (Figure 4.5C).

In addition to morphological appearance, NMR analysis were performed to characterize *Candida* ß-glucan structure. The results of this part were kindly analyzed by Assis. Prof. Dr. Panuwat Padungros (Green Chemistry for Fine Chemical Productions STAR, Department of Chemistry, Faculty of Science, Chulalongkorn University). The ^{1}H NMR spectra of both *C. albicans* ß-glucan and *C. tropicalis* ß-glucan were suggested that they were ß-(1,3)-glucan with ß-(1,6)-branching (Lowman et al., 2003; Sukumaran et al., 2010; Lowman et al., 2014). For the *C. krusei* ß-glucan, satisfying NMR spectra were obtained by performing measurement at room temperature. The simplicity of the ^{1}H NMR signals indicated that the *C. krusei* ß-glucan was a highly homogeneous ß-(1,3)-glucan without ß-(1, 6)-branching.

To determine the purity of extracted ß-glucan from any potential contamination of bacterial endotoxin LPS during ß-glucan preparation, this study pre-treated the *Candida* ß-glucan with polymyxin B, a potent LPS neutralizer (Cardoso et al., 2007; Noss et al., 2013), before BMDC stimulation followed by an assessment of DC maturation marker expression (CD40, CD80, CD86 and MHC-II) and inflammatory cytokine levels (IL-6 and TNF-α). The results showed no significant difference in these maturation markers and inflammatory cytokines between untreated and polymyxin B-treated groups (Figure 4.6 - 4.7). This indicated that there was no endotoxin contamination in *Candida* ß-glucans.

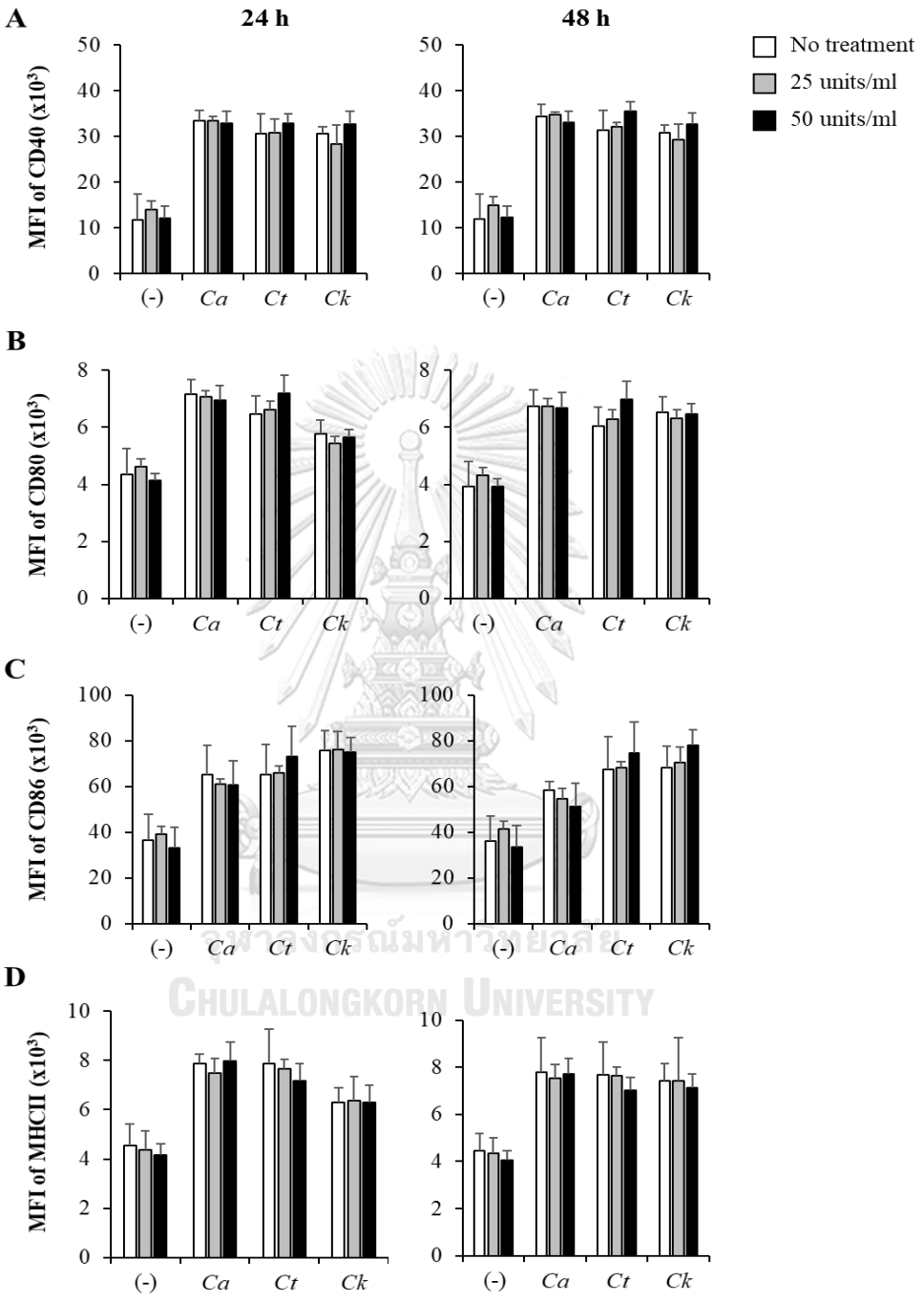


Figure 4.6 DC maturation after stimulation with *Candida* ß-glucans pre-treated with polymyxin B

Candida ß-glucans were pre-treated with 25 and 50 units/mL polymyxin B for 2 h at room temperature with rotation. The glucans were washed twice with PBS. BMDCs were stimulated with polymyxin B-treated ß-glucans (25 μg/mL) for 24 h and 48 h. DC maturation markers were assessed by flow cytometer. *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

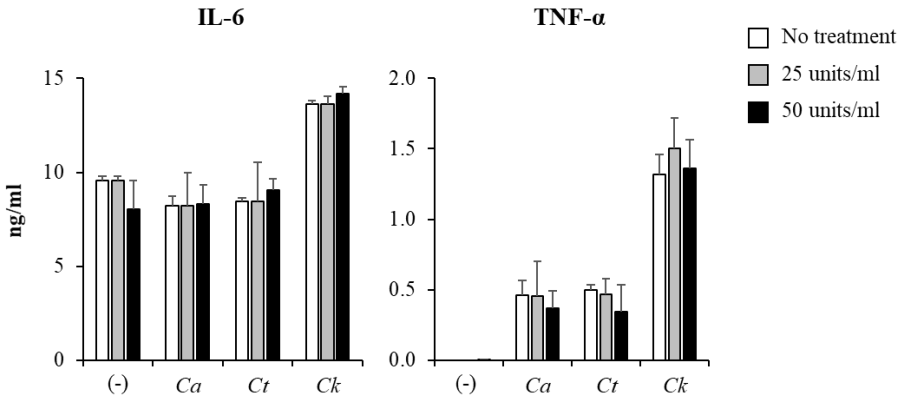


Figure 4.7 Cytokine expression after stimulation with *Candida* ß-glucans pre-treated with polymyxin B

Candida ß-glucans were pre-treated with 25 and 50 units/mL polymyxin B for 2 h at room temperature with rotation. The glucans were washed twice with PBS. BMDCs were stimulated with polymyxin B-treated ß-glucans (25 μg/mL) for 48 h. The inflammatory cytokine levels were assessed by ELISA. *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Effects of *Candida* ß-glucans on DC viability and CD11c expression

Candida ß-glucans are also PAMPs, which may transduce signals via PRRs and lead to cell death (Takeuchi & Akira, 2010; Kingeter & Lin, 2012; Ganesan et al., 2014). To observe the cytotoxicity of pathogenic fungal ß-glucan, an MTT assay was performed to test the viability percentage of BMDCs stimulated by various concentration mp-ß-glucan of *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. All kinds of mp-ß-glucan increased relatively high of BMDC viability up to 70% at 24 h and 48 h post-stimulation. The percentage of cell viability was approximately equal at all doses of all *Candida* ß-glucans (Figure 4.8). This possibly indicated that there was no significant difference on cytotoxicity among of these glucans at both time points of stimulation. However, DC viability at 48 h showed decreasing trend when BMDCs were stimulated with the highest concentration (50 μg/ml).

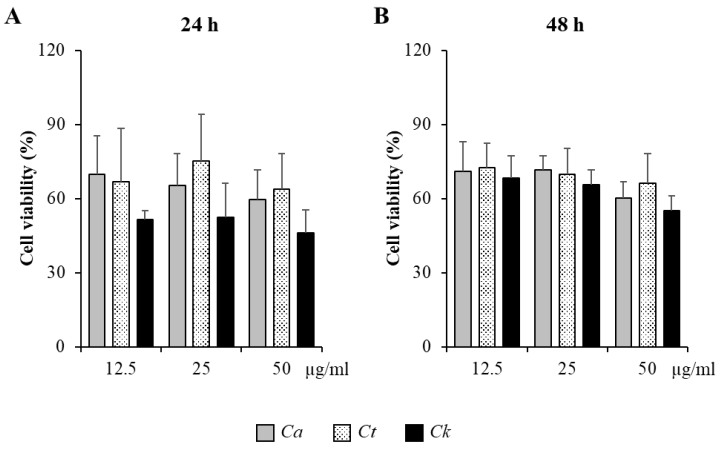


Figure 4.8 Effects of *Candida* ß-glucans on DC viability

BMDCs were stimulated with 12.5, 25 and 50 µg/ml of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for **(A)** 24 h and **(B)** 48 h. DC viability was evaluated using MTT assay. The percent cell viability was calculated by normalization to the negative control. *n* = 5; data are representative of two independent experiments. *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

CD11c has been known as a presentative marker of DC populations during their differentiation, which evaluated DC proportions in *Candida* ß-glucan-stimulated bone marrow cells by flow cytometry (Figure 4.9). At 24 h post-stimulation, *C. albicans* and *C. tropicalis* ß-glucan induced the DC proportion similarly to unstimulated cells, while ß-glucan of *C. krusei* tended to slightly impede the percent CD11c expression of BMDCs following its increased doses 25 and 50 µg/mL compared with this of unstimulated cells (Figure 4.9A). Moreover, the DC proportion of *C. krusei* ß-glucan-stimulated BMDCs was significantly lower than these of *C. tropicalis* and *C. albicans* ß-glucan stimulation (Figure 4.9A, left panel). At 48 h post-stimulation, all *Candida* ß-glucan reduced the percentages of $CD11c^+$ cells among BMDCs stimulated with doses 25 and 50 µg/mL compared with unstimulated cells (Figure 4.9A, right panel). Besides that, there was no difference of DC proportion among BMDCs stimulated with these three *Candida* ß-glucans at this time point (Figure 4.9A, right panel).

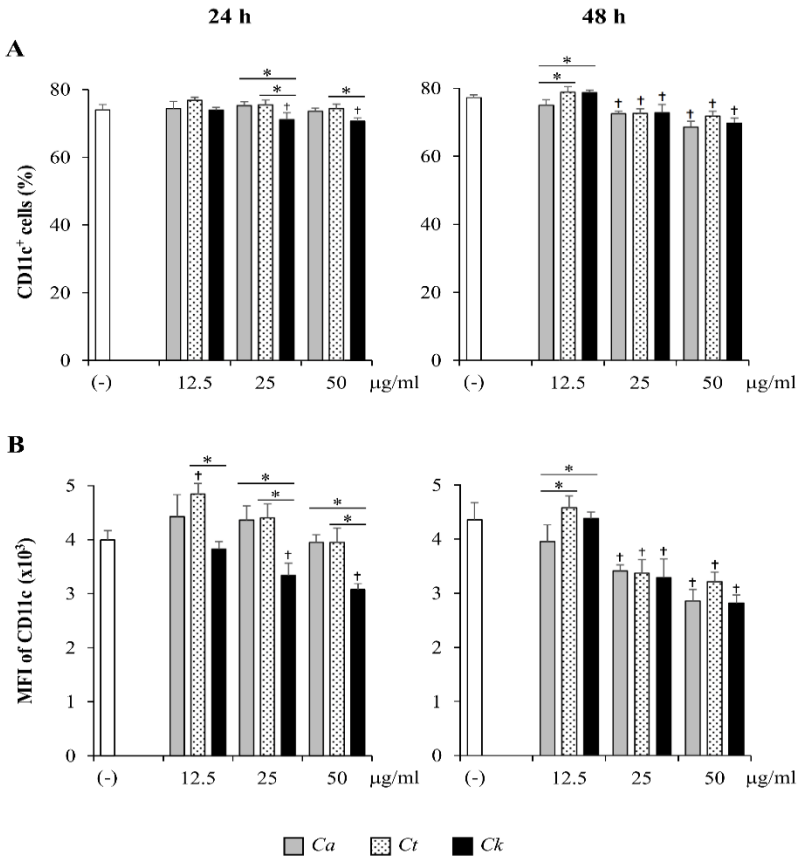


Figure 4.9 Changes of CD11c marker among BMDCs stimulated with *Candida* ß-glucans

BMDCs were stimulated with 12.5, 25 and 50 µg/mL of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. Cells were analyzed by flow cytometry. **(A)** The percentages of $CD11c^+$ cells were assessed using a dot pot analysis. **(B)** The geometric mean fluorescence intensity (MFI) of CD11c was determined using a histogram analysis. *n* = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Furthermore, the MFI levels of CD11c expression showed these alterations among *Candida* ß-glucan-stimulated BMDCs more clearly (Figure 4.9B). The CD11c expression apparently displayed a down-regulation due to mp-ß-glucan in species- and dose-dependent manner. At 24 h post-stimulation, CD11c expression levels of BMDCs stimulated with *C. albicans* and *C. tropicalis* ß-glucans were not altered, whereas stimulation with *C. krusei* ß-glucans at 25 and 50 µg/mL significantly diminished CD11c expression on BMDCs compared with expression on unstimulated BMDCs (Figure 4.9B, left panel). *C. krusei* ß-glucan-reduced CD11c expression of

BMDCs significantly more than *C. albicans* and *C. tropicalis* ß-glucans did (Figure 4.9B, left panel). In similar way to the percent CD11c levels, at 48 h post-stimulation, the expression of CD11c was significantly prevented on BMDCs stimulated with high doses 25 and 50 µg/mL of all *Candida* ß-glucans and no difference was shown on among BMDCs stimulated by distinct *Candida* ß-glucans (Figure 4.9B, right panel). These data also showed that *C. krusei* ß-glucan affected on CD11c expression of BMDCs more rapidly than other *Candida* ß-glucans did.

Overall, ß-glucans from *C. albicans*, *C. tropicalis*, and *C. krusei* did not significantly affect DC viability and DC proportion. However, ß-glucan from *C. krusei* downregulated CD11 expression on BMDCs.

C. krusei ß-glucans had differential impacts on DC maturation

Interestingly, the DC maturation was expressed differently among these *Candida* ß-glucans through expression of some common markers of $CD11c^+$ BMDCs at each time point of stimulation. All these kinds of *Candida* ß-glucans significantly enhanced the proportion of $CD40^+$, $CD80^+$, $CD86^+$ and MHC-II^+ cells higher than unstimulated cells at both 24 h and 48 h post-stimulation (Figure 4.10). In addition, the percentage levels of these molecules fluctuated among these ß-glucans. All ß-glucans of these species similarly affected on the percentage of $CD40^+$ cells at both time points (Figure 4.10A). *C. krusei* ß-glucan induced the proportion of $CD80^+$ cells lower than other ß-glucans at 24 h, while *C. albicans* ß-glucan induced this phenomenon at 48 h when compared with other ß-glucans (Figure 4.10B). The proportion of $CD86^+$ cells was not altered upon stimulation of these *Candida* ß-glucan at 24 h, but *C. krusei* ß-glucan mediated an increasing trend of CD86 percent higher than other ß-glucans did at 48 h (Figure 4.10C). For MHC-II percentage, there was likely no alteration of this molecules among stimulation of *Candida* ß-glucans at both time points (Figure 4.10D). However, *C. krusei* ß-glucan tended to induce lower proportion of MHC-II^+ cells than other ß-glucans did, while *C. albicans* ß-glucan at

low dose 12.5 μg/mL increased the percentage of MHC-II higher than other ß-glucans did at 24 h (Figure 4.10D). Although *Candida* ß-glucans seemed to have differential effects on the proportion of DC maturation markers, only slightly significant differences were observed among them.

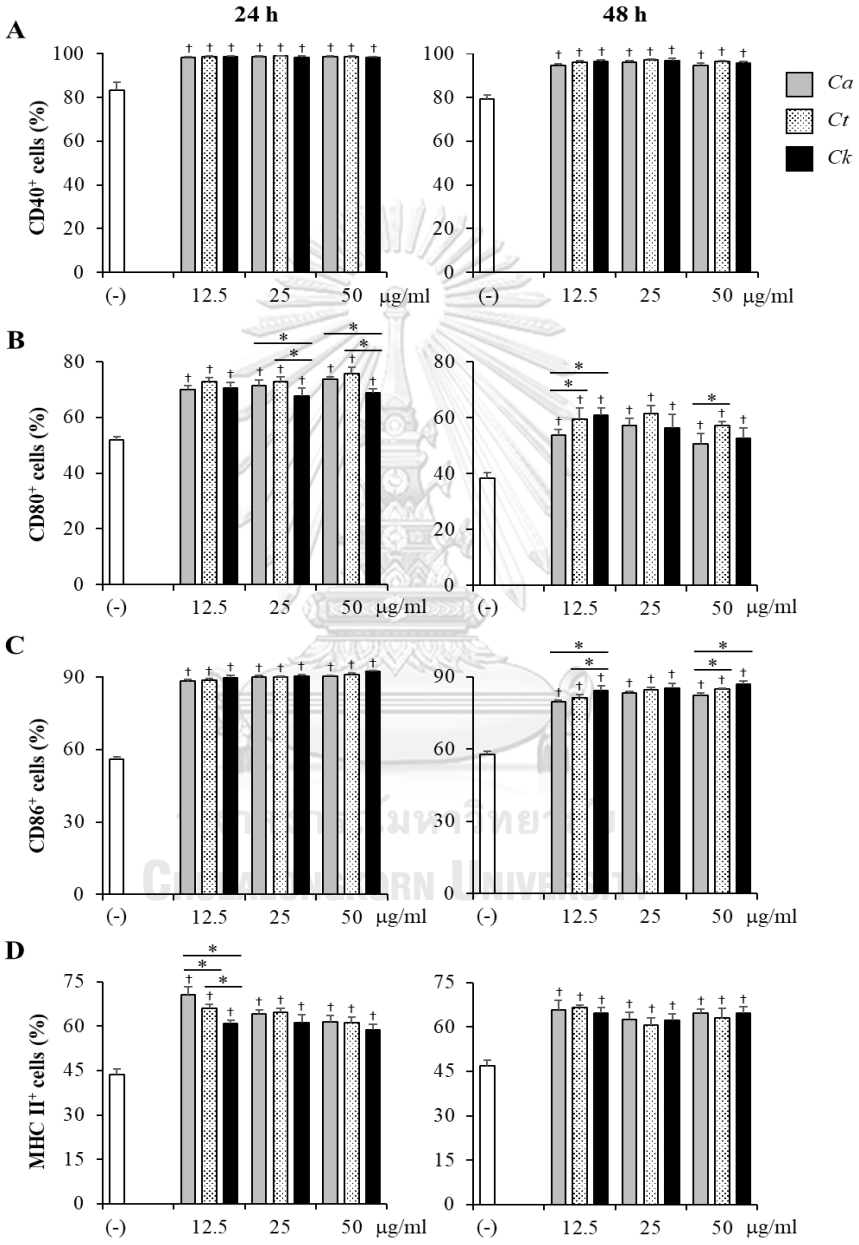


Figure 4.10 Effects of *Candida* ß-glucans on the proportions of mature DCs

BMDCs were stimulated with 12.5, 25 and 50 μg/mL of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. Cells were analyzed by flow cytometry. The percentages of **(A)** $CD40^+$ cells, **(B)** $CD80^+$ cells, **(C)** $CD86^+$ cells, and **(D)** MHC class II^+ cells in $CD11c^+$ population were assessed using dot plot analyses. *n* = 5; data are representative of two independent

experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

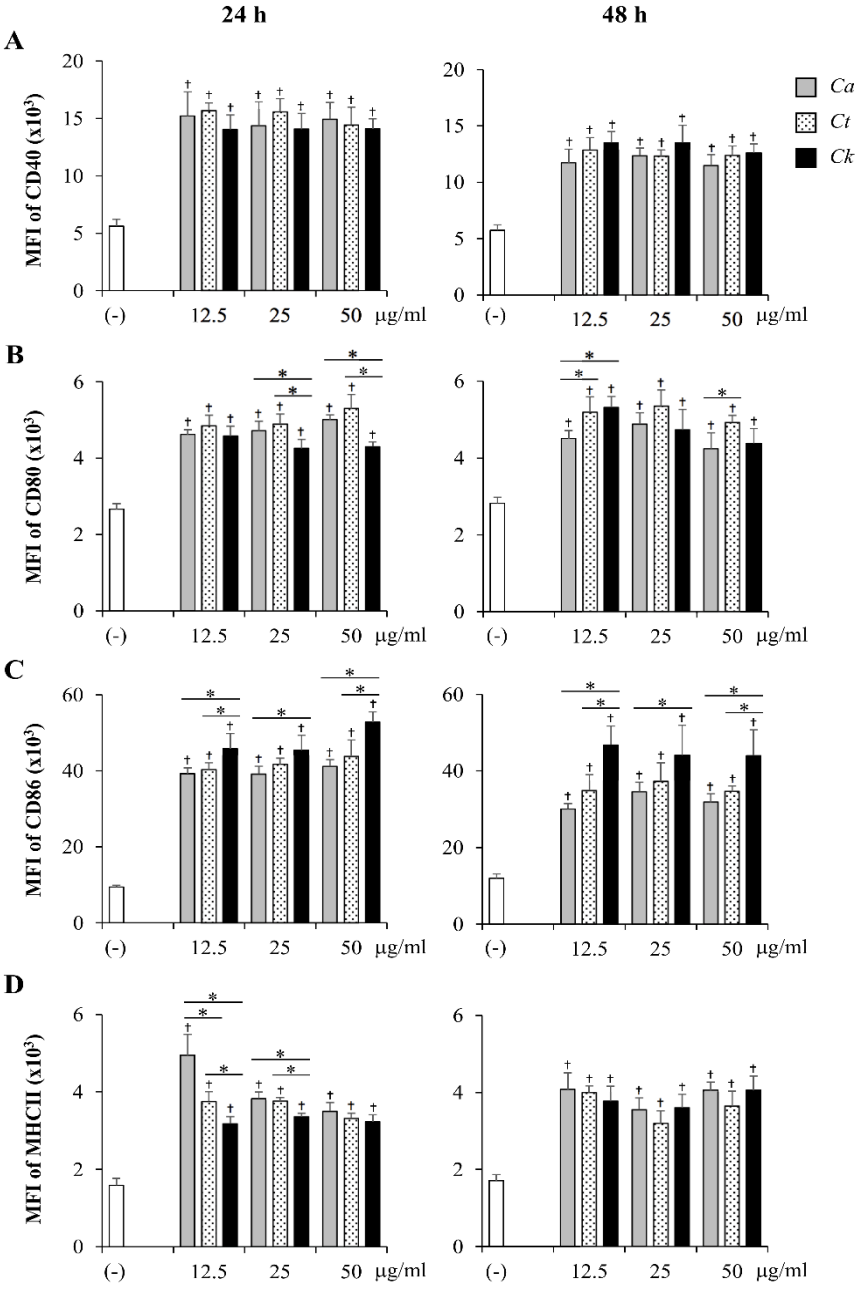


Figure 4.11 Different effects of *Candida* ß-glucans on the expression of DC maturation

BMDCs were stimulated with 12.5, 25 and 50 µg/mL of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h, and the geometric mean fluorescence intensity (MFI) of DC maturation markers **(A)** CD40, **(B)** CD80, **(C)** CD86, and **(D)** MHC class II on $CD11c^+$ cells were determined using histogram analyses. $n = 5$; data are representative of two independent experiments. †$p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Notably, the expression intensity of DC maturation molecules showed apparent differences among stimulation of *Candida* ß-glucans (Figure 4.11). Consistently, all *Candida* ß-glucans significantly elevated the expression levels of these molecules compared with unstimulated BMDCs. The expression levels of CD40, CD80 and MHC-II among stimulation of *Candida* ß-glucans at 24 h and 48 h (Figure 4.11A, 4.11B and 4.11D, respectively) corresponded with the proportions of cell positive with these markers (Figure 4.10A, 4.10B and 4.10D, respectively). At both time points, the CD40 expression was not distinct among these *Candida* ß-glucans at both time points (Figure 4.11A) and *C. krusei* ß-glucan-stimulated BMDCs expressed the lower levels of CD80 than BMDCs stimulated with other ß-glucans (Figure 4.11B). The expression levels of MHC-II only showed differences at 24 h, of which lower levels of this marker were induced by BMDCs stimulated with *C. krusei* ß-glucan (Figure 4.11D, left panel). By contrast, *C. krusei* ß-glucans notably elicited the highest levels of CD86 expression of BMDCs at both time points when compared with ß-glucans of *C. albicans* and *C. tropicalis* (Figure 4.11C). From these data, *C. krusei* ß-glucan had the distinct effects on BMDC maturation compared with other *Candida* ß-glucans.

During activating DCs, abundant molecules have been known to be up or down modulated by many intracellular signaling mechanisms in compatible to DC phenotype and functional differentiation (Ritprajak et al., 2019). The mature DCs closely associates with immunogenic DC phenotype, while immature DCs relates to tolerogenic or regulatory DC phenotype (Schmidt et al., 2012). Typically, mannose receptor CD206 and programmed death-ligand 1 (PD-L1 and PD-L2) play a role in DC immunomodulatory function (Serrano et al., 2018). Thus, this study further characterized these DC markers under *Candida* ß-glucan stimulations for better understanding how DC properties differ from each other. According to the results, the surface expression levels of CD206, PD-L1 and PD-L2 were significantly upregulated

upon all stimulations of *Candida* ß-glucans in dose-dependent manner when compared with unstimulated BMDCs (Figure 4.12). However, there was no notable difference among stimulations of *Candida* ß-glucans on the expression of these molecules at 24 h and 48 h (Figure 4.12). Collectively, distinct kinds of *Candida* cell wall ß-glucans differentially affected the maturation and phenotypes of DCs.

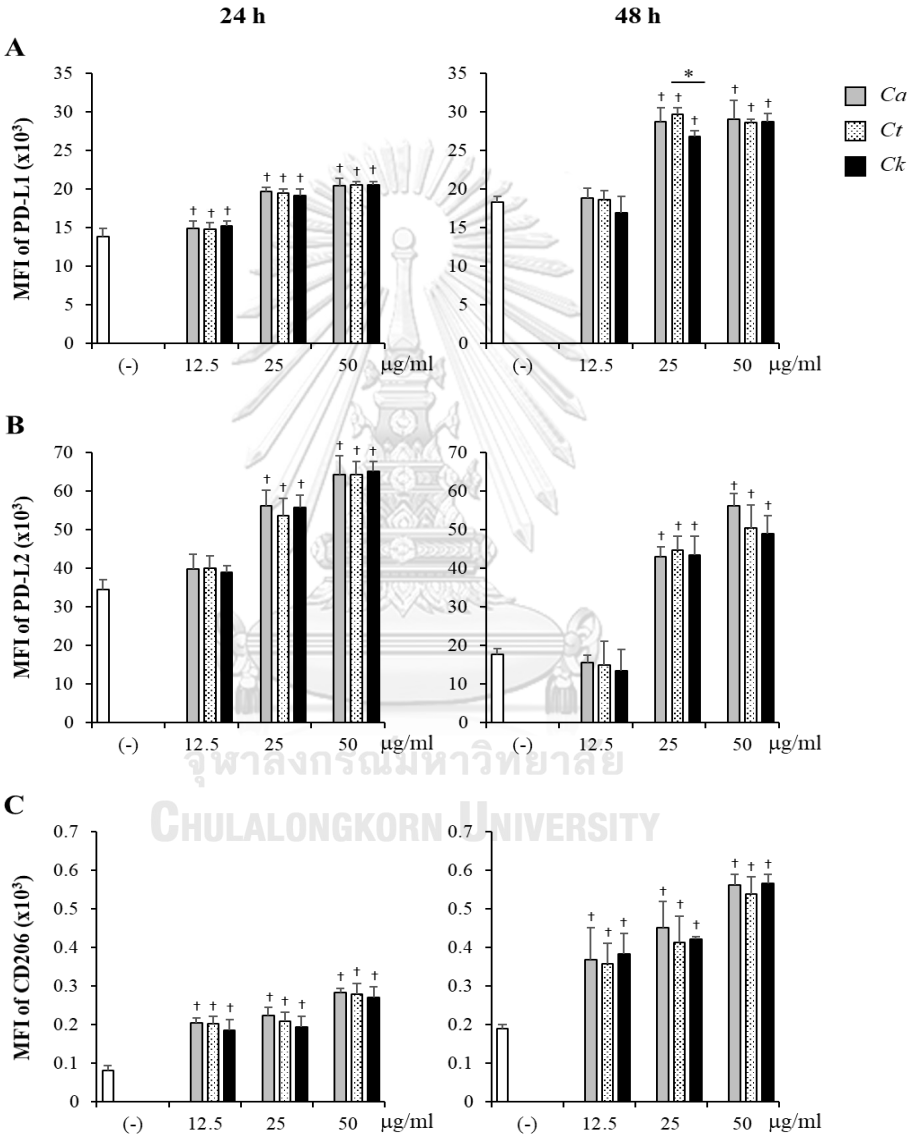


Figure 4.12 Expression levels of surface protein PD-L1, PD-L2, CD206 on BMDCs stimulated with *Candida* ß-glucans

BMDCs were stimulated with 12.5, 25 and 50 µg/mL of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h, and the geometric mean fluorescence intensity (MFI) of tolerogenic markers **(A)** PD-L1, **(B)** PD-L2, and **(C)** CD206 on $CD11c^+$ cells were determined using histogram analyses. n = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Massive pro-inflammatory and anti-inflammatory cytokine secretion of BMDCs upon stimulation of *C. krusei* ß-glucan

One of the most critical functions of DCs, the cytokine production, is necessary for DCs to fulfill their roles in a skewing of T cell responses (Leung et al., 2010). Under the stimulation of *Candida* ß-glucans, some presentative cytokines such as TNF-α, IL-1ß, IL-6, IL-23, IL-12, IFN-γ, and IL-10 secreted in cell supernatant was evaluated at 24 h and 48 h post-stimulation. As expected, BMDCs elevated these cytokine levels significantly in response to these kinds of ß-glucan compared to unstimulated cells (Figure 4.13 - 4.14). In general, there was an absolute difference of these cytokine levels under the stimulation of *C. krusei* ß-glucan in a dose-dependent manner at both time points, whereas *C. tropicalis* and *C. albicans* ß-glucan showed mostly similar effects on these cytokine levels. Besides, the variation of each cytokine level was also flexible following the time of stimulation. A high upregulation of almost cytokines was remained until 48 h post-stimulation, except for a decreasing trend of IL-23 and IL-10 cytokines (Figure 4.13 - 4.14).

In detail, *C. tropicalis* and *C. albicans* ß-glucan merely displayed a little difference (Figure 4.13 - 4.14), that the former induced a rising of IL-23 and IL-12 more than the latter at both time points (Figure 4.13D, 4.13E and Figure 4.14D, 4.14E). On the contrary, *C. krusei* ß-glucans induced the highest of almost cytokine levels compared with other *Candida* ß-glucans at both time point (Figure 4.13 - 4.14). The amount of pro-inflammatory cytokines such as TNF-α, IL-6 was promoted 2-fold up by *C. krusei* ß-glucan (Figure 4.13A, 4.13C and Figure 4.14A, 4.13C), while cytokine IL-1ß was elevated approximately up to 2-fold at doses 25 and 50 µg/mL by *C. krusei* ß-glucan compared with those levels of other two ß-glucans (Figure 4.13B and Figure 4.14B). For Th17-differentiated cytokines, besides IL-6 and IL-1ß cytokine, *C. krusei* ß-glucan elicited BMDCs to produce a large amount of IL-23, which was over 3-fold

more than *C. albicans* ß-glucan and over 2-fold more than *C. tropicalis* ß-glucan (Figure 4.13D and Figure 4.14D). Parallel, a similar increase was shown on Th1-priming cytokines consisting of IL-12 and IFN-γ (Figure 4.13E, 4.13F and Figure 4.14E, 4.13F). *C. krusei* ß-glucan impacted BMDC secretion of these cytokines reaching 2-fold at 48 h post-stimulation more than other ß-glucans. Especially, *C. krusei* ß-glucan induced a robust amount of anti-inflammatory cytokine IL10 of BMDCs which was shown approximate 3-fold at least and reached 4-6-fold at high doses of *C. krusei* ß-glucan in comparison with *C. albicans* ß-glucan and *C. tropicalis* ß-glucan respectively (Figure 4.13G and Figure 4.14G).

Overall, these results confirmed that diverse effects of ß-glucans on DC cytokine function depended on *Candida* species. *C. krusei* ß-glucan elicited the richest multi-cytokine production of DCs in consistent with the strong DC maturation previously observed. Interestingly, *C. krusei* ß-glucan induced a high production of anti-inflammatory cytokine, IL-10.

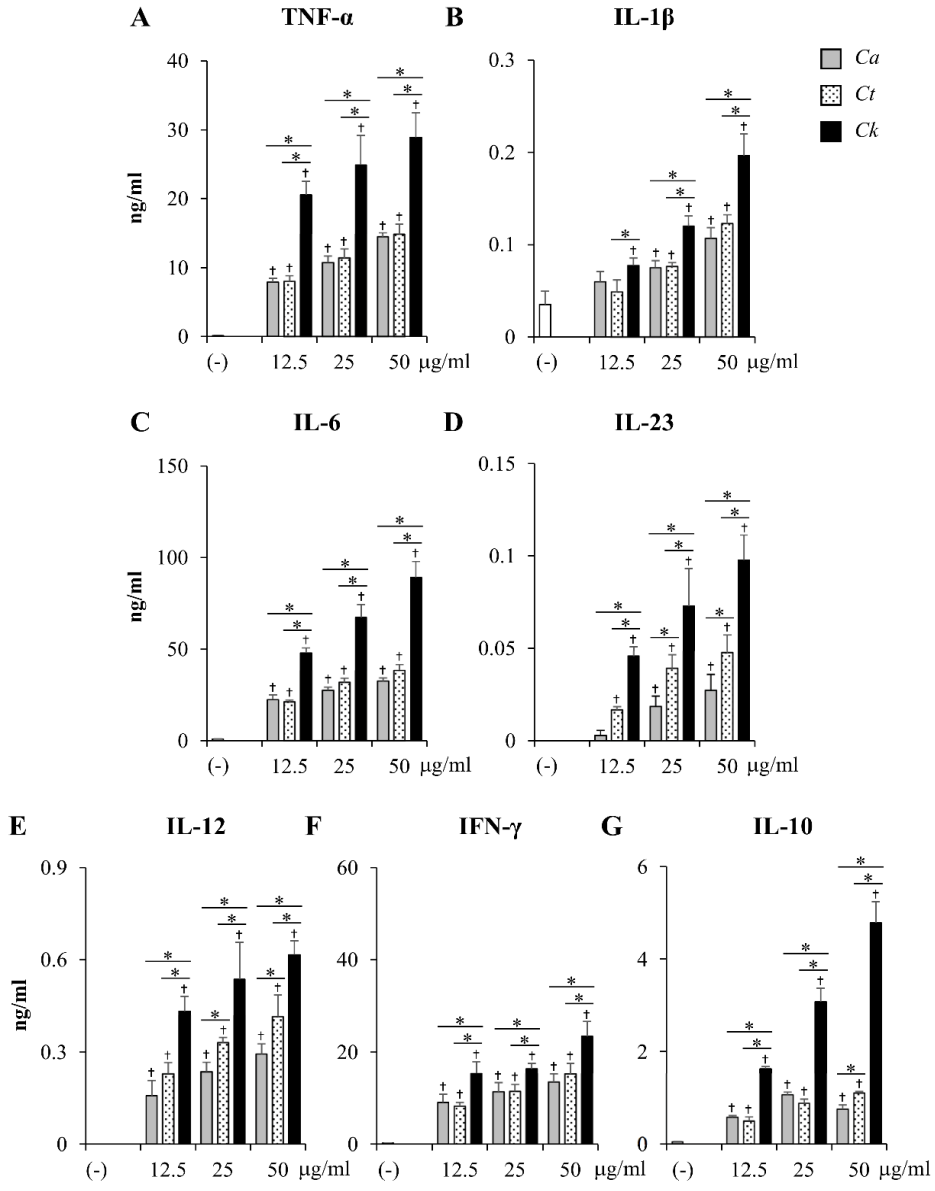


Figure 4.13 Excessive pro-inflammatory and anti-inflammatory cytokine production in *C. krusei* β-glucan-stimulated BMDCs at 24 h

BMDCs were stimulated with 12.5, 25 and 50 μg/mL of β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h. Subsequently, the culture supernatants were collected and levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were quantitated by ELISA. *n* = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

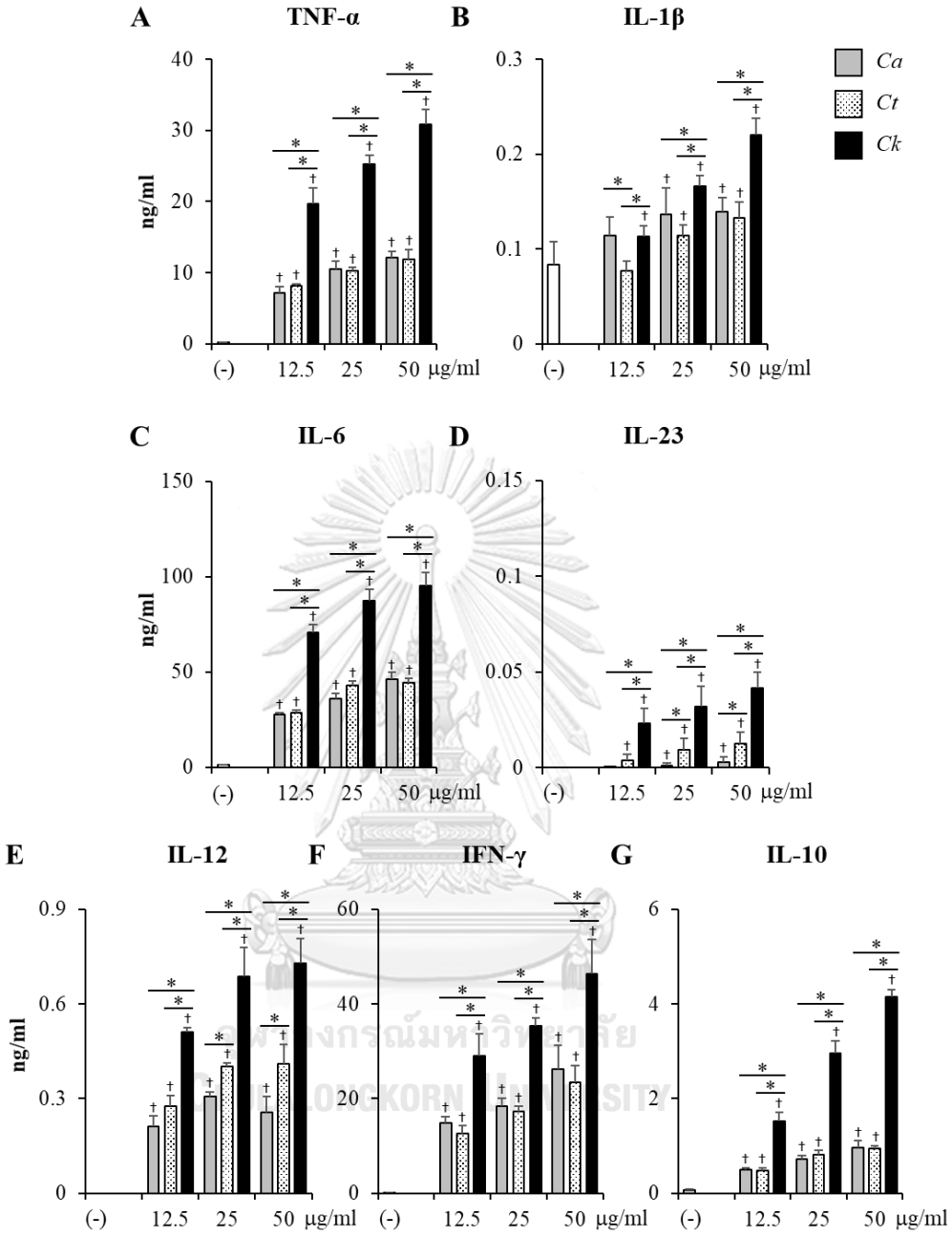


Figure 4.14 Cytokine profiles of *Candida* β-glucan-stimulated BMDCs at 48 h

BMDCs were stimulated with 12.5, 25 and 50 μg/ml of β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 48 h. Subsequently, the culture supernatants were collected, and levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were quantitated by ELISA. *n* = 5; Data are representatives of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

C. krusei ß-glucan enhanced IL-10-producing T cells, but not FoxP3$^+$ regulatory T cells in DC-T cell co-culture assay

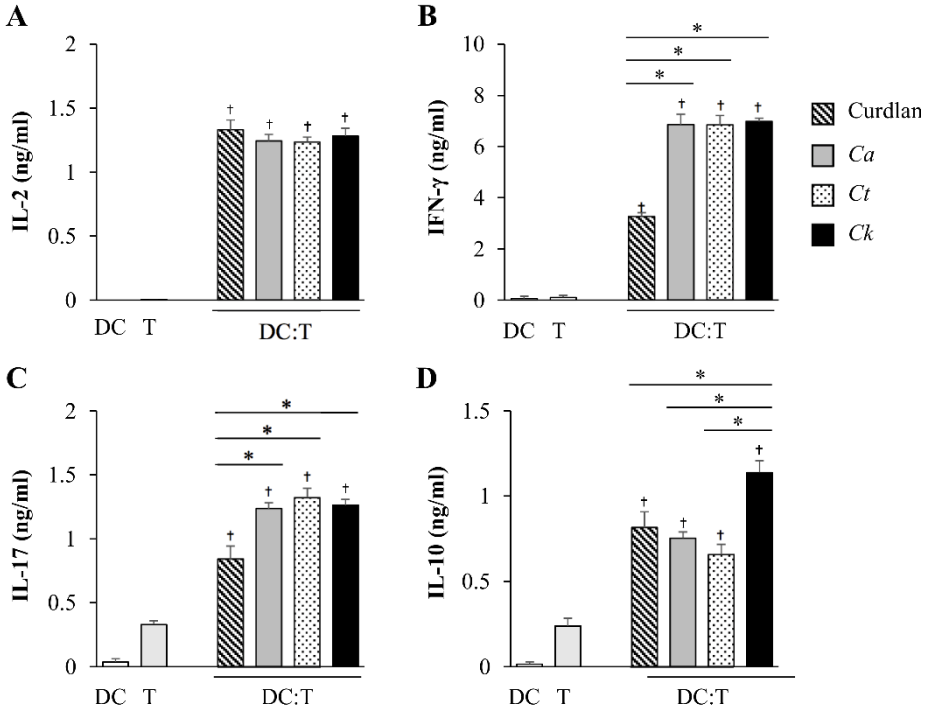


Figure 4.15 Effects of *Candida* ß-glucan-stimulated BMDCs on T cell-produced cytokines

BMDCs were stimulated with 25 μg/mL of curdlan and ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h. The stimulated BMDCs were co-cultured with murine splenic T cells at a DC:T cell ratio of 1:10 in the presence of soluble anti-mouse CD3 monoclonal antibody (30 ng/mL). Levels of T cell cytokines were assessed in culture supernatants. **(A)** IL-2 levels were measured following 24 h of co-culture. **(B)** IFN-γ, **(C)** IL-17, and **(D)** IL-10 levels were measured following 72 h of co-culture. BMDCs alone and T cells alone were incubated with the soluble anti-CD3 antibody as negative controls. n = 3; Data are representative of two independent experiments. † $p<0.05$ compared with negative controls, * $p<0.05$. DC, BMDCs; T, T cells, *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

To investigate the functions of *Candida* ß-glucan-stimulated BMDCs on T cell polarization, this study performed an assay of DC-T co-culture with using anti-soluble CD3 monoclonal antibody to first observe the outcomes of this direct interaction. Basing on the principle of specific signals required for T cell activation by DCs, anti-CD3 antibody binds with Fc receptors on DCs and Fab arms of this antibody subsequently binds with CD3 expressed on T cells. This connection of DCs and T cells through soluble anti-CD3 antibody replaces the signal of antigen-MHC-II

complex as known the first signals for T cell activation and proliferation (Clement et al., 1985) (Yixin Li & Roger J Kurlander, 2010). Thereby, BMDCs were exposed to *Candida* ß-glucan for 24 h prior to co-culture with spleen-derived T cells in the present of soluble anti-CD3 antibody. Since *C. krusei* ß-glucan-stimulated BMDCs enhanced the highest amount of anti-inflammatory cytokine IL-10 (Figure 4.13 - 4.14), this questioned how the regulatory T cells response in this case. Thus, the co-cultured T cells were evaluated FoxP3 and CD25 marker of $CD4^+$ T cells after 72 h co-culture (Figure 4.16) and the functional cytokines of T cells were quantitated in supernatants including IL-2, IL-10, IFN-γ and IL-17 (Figure 4.15). Negative controls were BMDCs alone and T cell alone in this experiment. Curdlan, a commercial ß-glucan, was used as a positive control.

For the results of cytokines (Figure 4.15), there was very low amount of IL-2, IFN-γ, IL-17 and IL-10 on control T cells alone and no cytokine secretion on control DCs alone. This showed that the low concentration of anti-CD3 antibody did not give any effect to interfere the results of *Candida* ß-glucan-primed DCs and T cells interaction. Interestingly, all cases of ß-glucan-primed BMDCs significantly promoted all T cell-secreted cytokines when compared with negative controls. Of note, IL-2 is known as a critical growth factor for T cell proliferation and is produced during T cell activation (Spolski et al., 2018). In addition, there was no difference of amounts of IL-2, IFN-γ and IL-17 among *Candida* ß-glucan-stimulated BMDCs (Figure 4.15A), whereas T cells co-cultured with *Candida* ß-glucan-stimulated BMDCs secreted higher amount of IFN-γ and IL-17 than T cells co-cultured with curdlan-stimulated BMDCs (Figure 4.15B and 4.15C). Intriguingly, BMDCs stimulated with *C. krusei* ß-glucan promoted the highest IL-10 production of T cells in this assay (Figure 4.15D). These results supported partly the assumption that Treg may be expanded highly under activation of *C. krusei* ß-glucan-stimulated BMDCs.

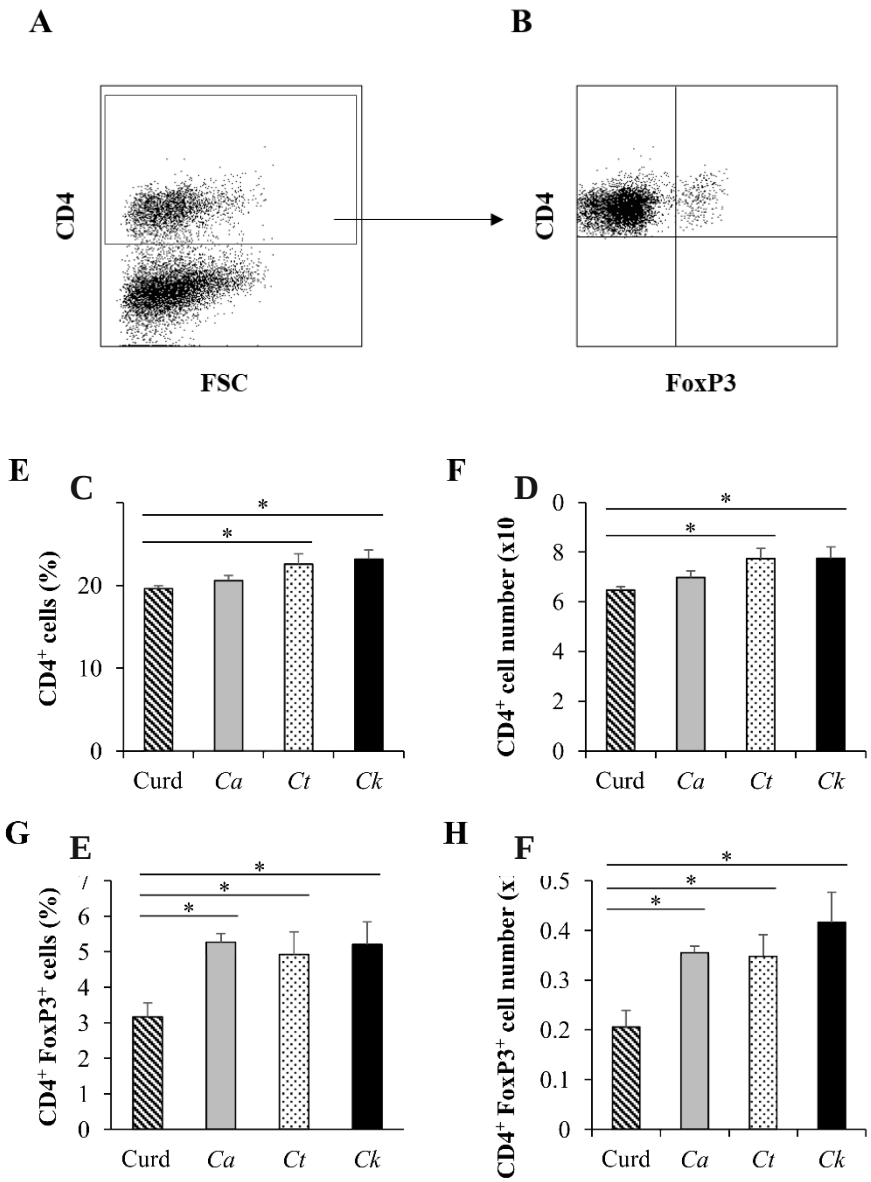


Figure 4.16 Similar effects of *Candida* ß-glucans-stimulated BMDCs on Treg cells

BMDCs were stimulated with 25 µg/mL of curdlan and ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h. The stimulated BMDCs were co-cultured with murine splenic T cells at a DC:T cell ratio of 1:10 in the presence of soluble anti-mouse CD3 monoclonal antibody (30 ng/mL). The percentages and numbers of $CD4^+$ T cells and $CD4^+FoxP3^+$ were determined following 72 h of co-culture by flow cytometry. *n* = 3; **(A)** CD4 T cells were first identified by gating on $CD4^+$ cells. **(B)** $FoxP3^+$ regulatory T cells in $CD4^+$ T cell population were next identified by gating on $CD4^+$ $FoxP3^+$ cells. **(C)** percentage and **(D)** number of $CD4^+$ cells, **(E)** percent and **(F)** number of $CD4^+FoxP3^+$ cells. Data are representative of two independent experiments. *$p<0.05$ compared between two kinds of ß-glucan, Curd, Curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Our study observed the signature markers of Treg in parallel of above cytokine examination (Figure 4.16A and 4.16B). The percentage and number of total

$CD4^+$ T cells were significantly higher under co-culture conditions of BMDCs stimulated with *C. tropicalis* and *C. krusei* ß-glucan when compared with the co-culturation in other ß-glucan stimulations (Figure 4.16C and 4.16D). Unexpectedly, although the percentage and number of $CD4^+FoxP3^+$ T cells significantly increased in all cases of T cells cocultured with *Candida* ß-glucan-stimulated BMDCs when compared with the co-culturation in curdlan stimulation, there was no difference of percentage and number of $CD4^+FoxP3^+$ T cells induced by among the co-culturation of *Candida* ß-glucan-stimulated BMDCs (Figure 4.16E and 4.16F). Overall, these data inferred that *C. krusei* ß-glucan-stimulated BMDCs highly promoted IL-10-producing T cells but not $FoxP3^+$ regulator T cells.

***In vivo C. krusei* ß-glucan immunization expanded IL-10-producing T cell population in antigen-specific manner**

To reinforce the outcomes of the interaction between *C. krusei* ß-glucan-stimulated DCs and T cells, our study performed the subcutaneous immunization of *C. krusei* ß-glucan plus with OVA antigen in parallel to compare with *C. albican* ß-glucan plus OVA antigen. Negative control was immunized with PBS plus OVA antigen in this experiment. Because DCs are known that they distribute mostly at the skin and can be highly exposed to the stimuli, these cells could increase the T cell polarization at local draining lymph nodes (Thu Ngoc Yen Nguyen, 2018). The T cells were isolated from the lymph nodes after those ß-glucan immunization were re-stimulated with specific antigen *in vitro* to evaluate T cell responses clearly. Then they were assessed the cytokine production in supernatants and surface target markers of Treg cells.

The findings showed that the responses of cytokine secretion were higher in cases of OVA re-stimulation than in case of non-OVA re-stimulation (Figure 4.17A-C). However, the response of IFN-γ and IL17 were similar between *C. albicans* ß-glucan and *C. krusei* ß-glucan immunization, and both these responses were significantly

increased in relevant to negative control (Figure 4.17A and 4.17B). Interestingly, *C.krusei* ß-glucan immunization induced the highest amount of IL-10 cytokine of T cell response in OVA-specific manner when compared with the counterparts (Figure 4.17C). However, the $CD4^{+}FoxP3^{+}$ T cells were not different between OVA re-stimulation and non-OVA re-stimulation, and this population was also not different between the immunization of *C. krusei* ß-glucan and *C. albicans* ß-glucan (Figure 4.17D). These data were compatible with the results of high IL-10-producing T cells mediated by *C. krusei* ß-glucan-stimulated BMDCs in the co-culture system.

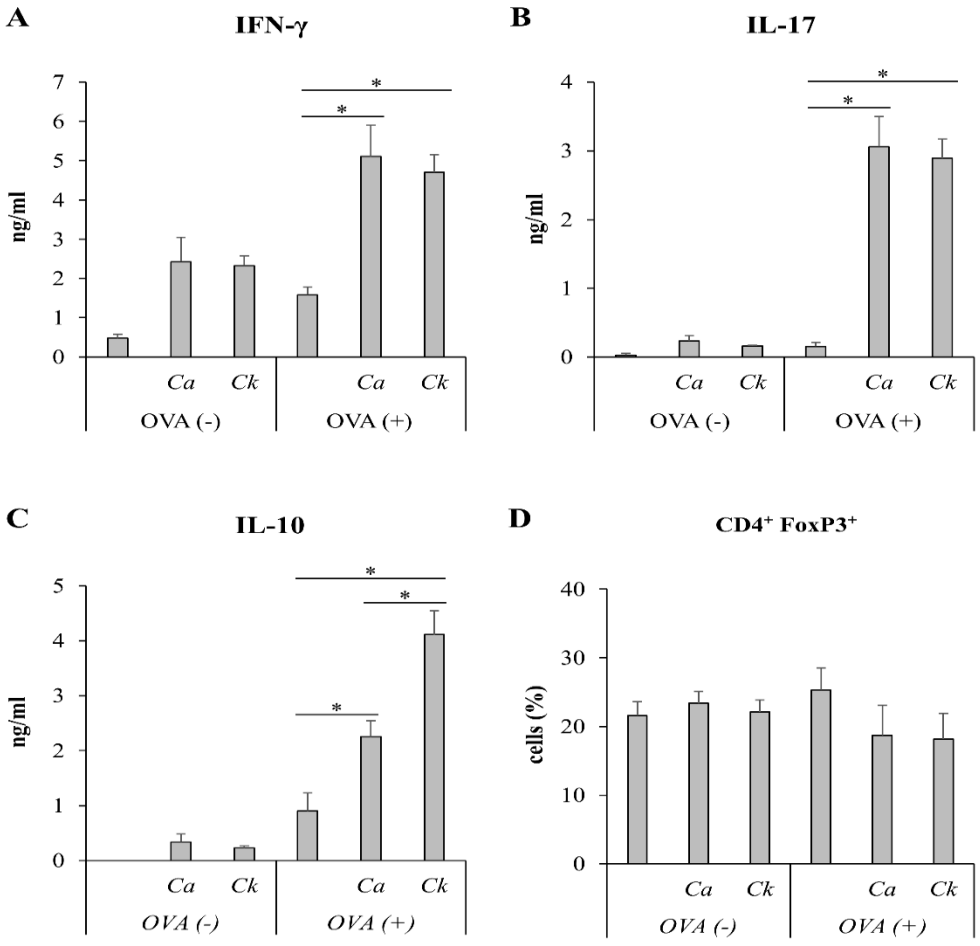


Figure 4.17 High IL-10-producing T cells in in vivo *C. krusei* ß-glucan immunization

Mice were subcutaneously immunized with a mixture of *C. albicans* or *C. krusei* ß-glucans and OVA at day 0 and day 7. On day 14, the draining LNs cells were isolated and were *ex vivo* re-stimulated with OVA for 48 h. Culture supernatants were measured for

(A) IFN-γ, **(B)** IL-17, and **(C)** IL-10 by ELISA. **(D)** $CD4^+FoxP3^+$ T cells were assessed by flow cytometry. *n* = 6. * $p<0.05$. *Ca*, *C. albicans*; *Ck*, *C. krusei*.

High expansion of IL-10-producing T cell population in systemic *C. krusei* infected-mice

C. krusei ß-glucan had many distinct effects observed on BMDCs and T cells polarization and that was the reason to investigate whether ß-glucan could contribute to the immunological pathogenesis mechanisms of *C. krusei* infection. To illustrate the immunosuppression condition of invasive candidiasis patients, the immunosuppression mice model for *C. krusei* infection were performed by using dexamethasone treatment prior doing the systemic *C. krusei* infection (Jacobsen et al., 2010). Negative control mice were intravenously injected with PBS buffer. Systemic *C. krusei* infection were generated by intravenously injection of low dose and high dose of live *C. krusei* yeasts. After 7 days post-infection, splenocytes excised from these mice were cultured in two conditions *ex vivo*, one was in the absence of immobilized anti-CD3 re-stimulation and another was in the presence of anti-CD3 re-stimulation. Our study evaluated the cytokine responses in blood sera and in supernatants of anti-CD3-boosted splenocytes (Figure 4.18A and 4.18B). Besides that, the markers of splenic Treg population were continuously assessed at 48 h post-culture *in vitro* and compared with splenic Treg cells plus the anti-CD3 antibody boosting (Figure 4.18C and 4.18D).

Interestingly, the cytokine responses in sera were rather similar those responses in supernatant of anti-CD3 re-stimulated splenocytes. On the one hand, in sera, amount of IFN-γ was significantly decreased and amount of IL-17 was highly increased in *C. krusei*-infected mice compared with negative control mice (Figure 4.18A, left and middle panels). *C. krusei*-infected mice likely produced the increased amount of IL-10 higher than negative control, but this was not statistical significance (Figure 4.18A, right panel). In addition, there was no difference of these cytokines

between two doses of *C. krusei*-infected mice (Figure 4.18A). On the other hand, in supernatant of anti-CD3 re-stimulation splenocytes, amount of IFN-γ was secreted similarly among infected and non-infected mice (Figure 4.18B, left panel). The high amount of IL-17 was significantly different between infected mice and non-infected mice under this condition (Figure 4.18B, middle panel). Notably, *C. krusei* infected mice produced significantly high amount of IL-10 in dose-dependent manner and higher than this of non-infected mice (Figure 4.18B, right panel). Thus, these results highlighted that *C. krusei* ß-glucan profoundly influenced on the immunomodulator, IL-10 production of host immune cells in case of systemic *C. krusei* infection.

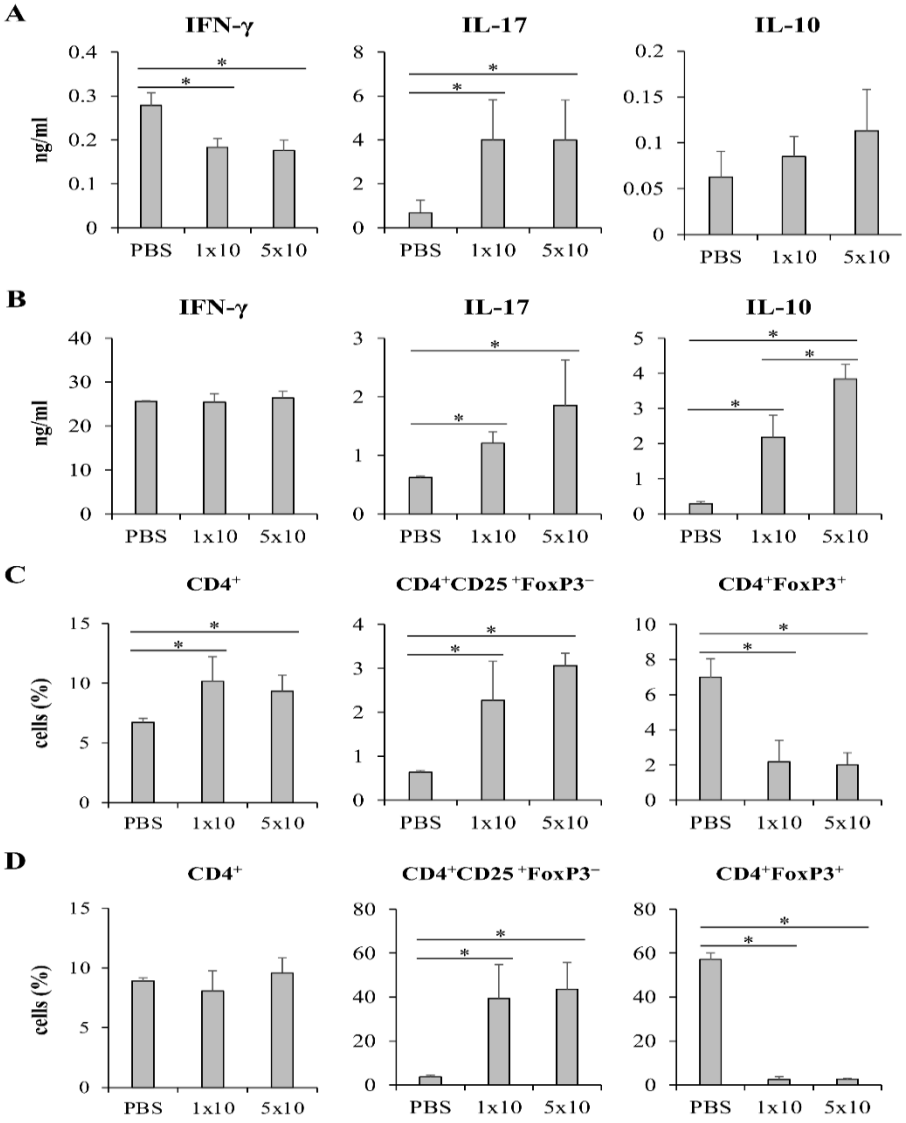


Figure 4.18 Systemic *C. krusei* infection possibly promoted IL-10-producing T cells

Mice were treated with dexamethasone to induce immunosuppression and were systemically infected with $1x10^6$ and $5x10^6$ of *C. krusei*. Serum and spleens were collected on day 7 post-infection. Splenocytes were re-stimulated with immobilized anti-CD3 for 48 h. **(A)** Serum cytokines. **(B)** Cytokines from supernatant from anti-CD3-stimulated splenocytes. **(C)** T cells from unstimulated splenocytes. **(D)** T cells from anti-CD3 stimulated splenocytes. $n = 5$. * $p<0.05$.

For T cell polarization, our study found that the $CD4^+$ T cell enhanced in *C. krusei* infected mice significantly higher than non-infected mice (Figure 4.18C, left panel), although this did not show in the presence of anti-CD3 re-stimulation (Figure 4.18D, left panel). Notably, *C. krusei*-infected mice did not increase the $CD4^+FoxP3^+$ T cells when compared with non-infected mice (Figure 4.18C, right panel). Anti-CD3 re-

stimulated splenocytes from *C. krusei*-infected mice also did not enhance the Treg population (Figure 4.18D, right panel). Nevertheless, *C. krusei*-infected mice remarkably increased the $CD4^{+}CD25^{+}FoxP3^{-}$ T cells at any dose of *C. krusei* yeasts higher than those cells of non-infected mice (Figure 4.18C, middle panel). Furthermore, the notable phenomenon of increased $CD4^{+}CD25^{+}FoxP3^{-}$ T cell population showed similarly in anti-CD3 re-stimulated splenocytes from *C. krusei*-infected mice (Figure 4.18D, middle panel). Overall, *C. krusei* yeasts possibly influenced on DC properties to dictate the fate of T cell responses toward IL-10-producing T cells.

C. krusei ß-glucan-induced DC maturation and cytokine production partially depends on dectin-1 receptor

Dectin-1 receptor plays the main role in interaction of host immune cells with fungal organisms through detecting the structure ß-glucan on the fungal cell wall (Brown et al., 2002; Taylor et al., 2007). Presumably, these kinds of *Candida* ß-glucan could produce different effects on DCs that may be result from dissimilar interactions with dectin-1 receptor. Thereby, to test this hypothesis, this work used dectin-1 antagonist to block dectin-1 receptor and evaluate the reverse effects of these ß-glucans on BMDCs at 24 h and 48 h post-stimulation. Untreated and ß-glucan-unstimulated cells or alone dectin-1-treated cells were negative controls. Curdlan was used as a positive control in dectin-1 blockade experiments, because it is a well-known ligand of human and mouse dectin-1 receptor (Palma et al., 2006; Takano et al., 2017). Under dectin-1 inhibition, the proportion and CD11c expression was examined by flow cytometry (Figure 4.19). The results showed that the proportion and CD11c expression levels were not altered among *Candida* ß-glucan stimulations between untreated and treated dectin-1 antagonist. Thus, dectin-1 blockade did not affect DC proportion and CD11c expression. In consistence to previous data (Figure 4.9), *C. krusei* ß-glucan still down-regulated CD11c expression of BMDCs lower than

that of other ß-glucans, even though treating cells with dectin-1 antagonist before (Figure 4.19). This suggested that the decrease of CD11c marker due to *C. krusei* ß-glucan was independent on dectin-1 receptor.

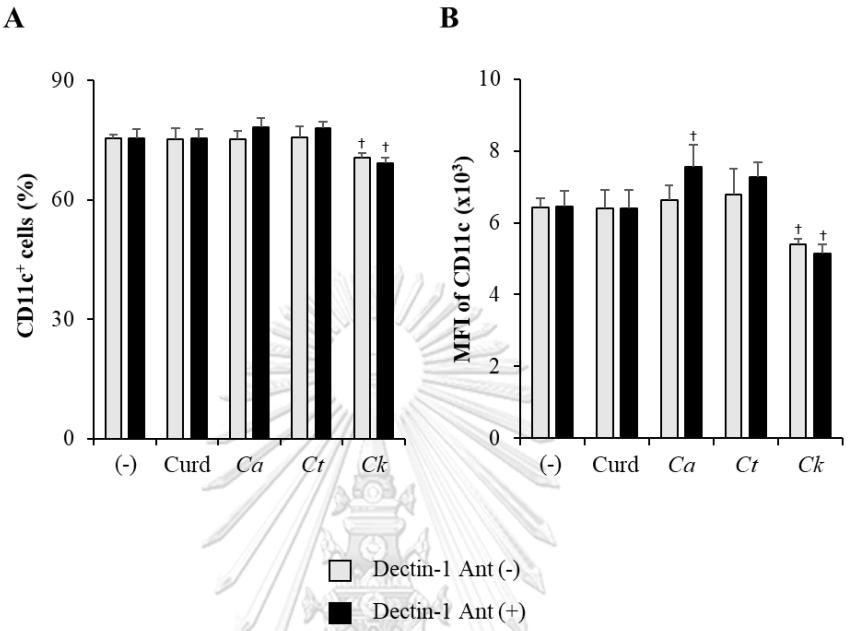


Figure 4.19 Impact of dectin-1 blockade on DC proportion and CD11c expression

BMDCs were pre-treated with 25 µg/mL of dectin-1 antagonist for 2 h, then cells were stimulated with 25 µg/mL of curdlan and ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h. **(A)** The percentages of $CD11c^+$ cells were assessed using a dot pot analysis. **(B)** The geometric mean fluorescence intensity (MFI) of CD11c was determined using a histogram analysis. $n = 5$; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

For DC maturation markers, the impact of *Candida* ß-glucan showed some apparent alterations of these markers between before and after blocking cells with dectin-1 antagonist (Figure 4.20). At 24 h post-stimulation, dectin-1 antagonist suppressed similarly the expression level of CD40, CD80 and CD86 of BMDCs when compared between the stimulation of *C. albicans* and *C. tropicalis* ß-glucan (Figure 4.20A, B and C, left panels). With these two stimuli, the MFI expression levels of those molecules were reduced 30%, 18%, 21% respectively (Table 4.1). Meanwhile, MHC-II expression was reduced a little more in *C. tropicalis* ß-glucan-stimulated BMDCs (25%) than *C. albicans* ß-glucan-stimulated BMDCs (18%) (Figure 4.20D, left

panel and Table 4.1). Oppositely, effects of *C. krusei* ß-glucan was inhibited by dectin-1 antagonist only on CD40 and CD80 expression of these cells at that time, 17% and 12% respectively (Figure 4.20A, 4.20B and Table 4.1), while this ß-glucan continuously triggered BMDCs to remain the expression of CD86 and MHC-II at relatively same level between before and after blocking dectin-1 receptor (Figure 4.20C, 4.20D and Table 4.1). The blocking effects of dectin-1 antagonist on *C. krusei* ß-glucan-stimulation BMDCs were significantly lower than those of BMDCs stimulated by *C. albicans* and *C. tropicalis* ß-glucan (Figure 4.20 and Table 4.1). The effects of dectin-1 blockade on curdlan-stimulated BMDCs were similar in case of *C. krusei* ß-glucan. At 48 h post-stimulation, the blocking effects on maturation markers were likely to persist under dectin-1 blockade (Figure 4.20, right panels). Especially, CD40 and CD80 makers were reduced among all kinds of ß-glucan stimulation under dectin-1 blockade condition (Figure 4.20A and 4.20B, right panels). There was less difference of these blocking effects among ß-glucan-stimulated BMDCs at 48 h (Table 4.1). These findings highlighted that BMDC maturation in response to ß-glucan of *C. albicans* and *C. tropicalis* was highly specific to dectin-1-related recognition, whereas ß-glucan of *C. krusei* promoted those responses separately which were not totally dependent on dectin-1 receptor.

For DC cytokine production, the cytokine responses of ß-glucan-triggered cells were also inhibited by dectin-1 antagonist at different levels depending on *Candida* species at 24 h and 48 h (Figure 4.21 - 4.22). The inhibitory ability of this antagonist to ß-glucan effects were efficiently firmed through decreased cytokine effects of curdlan control on treated BMDCs (Figure 4.21 - 4.22), of which, remarkably complete abolishment of IL-1ß and IL-23 secretion (Figure 4.21B, 4.21D and Figure 4.22B, 4.22D). In line with that general tendency, all observed cytokines were significantly decreased their amounts because of dectin-1-inhibited cells prior to exposure of any kind of *Candida* ß-glucan (Figure 4.21 - 4.22). Interestingly, *C. tropicalis* and *C.*

albicans ß-glucan were similarly decreased their effects on DC cytokine induction by dectin-1 antagonist (Figure 4.21 - 4.22 and Table 4.1). By contrast, *C. krusei* ß-glucan still elicited great differences on BMDC cytokine secretion when compared to *C. albicans* and *C. tropicalis* ß-glucan at both time points, even if in dectin-1-blockade condition (Figure 4.21 - 4.22 and Table 4.1).

In details, dectin-1-inactivated BMDCs stimulated by *C. albicans* and *C. tropicalis* ß-glucan sharply dropped over 70% of the amount of TNF-α, IL-6 and IFN-γ, minimized nearly 80% of IL-10 amount, and failed to produce cytokine IL-1ß, IL-23 and IL-12 following the time (Table 4.1). Even though TNF-α, IL-6 and IFN-γ of dectin-1-treated BMDCs were released due to *C. albicans* ß-glucan a little more different than *C. tropicalis* ß-glucan (Table 4.1). These findings implied ß-glucan of these two species were recognized largely by dectin-1 receptor of BMDCs and were highly specified to dectin-1 signaling pathway in production of IL-1ß, IL-23 and IL-12.

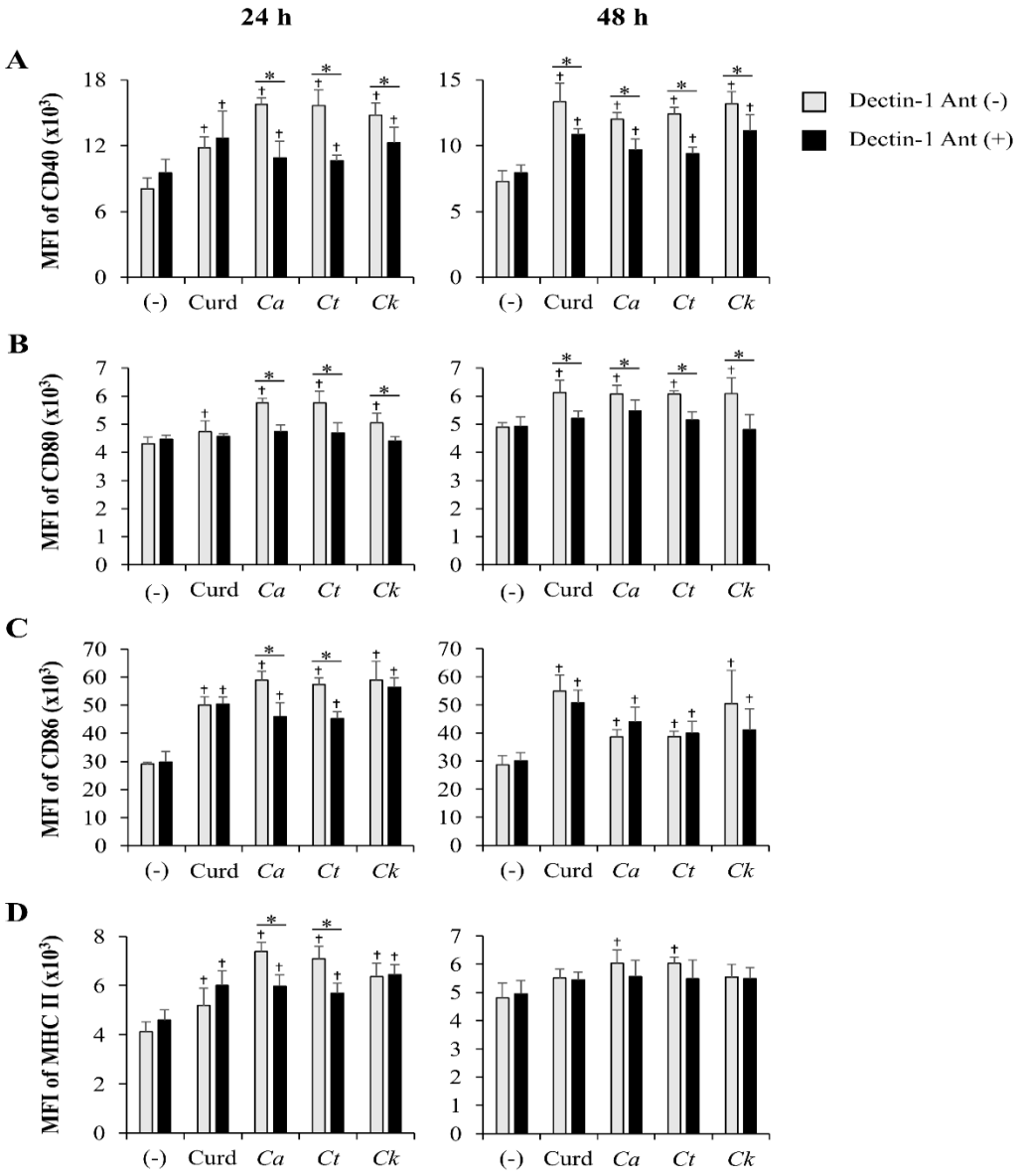


Figure 4.20 Inhibition of DC maturation by dectin-1 blockade

BMDCs were pre-treated with 25 μg/mL of dectin-1 antagonist for 2 h, and then the cells were stimulated with 25 μg/mL of curdlan and β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. The geometric mean fluorescence intensity (MFI) of DC maturation markers **(A)** CD40, **(B)** CD80, **(C)** CD86, and **(D)** MHC class II on $CD11c^+$ cells were determined using histogram analyses. *n* = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Table 4.1 Blockade of DC maturation and cytokine production by dectin-1 antagonist

	% Blocking[1, 2] ± SD			
	Curdlan	*C. albicans*	*C. tropicalis*	*C. krusei*
24h				
CD40	2.9 ± 4.0 [b, c, d]	30.9 ± 9.5 [a, d]	32.2 ± 3.3 [a, d]	17.1 ± 9.7 [a, b, c]
CD80	3.5 ± 2.0 [b, c]	17.9 ± 4.3 [a]	18.6 ± 6.1 [a]	12.6 ± 7.1
CD86	1.6 ± 2.3 [b, c, d]	21.8 ± 8.3 [a, d]	21.2 ± 4.3 [a, d]	4.5 ± 5.3 [a, b, c]
MHC-II	0.0 ± 0.0 [b, c, d]	18.9 ± 6.2 [a, d]	25.8 ± 5.3 [a, d]	2.0 ± 2.7 [a, b, c]
48h				
CD40	18.5 ± 3.2	19.4 ± 6.9	24.3 ± 4.0	15.6 ± 9.2
CD80	15.0 ± 4.2	9.6 ± 6.3	15.2 ± 4.8	21.2 ± 10.5
CD86	6.1 ± 7.7	1.1 ± 2.5 [d]	2.1 ± 2.4 [d]	18.6 ± 14.8 [b, c]
MHC-II	2.7 ± 2.9	7.8 ± 9.2	10.8 ± 8.0	3.8 ± 3.5
24h				
TNF-α	45.1 ± 3.4 [b, c, d]	76.4 ± 2.5 [a, d]	83.1 ± 2.2 [a, d]	24.7 ± 6.9 [a, b, c]
IL-1β	92.2 ± 17.5 [d]	73.3 ± 36.9 [d]	80.8 ± 16.9 [d]	4.0 ± 9.0 [a, b, c]
IL-6	54.5 ± 4.5 [b, c, d]	77.0 ± 2.6 [a, c, d]	85.2 ± 2.0 [a, b, d]	34.2 ± 4.9 [a, b, c]
IL-23	N.D	100.0 ± 0.0	100.0 ± 0.0	99.1 ± 2.0
IL-12	53.0 ± 10.8 [b, c, d]	99.6 ± 0.9 [a, d]	100.0 ± 0.0 [a, d]	74.3 ± 3.7 [a, b, c]
IFN-γ	0.3 ± 0.6 [b, c, d]	71.5 ± 3.5 [a, c, d]	59.4 ± 4.5 [a, b, d]	34.6 ± 3.8 [a, b, c]
IL-10	45.9 ± 7.3 [b, c, d]	78.4 ± 4.7 [a, d]	84.4 ± 2.7 [a, d]	61.3 ± 2.0 [a, b, c]
48h				
TNF-α	42.6 ± 6.1 [b, c, d]	74.4 ± 1.5 [a, d]	81.5 ± 0.9 [a, d]	28.9 ± 8.6 [a, b, c]
IL-1β	100.0 ± 0.0 [d]	100.0 ± 0.0 [d]	100.0 ± 0.0 [d]	59.6 ± 9.4 [a, b, c]
IL-6	51.4 ± 2.6 [b, c, d]	71.5 ± 1.8 [a, c, d]	79.7 ± 1.7 [a, b, d]	40.5 ± 4.7 [a, b, c]
IL-23	N.D	100.0 ± 0.0	100.0 ± 0.0	99.8 ± 0.6
IL-12	64.0 ± 7.9 [b, c, d]	100.0 ± 0.0 [a, d]	100.0 ± 0.0 [a, d]	75.9 ± 4.9 [a, b, c]
IFN-γ	30.3 ± 6.2 [b, c]	68.8 ± 1.9 [a, d]	66.8 ± 1.5 [a, d]	18.2 ± 11.8 [b, c]
IL-10	48.6 ± 7.5 [b, c, d]	79.6 ± 4.2 [a, d]	84.6 ± 3.7 [a, d]	60.0 ± 4.4 [a, b, c]

[1] % Blocking of maturation molecules was calculated by the following formula; (average MFI of molecule A expressed on glucan-stimulated DCs – MFI of molecule A expressed on dectin-1 antagonist treated glucan-stimulated DCs) x 100/average MFI of molecule A expressed on glucan-stimulated DCs).

[2] % Blocking of cytokine level was calculated by the following formula; (average amount of cytokine A secreted from glucan-stimulated DCs – amount of cytokine A secreted from dectin-1 antagonist treated glucan-stimulated DCs) x 100/average amount of cytokine A secreted from glucan-stimulated DCs).

Data of n = 5; [a]$p < 0.05$ compared with Curdlan, [b]$p < 0.05$ compared with *C. albicans* glucan, [c]$p < 0.05$ compared with *C. tropicalis* glucan, [d]$p < 0.05$ compared with *C. krusei* glucan. N.D, not determined.

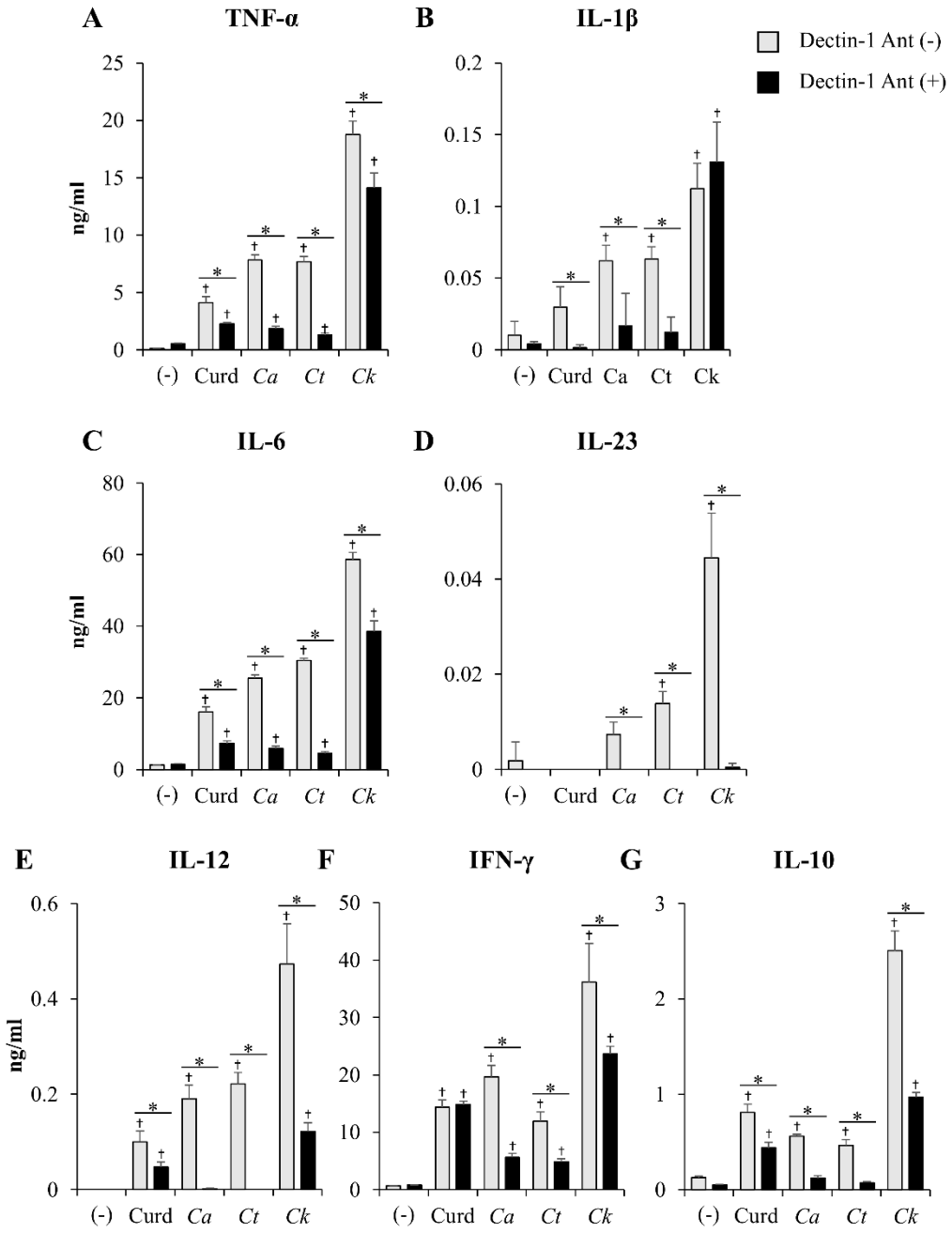


Figure 4.21 Contribution of dectin-1 blockade to DC cytokine production at 24 h

BMDCs were pre-treated with 25 μg/mL of dectin-1 antagonist for 2 h, and then the cells were stimulated with 25 μg/mL of curdlan and β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h. Levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were measured in culture supernatants by ELISA. *n* = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

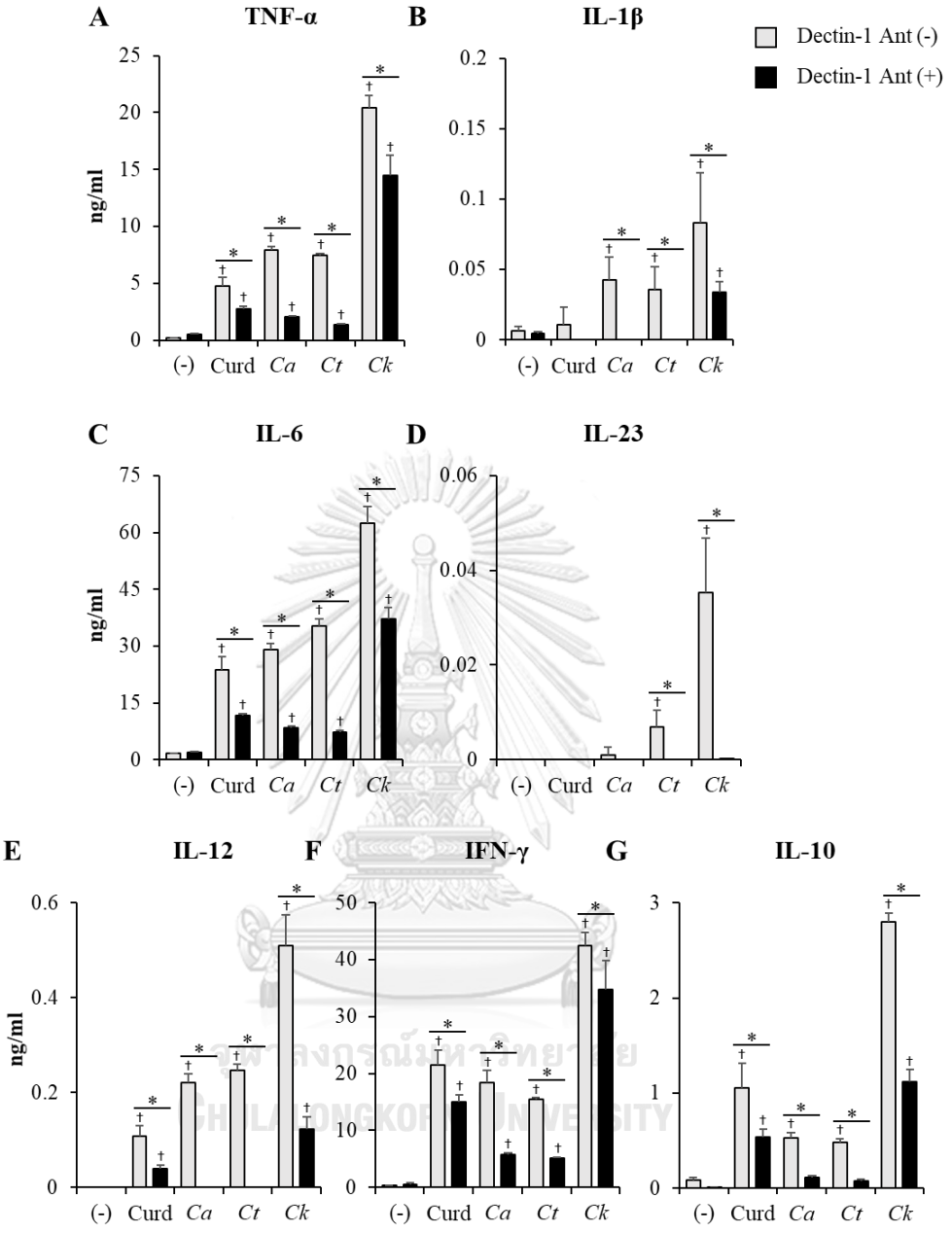


Figure 4.22 Effects of dectin-1 blockade on DC cytokine production at 48 h

BMDCs were pre-treated with 25 μg/ml of dectin-1 antagonist for 2 h, and then the cells were stimulated with 25 μg/ml of curdlan and β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 48 h. Levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were measured in the culture supernatants by ELISA. *n* = 5; Data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

On the contrary, in case of *C. krusei* β-glucan-stimulated DCs plus the antagonist pretreatment, merely a disappearance of cytokine IL-23 occurred at both

time points (Figure 4.21D and Figure 4.22D), while the residue cytokines were not reduced as much as in stimulation of other *Candida* ß-glucans (Figure 4.21 - 4.22). The amount of TNF-α and IL-6 were decreased less than 30% and around 40% respectively in dectin-1 blockade, whereas around 75% of IL-12 amount and 60% of IL-10 amount were suppressed at both time points (Table 4.1). In addition, there were special fluctuations of cytokine IL-1ß and IFN-γ following the time in this case. The amount of IL-1ß was unchanged at 24 h but reduced 60% at 48 h (Figure 4.21B, Figure 4.22B and Table 4.1), and the amount of IFN-γ was decreased about 35% at 24 h but only 18% at 48 hour later (Figure 4.21F, Figure 4.22F and Table 4.1). Thus, these data also re-confirmed that BMDCs activated by ß-glucan of *C. krusei* promoted the highest amount of almost presentative cytokines in both dectin-1-dependent and -independent manner.

Collectively, combining all effects of ß-glucan on DC maturation and cytokine induction, these results indicated that ß-glucan of *C. krusei* influenced differently from ß-glucan of both *C. tropicalis* and *C. albicans* on DC responses.

Differential DC activation via Syk-dependent signaling pathway of *Candida* ß-glucan

Engagement of ß-glucan to dectin-1 receptor mainly operates its downstream signaling pathway through activities of classical spleen tyrosine kinase (Syk) adaptor, which is typical mechanism of *C. albicans* ß-glucan effects (Rogers et al., 2005; Thwe et al., 2019). Thus, this study investigated whether the different impacts of mentioned NACs ß-glucans on BMDCs go through Syk signaling pathway. Using Syk inhibitor pre-incubates BMDCs prior to contact with ß-glucans of *C. tropicalis*, *C. krusei* and *C. albicans* aiming to evaluate these relations. Maturation markers of BMDCs were evaluated their expression by flow cytometry (Figure 4.23) and DC

cytokine were quantitated by ELISA at 24 h and 48 h post-stimulation (Figure 4.24 - 4.25).

The influences of *Candida* ß-glucan on BMDCs maturation were efficiently suppressed by Syk inhibitor with a few differences among these ß-glucans (Figure 4.23). This inhibition efficiency on these molecules was altered following the time. In which, the MFI expression levels of CD80, CD86 and MHC-II marker were dropped obviously due to blocking Syk signaling on ß-glucan-stimulated BMDCs as early as 24 h post-stimulation (Figure 4.23B-D). An exception, CD40 expression level was reduced among ß-glucan- stimulated BMDCs plus Syk inhibition at 48 h post-stimulation when compared with single ß-glucan-stimulated cells (Figure 4.23A). Interestingly, among three of *Candida* ß-glucans, BMDCs stimulated with *C. krusei* ß-glucan likely induced higher expression levels of these molecules such as CD40 and CD86 in the absence of Syk signaling (Figure 4.23A and 4.23C), while BMDCs stimulated with *C. tropicalis* or *C. albicans* ß-glucan mediated the similar effects on all maturation markers in that condition (Figure 4.23).

For cytokine secretion, curdlan and *Candida* ß-glucan-stimulated BMDCs were largely impeded the production of pro-inflammatory and anti-inflammatory cytokines in case of Syk inhibitor treatment (Figure 4.24 - 4.25). It appeared that those reduction of cytokines also varied depending on time, cytokine type and ß-glucan type. Under stimulation of ß-glucans plus pre-treated Syk inhibitor, amount of IL-1ß, IL-23 and IL-10 tended to reduce slowly because the blocking effects of these cytokines revealed clearly at 48 h of post-stimulation (Figure 4.24B, 4.24D, 4.24G and Figure 4.25B, 4.25D, 4.25G). On the other hand, amount of TNF-α, IL-6, IL-12 and IFN-γ were decreased sharply from 24 h (Figure 4.24A,4.24C, 4.24E and 4.24F) and persisted to 48 h in the same condition (Figure 4.25A, 4.25C, 4.25E and 4.25F). Besides, in the absence of Syk activity, *C. tropicalis* ß-glucan effects showed on

BMDCs in a similar pattern of all cytokine reductions relative to *C. albicans* ß-glucan effects (Figure 4.24 - 4.25). By contrast, under the same condition, *C. krusei* ß-glucan possibly persisted higher levels of DC cytokine production at both time points when compared with other *Candida* ß-glucans, typically IL-1ß and IL-10 (Figure 4.24 - 4.25).

Hence, it possibly inferred that the influences of *Candida* mp-ß-glucan on DCs mainly converged at downstream Syk signaling pathway of dectin-1 receptor. Nevertheless, *C. krusei* ß-glucan still drove its unique impacts on BMDC properties in certain different manner, which was Syk-independent pathway and distinct from those of *C. tropicalis* and *C. albicans* ß-glucan.

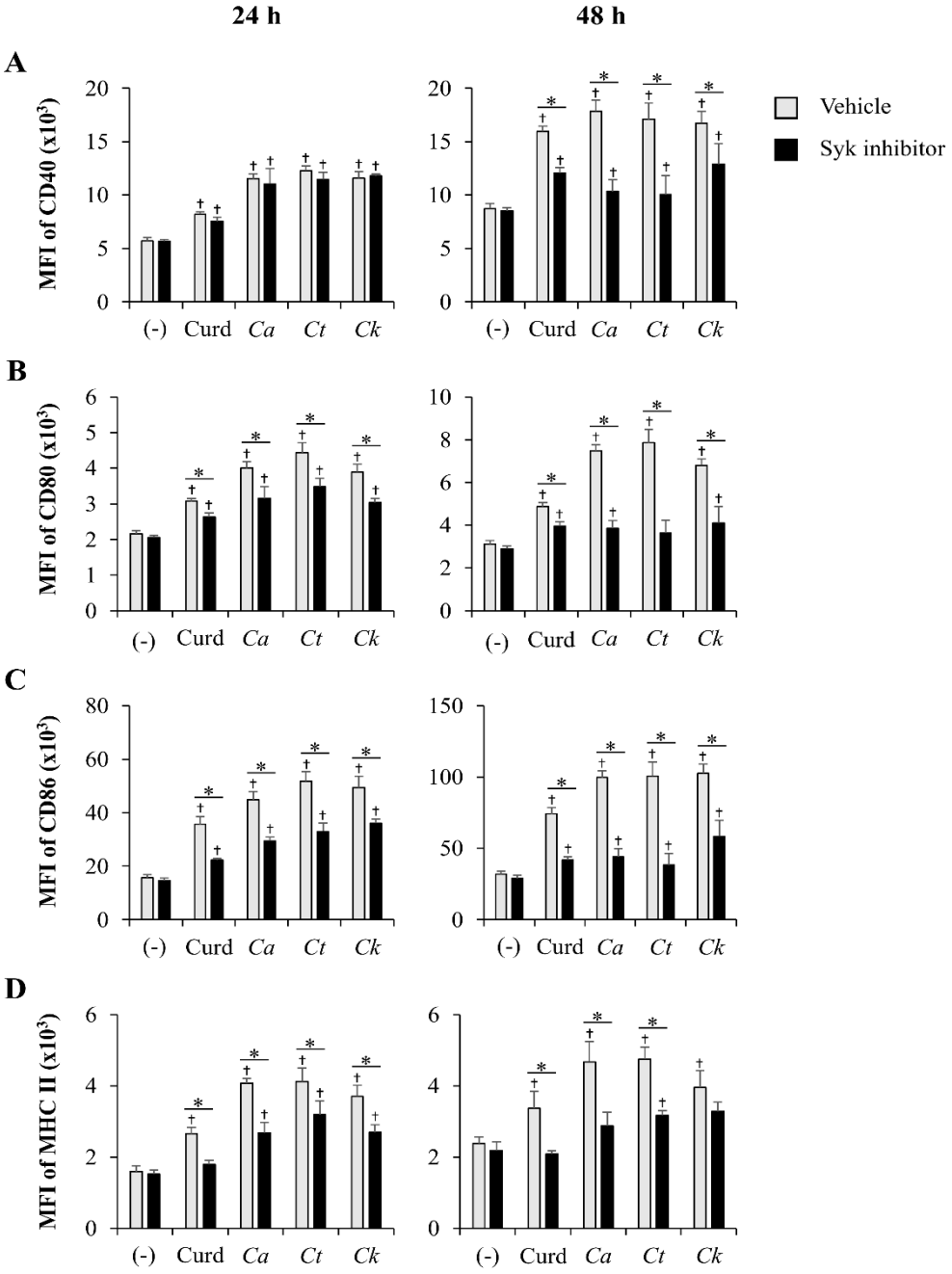


Figure 4.23 Inhibition of DC maturation by Syk inhibitor

BMDCs were pre-treated with 1 μM of Syk inhibitor for 30 min, and then the cells were stimulated with 25 μg/mL of curdlan and ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. The geometric mean fluorescence intensity (MFI) of DC maturation markers **(A)** CD40, **(B)** CD80, **(C)** CD86, and **(D)** MHC class II on $CD11c^+$ cells were determined using histogram analyses. n = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

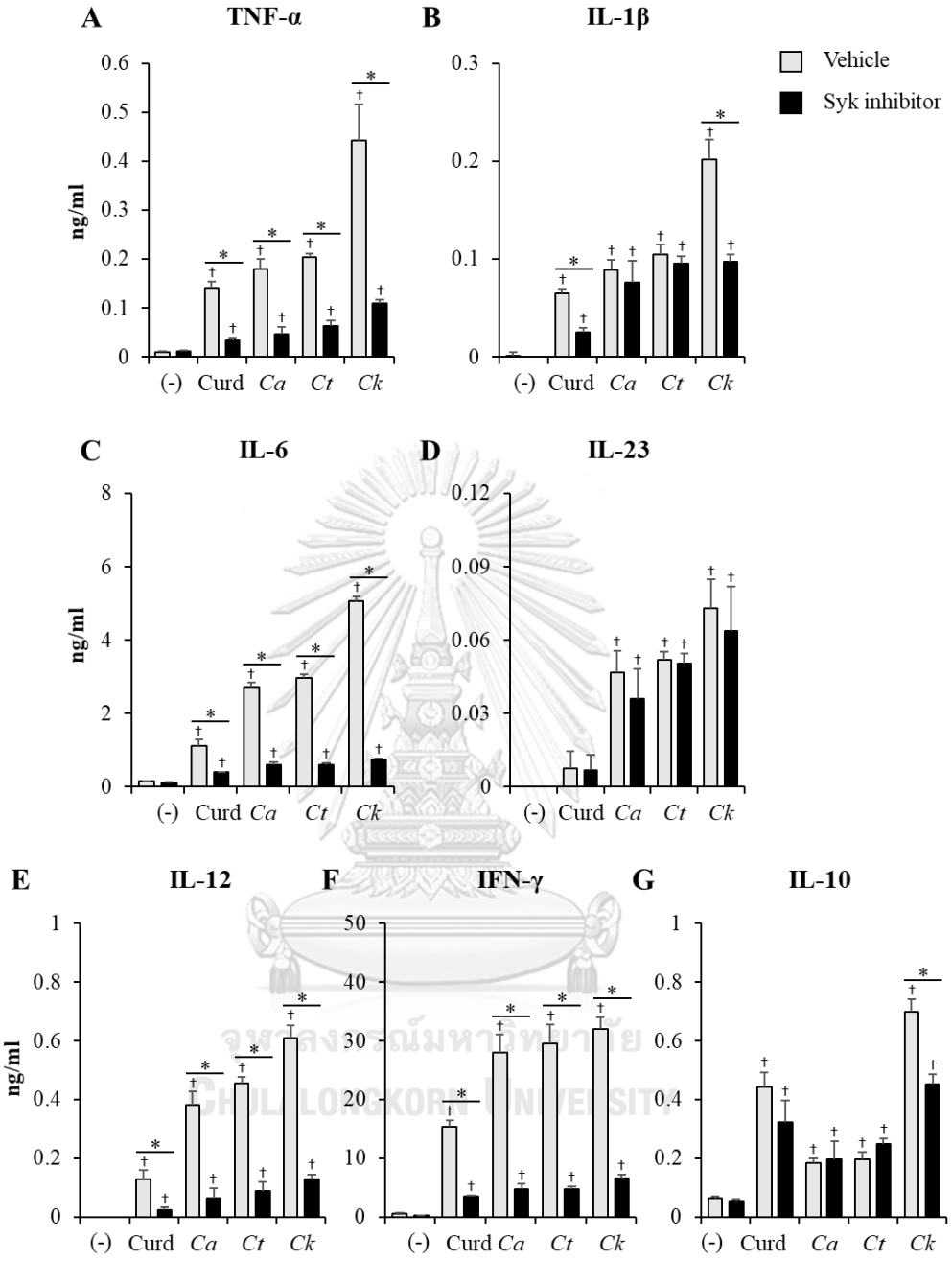


Figure 4.24 Effects of Syk inhibitor on DC cytokine production at 24 h

BMDCs were pre-treated with 1 μM of Syk inhibitor for 30, and then the cells were stimulated with 25 μg/ml of curdlan and β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 48 h. Levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were measured in the culture supernatants by ELISA. *n* = 5; Data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, *$p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

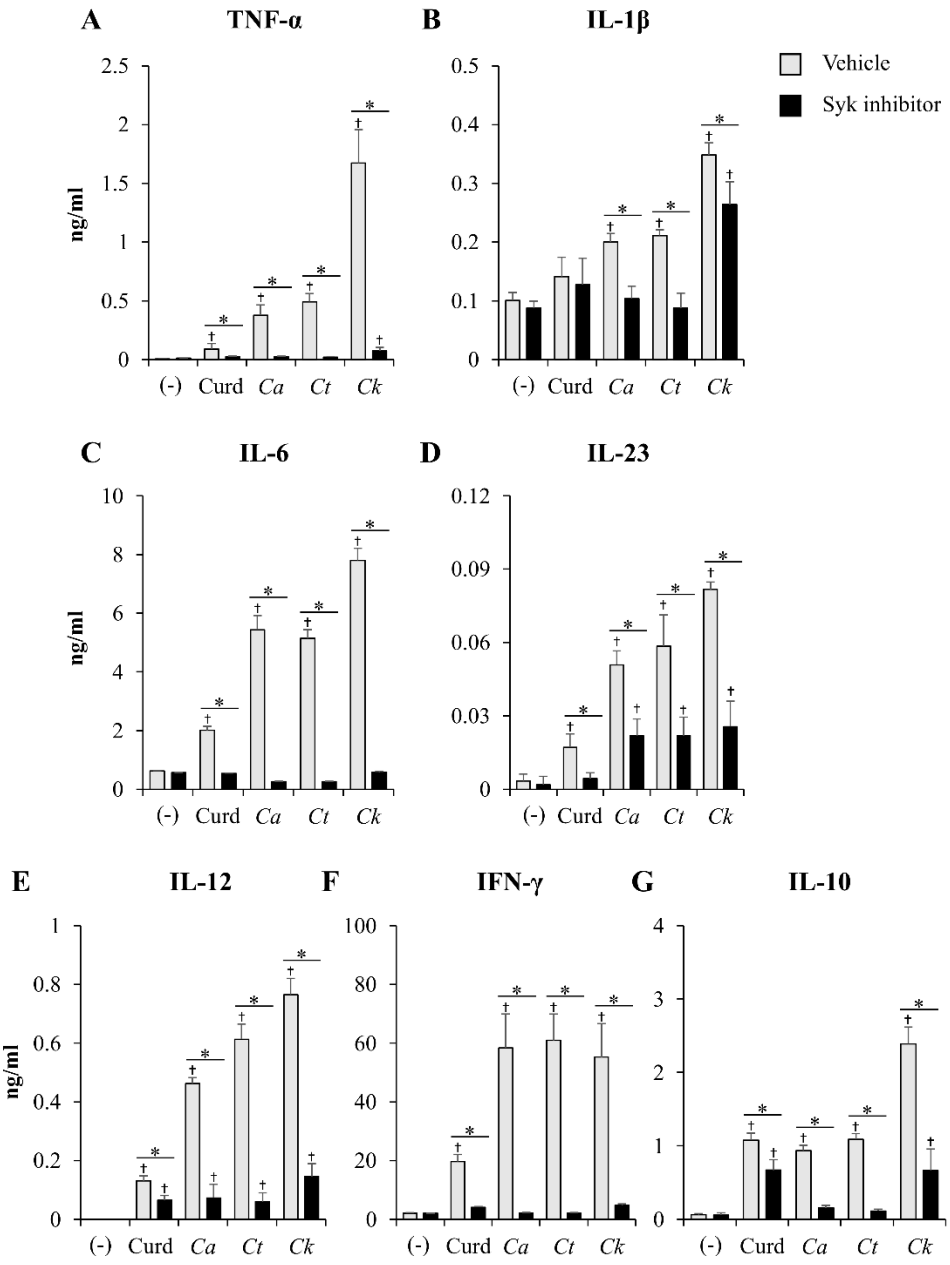


Figure 4.25 Effects of Syk inhibition on DC cytokine production at 48 h

BMDCs were pre-treated with 1 μM of Syk inhibitor for 30 min, and then the cells were stimulated with 25 μg/mL of curdlan and β-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 48 h. Levels of **(A)** TNF-α, **(B)** IL-1β, **(C)** IL-6, **(D)** IL-23, **(E)** IL-12, **(F)** IFN-γ, and **(G)** IL-10 were measured in culture supernatants by ELISA. *n* = 5; data are representative of two independent experiments. † $p<0.05$ compared with unstimulated BMDCs, * $p<0.05$. (-), unstimulated BMDCs; Curd, curdlan; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Different expression of surficial dectin-1 receptor on *Candida* ß-glucan-stimulated BMDCs

Having found that *Candida* ß-glucans and dectin-1 interactions mainly regulated Syk-mediated cytokine production. Next, to understand more whether the altered DC responses associates with expression of dectin-1 receptor on their surface under stimulations of mentioned *Candida* ß-glucans, this study observed the expression of dectin-1 receptor under stimulation of those ß-glucans by flow cytometry method. Using anti-CLEC7A monoclonal antibody tagged fluorescence detects the expression of this receptor on $CD11c^{+}$ BMDC surface during the interaction with ß-glucans at 24 h and 48 h (Figure 4.26A). As predicted, the results showed there were different expression levels of dectin-1 receptor on DC surface among stimulations of *Candida* ß-glucans (Figure 4.26B). In details, *C. albicans* ß-glucan-stimulated BMDCs expressed dectin-1 receptor significantly lower than glucan-unstimulated cells and this phenomenon was observed apparently at 48 h. Meanwhile, *C. tropicalis* ß-glucan-stimulated BMDCs did no change surface expression level of dectin-1 receptor at 24 h and rather downregulated this receptor at 48 h. Intriguingly, *C. krusei* ß-glucan elicited BMDCs express the highest level of dectin-1 receptor as early as 24 h post-stimulation compared with other *Candida* ß-glucan and then significantly decreased the expression level of this receptor at 48 h compared with unstimulated cells (Figure 4.26A and 4.26B). Hence, these data indicated that different expression of dectin-1 receptor on BMDC surface possibly related to distinct impacts of *Candida* ß-glucan through dectin-1/Syk signaling pathway axis.

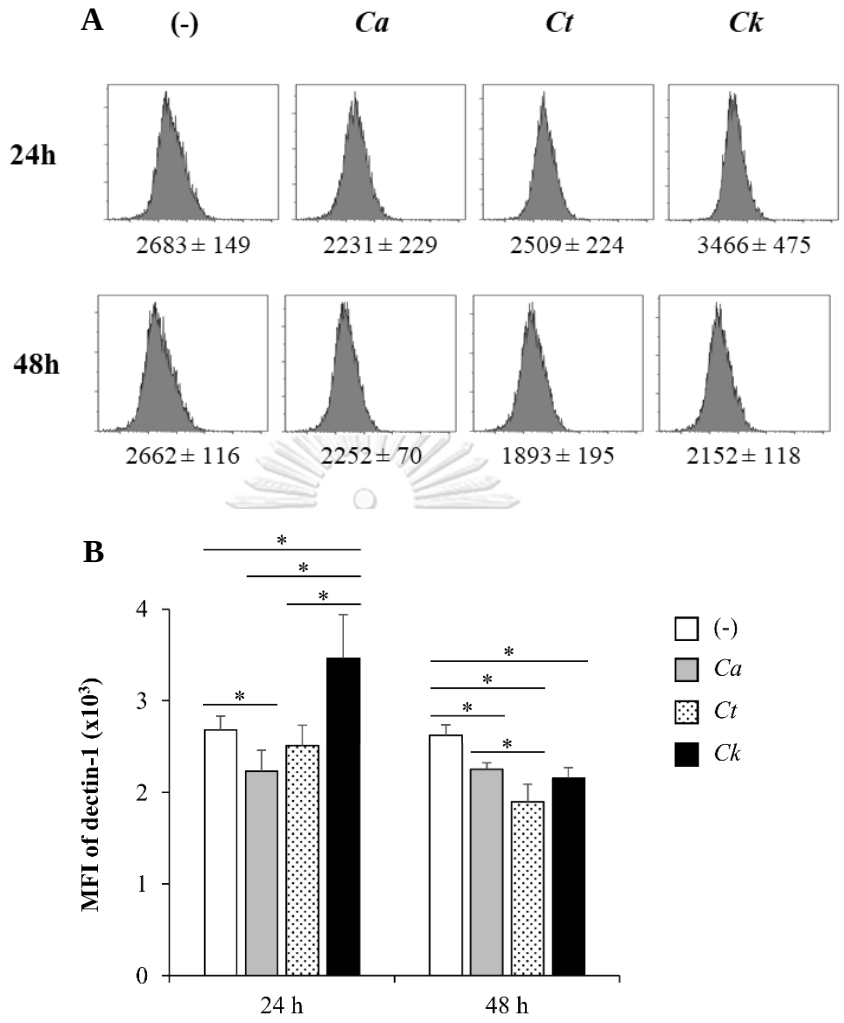


Figure 4.26 Differential dectin-1 expression on *Candida* ß-glucan-stimulated BMDCs

BMDCs were stimulated with 25 µg/mL of ß-glucans isolated from *C. albicans*, *C. tropicalis*, and *C. krusei* for 24 h and 48 h. The expression of dectin-1 was determined by a flow cytometric analysis. DCs were first identified by gating on CD11c, and (**A**) the expression of dectin-1 in $CD11c^+$ population was subsequently assessed by histogram analyses with the values of MFI (mean ± S.D.), (**B**) the geometric mean fluorescence intensity (MFI) of dectin-1 on $CD11c^+$ cells were determined among *Candida* ß-glucan. n = 5; data are representative of two independent experiments. * p<0.05. (-), unstimulated BMDCs; *Ca*, *C. albicans*; *Ct*, *C. tropicalis*; *Ck*, *C. krusei*.

Discussion

With some similarities in virulence factors to *C. albicans*, *C. tropicalis* and *C. krusei* were selected as representatives of NACs aiming to compare and find out different interactions with host immune cells possibly related to the cell wall components. *C. tropicalis* has similar pathogenicity to *C. albicans*, while the pathogenicity of *C. krusei* is lower than those two species (Papon et al., 2013; Whibley & Gaffen, 2015; Ortega-Riveros et al., 2017; Gómez-Gaviria & Mora-Montes, 2020). However, an intracellular survival phenomena of *C. krusei* and its escape from the killing of macrophages possibly suggested a complex interaction of the *Candida* cell wall components and host immune cells could initially mediate that outcome of interaction as well as of an infection afterwards (García-Rodas et al., 2011). Besides, *C. krusei* and *C. tropicalis* can cause candidiasis with severe invasive infections in immunocompromised patients (Lamoth et al., 2018; Gómez-Gaviria & Mora-Montes, 2020). ß-glucan is well-known as a major carbohydrate structure on yeast *Candida* cell wall (Gow & Hube, 2012; Free, 2013) and could be hidden under mannan layer of yeast cell wall (Bain et al., 2014; Davis et al., 2014; Yadav et al., 2020). Cell wall ß-glucan is probably responsible for the induction of antifungal immune responses in early stage of *Candida* yeast infection (Gow & Hube, 2012; Mihai G. Netea, 2015). Possibly, the different impacts of cell wall ß-glucans may link to the different immunopathogenesis mechanisms in those *Candida* yeasts-infected patients. Herein, this study first characterized DC properties including maturation and cytokine production altered differently in response to ß-glucans of *C. tropicalis* and *C. krusei* versus *C. albicans*. The findings showed that *C. krusei* ß-glucan remarkably influenced DC activation, whereas *C. tropicalis* and *C. albicans* ß-glucan impacted on almost DC responses in a similar way which was lower than those effects of the former. In addition, the T cell responses were resulted from these distinct *Candida* ß-glucan-triggered DC alterations. Moreover, it found that *C. krusei* could survive and

replication inside innate immune cells with unclear mechanisms (García-Rodas et al., 2011). There is little known about pathogenesis strategies of these species (Gómez-Gaviria & Mora-Montes, 2020).

The different effects of ß-glucans on DCs were supposed to be directly related to the variation of ß-glucan structures among *Candida* species through some evidences. *C. albicans* ß-glucan structurally differs between yeast and hyphae form. This difference leaded to induce distinct pro-inflammatory and anti-inflammatory cytokine levels in human monocyte-derived macrophages (Lowman et al., 2014). Despite of being same *C. tropicalis* species, the different ß-glucan contents of distinct strains of this species elicited a variation of immune responses from peripheral blood mononuclear cells (Mesa-Arango et al., 2016). Since the different levels of ß-(1,3)-glucan and ß-(1,6)-glucan were detected in culture supernatants of *Candida* spp. by using ß-glucan-specific antibodies, this implied the inter-species diversity of ß-glucan contents and structures on yeast cell wall (Matveev et al., 2019; Yamanaka et al., 2020). In addition, the NMR analysis was in favor of above assumption. ß-glucans of *C. albicans and C. tropicalis* composed of ß-(1,3)-glucan with ß-(1,6)-branching. These results of *C. albicans* ß-glucan structures were concordant with other studies (Lowman et al., 2003; Sukumaran et al., 2010; Lowman et al., 2014). Meanwhile, ß-glucan structure of *C. krusei* mainly contained ß-(1,3)-glucan which was distinct from other *Candida* ß-glucans. Moreover, one parameter that may also contribute to the variation in immune responses is the particle size of ß-glucans. Particulate ß-glucans with larger size had stronger effects in the cytokine responses of human monocyte-derived DCs via regulation of dectin-1 expression (Elder et al., 2017). In our study, the results of SEM described that morphology of *C. albicans* and *C. tropicalis* ß-glucan particles were quite similar in shape and size, whereas the morphology and size of *C. krusei* ß-glucan particles were larger and formed different shape (Figure 4.5). The resemblance in morphology and ß-glucan structure of *C. albicans* and *C. tropicalis*

may explain for the similarity in DC immune responses observed throughout this study.

In addition, it has presumed that difference of glucan exposure on yeast surface also associated to the different responses of innate immune cells. Since ß-glucan is exposed more on yeast surface after heating treatment (Benjamin N Gantner, 2005; Gow et al., 2007), thus this simple method was used to observe the interaction of immune cells and cell wall ß-glucan in some studies. BMDCs secreted higher level of pro-inflammatory cytokines and anti-inflammatory cytokines under incubation with heat-killed *C. albicans* than heat-killed *C. tropicalis* (Thompson et al., 2019). This is not consistent with our results, expression of maturation markers and amount of DC cytokine TNF-α, IL-6, IL-1ß, IFN-γ and IL-10 were notably homogenous in ß-glucans from these two species, meanwhile, amount of IL-12 and IL-23 were little higher in *C. tropicalis* ß-glucan (Figure 4.10 - 4.11, Figure 4.13 - 4.14). Another study found that heat-killed *C. krusei* yeast triggered human monocytes to secrete amount of TNF-α, IL-6 and IL-1ß larger than heat-killed *C. albicans* and *C. tropicalis* yeast (Navarro-Arias et al., 2019). This is in line with our results of the strongest effects of *C. krusei* ß-glucan observed on DC maturation and cytokine induction (Figure 4.10 - 4.11 and Figure 4.13 - 4.14). Nevertheless, the different level of ß-glucan exposure on yeast surface may not explain for all differences among these studies. A surface exposure of ß-glucan in *C. krusei* was higher than this in *C. albicans* yeast, that was considered to explain for the stronger effects of heat-killed *C. krusei* yeast (S. M. Chen et al., 2019; Navarro-Arias et al., 2019). Meanwhile, although *C. tropicalis* yeast exposed ß-glucan on yeast surface at equal or higher degree than *C. albicans* yeast, *C. tropicalis* yeast affected DC responses lower than *C. albicans* yeast did (Navarro-Arias et al., 2019; Thompson et al., 2019). Collectively, the structural alterations among ß-glucans of three *Candida* species may be of the root factors to

explain for different immune responses among these studies. Presumably, structural diversity and variation in size of *Candida* ß-glucans affected their ability to induce DC maturation and cytokine production.

C. krusei ß-glucan induced distinct nuance of DC maturation and cytokine function, of which, this ß-glucan downmodulated the percentage and expression of $CD11c^{+}$ BMDCs lower than other *Candida* ß-glucans but increased the maturation and massive cytokine release (Figure 4.9). However, DC viability was not different among *Candida* ß-glucan (Figure 4.8), so CD11c down-regulation seems to be independent of DC viability (Griffiths et al., 2014). This suggest that CD11c was not only a signature of BMDCs, but also might be a typical marker for DC activation in response to certain dangerous stimuli and inflammatory setting. In favor of this inference, CD11c level was down-regulated in DCs stimulated by lipopolysaccharide which has known a strong activator of TLR4 receptor, but DCs remained highly increased pro-inflammatory cytokine and maturation (Griffiths et al., 2014). Consistently, other studies found this phenomenon of murine DCs under stimulation of TLR 3/4/9 agonists, *Mycoplasma* (Chen & Chang, 2005; Singh-Jasuja et al., 2013). Moreover, CD11c down regulation may be a predictor of phenotypical and functional alteration of DCs, because $CD11c^{low}$ DCs possibly exhibited an immature-like phenotype that could govern regulatory type 1 (Tr1) T cell differentiation (Wakkach et al., 2003).

DCs are the most potential antigen-presenting cells since they can determine the direction of effector T cell differentiation through surface molecules and soluble substances as co-stimulatory markers and secreted cytokines (Zhou et al., 2009; Leung et al., 2010; Eisenbarth, 2019; Saravia et al., 2019). The proliferation of T lymphocytes were increased in DC:T cell co-culture assay (Figure 4.15 - 4.16) and *in vivo* immunization of *Candida* ß-glucan (Figure 4.17) along with T cells-secreted functional cytokines. These results inferred that *Candida* ß-glucans altered DC

properties leading to govern T cell proliferations distinctly depending on *Candida* species. Especially, T helper cell differentiation consistently interrelated to cytokine signals from BMDCs stimulated by ß-glucans. Curdlan-stimulated BMDCs governed the lowest T cell responses (Figure 4.16C and D), which was in line with the low cytokine responses of BMDCs (Figure 4.21 – 4.22). Meanwhile, *Candida* ß-glucan activated stronger cytokine responses of BMDCs (Figure 4.21 – 4.22) and stronger T cells responses compatibly (Figure 4.16C and 4.16D). As known, cytokines of BMDCs could suggested the direction of T cell responses. Of which, IFN-γ and IL-12 link to Th1, IL-6, IL-1ß and IL-23 are required for Th17, and IL-10 is for Treg differentiation (Zhou et al., 2009; Leung et al., 2010; Eisenbarth, 2019; Saravia et al., 2019). In consequences, cytokine responses of *Candida* ß-glucan stimulated BMDCs promoted the Th1, Th17 and Treg differentiation, which produced IFN-γ, IL17 and IL-10 respectively (Figure 4.15B-D and Figure 4.17A-C). Interestingly, the high IL-10 level of *C. krusei* ß-glucan-BMDCs (Figure 4.13G and Figure 4.14G) consistently promoted the high IL-10 production of T cell response, not only $FoxP3^+$ Treg cells (Figure 4.15D and Figure 4.17C). Several evidences indicated that IL-10 secretion of DCs plays an important role in promoting IL-10-secreting regulatory T cells, that are in line with our results (Wakkach et al., 2003; Hsu et al., 2015; Brockmann et al., 2017; Comi et al., 2018). In our study, there was no difference of increased $CD4^+FoxP3^+$Treg cells among *Candida* ß-glucans-exposed DCs (Figure 4.16E-F and Figure 4.17D). However, it has suggested that the high IL-10 production of T cells could be resulted from functional activation of both $CD4^+FoxP3^+$Treg cells and $FoxP3^-$ IL-10-secreting Tr1 cells (Schmidt et al., 2012). Meanwhile, other $CD4^+$ helper T cells have been recorded that they are also capable of secreting IL-10 as a self-protective mechanism to avoid tissue damages in response to pathogens (Jankovic et al., 2010). Th1, Th17 and Th2 can produce IL-10 in private signaling pathways that may be independent of IL-10

signaling from DCs (Ng et al., 2013). Since functional cytokines (IFN-γ, IL-17) from these T cells were not different in response to *Candida* ß-glucans (Figure 4.15B-C and Figure 4.17A-B), the level of IL-10 from those effector T cells probably exhibited same background amount in responses to *Candida* ß-glucans. Moreover, upregulated costimulatory molecules of BMDCs such as CD80, CD86 tightly associated with the induction of IL-10-secreting T cells (Pletinckx et al., 2011). Consistently, *C. krusei* ß-glucan-stimulated BMDCs expressed higher level of CD86 molecules compared with other *Candida* ß-glucan stimulations (Figure 4.10C and Figure 4.11C). These evidences support that the high IL-10 secretion and costimulatory molecules expression of *C. krusei* ß-glucan-activated BMDCs possibly mediated a functional enhancement of $CD4^{+}FoxP3^{+}$T cells and IL-10-producing T cells and IL-10 production of these T cells in our results. On the other hand, IL-10 is able to downmodulate the proliferation of effector T cells (S. Wang et al., 2016; Neumann et al., 2019). The high IL-10 secretion of T cells in response to *C. krusei* ß-glucan could prevent the proliferation of Th1 and Th17 cells, that revealed similar effects of IFN-γ and IL-17 production of these T cells (Figure 4.15B-C and Figure 4.17A-B) in despite of a strong secretion of Th1- and Th17-differentiating cytokines from *C. krusei* ß-glucan-stimulated BMDCs (Figure 4.13 – 4.14).

Since a relative consistence of the results from DC:T cell co-culture assay (Figure 4.15-4.16), in vivo immunization of *C. krusei* ß-glucan (Figure 4.17) and in vivo systemic *C. krusei* infection (Figure 4.18) was observed that notably high amount of IL-10 was produced by T cell responses. These data suggested that ß-glucan of *C. krusei* may mediate its effects on IL-10 production of host adaptive immunity in murine systemic *C. krusei* infection. Some evidences also supported this notion when surface ß-glucan exposure plays a crucial PAMP factor interacting with host immunity in fungal infection (Taylor et al., 2007; Gow & Hube, 2012; Mihai G. Netea, 2015). For

C. krusei yeast, ß-glucan layer accounted a large amount of cell wall mass (Navarro-Arias et al., 2019) and highly exposed on the outer surface of *C. krusei* cell wall (S. M. Chen et al., 2019). It reported that there was the presence of ß-glucan in serum of *Candida* infected mice (Yamanaka et al., 2020). In addition, the circulation of shed ß-glucan in serum of candidiasis patients negatively correlated with severity of infection in some studies (Sims et al., 2012; Giacobbe et al., 2015), that suggested the important impacts of ß-glucan on host immune responses. Besides, our study also observed that IL-17 production of Th17 cells was increased in dose-dependent manner of *C. krusei* infection (Figure 4.18A and 4.18B, middle panels). This is consistent with other studies that Th17 response was essentially promoted in *Candida* infections (LeibundGut-Landmann et al., 2007; Conti & Gaffen, 2015). Mannan of *C. krusei* possibly contributed to the high induction of Th17 response through triggering a massive response of Th17-priming cytokines (IL-6 and IL-23) from DCs (Thu Ngoc Yen Nguyen, 2018). Moreover, our study found that *C. krusei* yeasts did not enhance the $CD4^+FoxP3^+$ Treg cells, although there was a high IL-10 production in T cell responses (Figure 4.18C and 4.18D, right panels). A high number of $CD4^+FoxP3^+$ Treg cells was in the control uninfected group may be due to the dexamethasone impacts on increased $CD4^+FoxP3^+$ Treg cell expansion (Engler et al., 2017). Other study also found that dexamethasone inhibited the dectin-1 activation of ß-glucan-stimulated DCs and enhanced anti-inflammatory cytokine (IL-10) production of these DCs (Willment et al., 2003; Kotthoff et al., 2017). However, a reduced number of $CD4^+FoxP3^+$Treg cells and an increased number of $CD4^+CD25^+FoxP3^-$ Treg cells were significantly changed among *C. krusei* infection mice compared with control uninfected mice, despite of same dexamethasone-pretreated condition (Figure 4.18C and 4.18D, middle panels). This also implied that ß-glucan of *C. krusei* probably had certain private mechanism affecting on immune cells in *C.*

krusei infection aside from interacting with dectin-1 receptor. Therefore, it is possibly conceivable that high IL-10 production was mediated in *C. krusei*-specific response.

As well-known, dectin-1 receptor plays a crucial role from recognizing ß-glucan to elicit the host antifungal immune responses via dectin-1/Syk axis pathway to eradicate *Candida* infection (Taylor et al., 2007; Whitney et al., 2014). To decipher how the role of dectin-1 receptor and its signaling pathway determine the different impacts of distinct *Candida* ß-glucans in our study, BMDCs were respectively pre-treated by dectin-1 antagonist (Figure 4.19 - 4.22) or inhibitor of Syk adaptor (Figure 4.23 - 4.25) prior to stimulation of *Candida* ß-glucans. A few recent studies found that various *Candida* species had different impacts on innate immune cells due to different dependence on dectin-1 pathway(Navarro-Arias et al., 2019; Thompson et al., 2019), and dectin-1-knockout mice exhibited various levels of infectious susceptibility to distinct *Candida* spp. (S. M. Chen et al., 2019; Thompson et al., 2019). In consistent with these studies, the different responses of dectin-1-blocked BMDCs were occurred in relevant to distinct *Candida* ß-glucans (Figure 4.20 - 4.22). *C. krusei* ß-glucan continued to affect the maturation and cytokine release of dectin-1-blocked BMDCs, which were still the strongest influence compared with other *Candida* ß-glucans. Meanwhile, the similar effects of ß-glucans between *C. albicans* and *C. tropicalis* were displayed in dectin-1-blocked BMDCs (Figure 4.20 - 4.22). Once again, different properties of BMDCs in response to distinct *Candida* ß-glucan were highlighted through the different outcomes of Syk-inhibited BMDCs stimulated by these ß-glucans (Figure 4.23 - 4.25). These phenomena are possibly reasoned by the different interaction between dectin-1 receptor and ß-glucans of distinct *Candida* spp. It has investigated that dectin-1 dependent immune responses differed among *Candida* spp. in association with the exposure of ß-(1,3)-glucan on yeast cell wall surface (Navarro-Arias et al., 2019; Thompson et al., 2019). In addition, the different surficial expression of dectin-1 receptor on innate cells may involve in changes of

immune responses to various ß-glucans (Walachowski et al., 2016). Thereby, the expression of dectin-1 receptor on DCs possibly contributes a first requisite of the different DC reactions to distinct ß-glucans. Our data was in favor of this assumption because of the different expression of BMDC dectin-1 receptor following the time of *Candida* ß-glucan stimulations (Figure 4.26). Of which, ß-glucan of *C. krusei* triggered the highest expression of dectin-1 receptor on BMDCs at early time point, which may involve with the strongest effects of this ß-glucan particles. Moreover, the binding affinity of ß-glucan to dectin-1 receptor that is possible the second requisite of the different DC reactions to distinct ß-glucans, depends on the structure of polymer chain length and side-chain branching in ß-glucan structure (Adams et al., 2008). The bioactivity and receptor binding efficiency are also affected by the structural conformation of ß-glucan (Sletmoen & Stokke, 2008). Dectin-1 receptor revealed a high affinity to ß-(1,3)-glucan polymer plus branching ß-(1,6)-side chain, while it showed a low affinity to linear ß-(1,3)-glucan polymer (Adams et al., 2008). Comparing the NMR results of *Candida* ß-glucan, *C. albicans* and *C. tropicalis* ß-glucans had obviously high binding affinity to dectin-1 receptor in opposite of *C. krusei* ß-glucan. Furthermore, the different biological responses induced by the interaction of dectin-1 and *Candida* ß-glucan could be affected by the additional, synergistic or suppressive reciprocal of dectin-1 and other PRRs such as TLR2, TLR4 (Ferwerda et al., 2008; Kanjan et al., 2017; Ostrop & Lang, 2017), galectin-3, SIGN-R1 (Esteban et al., 2011; Takahara et al., 2011).

The difference of DC responses to distinct *Candida* ß-glucans could be mediated by other receptors of ß-glucan recognition. a co-existence and co-operation of many other receptors may be occurred during dectin-1 binding to this glucan (Heinsbroek et al., 2008). This possibility was not excluded in this study. CR3 is known as the first receptor of ß-glucan recognition (Ross & V̌etvicka, 1993), and it is expressed on many innate immune cells and involves in the innate and adaptive

immune responses (Ehirchiou et al., 2007; Goyal et al., 2018). A recent study found that CR3 activation was also an essential mechanism to eliminate *Candida* infection (Li et al., 2019). In addition, CR3 can be activated depending on the structure and composition of ß-glucan (Legentil et al., 2015). In part, CR3 showed higher affinity to ß-(1, 6)-glucan than ß-(1,3)-glucan (Rubin-Bejerano et al., 2007; Tang et al., 2018). Moreover, although other receptors such as lactosylceramide receptor or scavenger receptors expressed on innate immune cells can recognize ß-glucan (Zimmerman et al., 1998; Sato et al., 2006; Józefowski et al., 2012). A novel C-type lectin receptor, CD23 has been described that it involves in ß-glucan recognition on myeloid cells and contributes to *Candida* clearance (Zhao et al., 2017; Salazar & Brown, 2018).

Furthermore, in our study, an immunoregulatory response was possibly elicited via the high level of IL-10 production in *C. krusei* infection model, aside from high IL-17 production of Th17 response induction. Our results probably differ from the results of a recent study which described that dectin-1 receptor was crucially important for Th1 and Th17 responses of host protective immunity against *C. krusei* infection (S. M. Chen et al., 2019). The reduction of pro-inflammatory cytokines was occurred in host myeloid cells of dectin-1 knock-out mice. Besides, dectin-1 knock-out mice exhibited a high burden of *C. krusei* in internal organs, and increased susceptibility of *C. krusei* infection along with reduced levels of IFN-γ and IL-17 in Th1 and Th17 responses (S. M. Chen et al., 2019). The different strain of *C. krusei* were used in our study that could explain for this discrepancy. Another study, the genetic diversity of two distinct strain clusters was found among *C. krusei* (Gong et al., 2018). In addition, the structural diversity of cell wall ß-glucans could be noticed among distinct strains of one *Candida* species in a few studies. Different strains of *C. albicans* exhibited the structural variation of ß-glucan content in ^{13}C-NMR analysis (Miura et al., 2003). Cell wall ß-glucan changed in different strains of *C. tropicalis* leading to distinct immune responses of human PBMCs (Mesa-Arango et al., 2016). In

our study, only one strain of each *Candida* spp. was used to investigate the effects of cell wall ß-glucan. The observed distinct effects reflected the diversity of ß-glucan structure in inter-species fashion. Therefore, it may need to consider and further analyze the intra-species diversity of ß-glucan structure.

In summary, our study found that immunological nuances of DCs varied depending on distinct type of *Candida* ß-glucans which may function disparately on induction of adaptive immune responses in *Candida* infection. It also implied that cell wall ß-glucan may involve in the *Candida* virulence and pathogenesis mechanisms in species-dependent manner. Probably, for *C. krusei* pathogenicity, this species contains less virulent factors, but cell wall expose of ß-glucan may give a benefit from downregulating immune responses contributing to *C. krusei* avoid the elimination of the host immune cells. However, this study still has some limitations including incomplete characterizing of Treg cell population in IL-10-producing T cells, the signaling pathways of ß-glucan effects on DCs, and the interplay of these cells in NACs infections. Hence, our study lay the groundwork for further exploring the interaction of host immunity and non-*albicans Candida* infections in candidiasis patients.

CHAPTER V

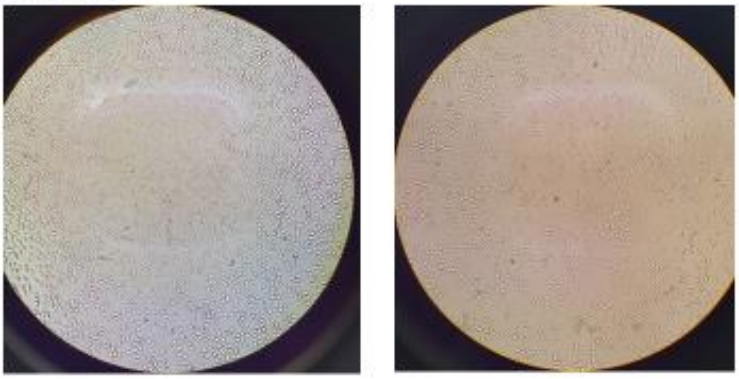
CONCLUSION AND PERSPECTIVE

Dendritic cells are known to be the most magnitude innate cells of antifungal immunity. DCs are capable of processing pathogens as far as shaping specific adaptive immune responses to eliminate fungal invasion. The presence of ß-glucan is one of core factors on yeast cell wall, since it determines the changes of host immunity triggered by ß-glucan recognition of the main surficial receptor, dectin-1, on innate cells. In this work, DC properties altered by ß-glucan effects at various degrees in *Candida* species-dependent manner. It is possible that the different structural and morphology of three presentative *Candida* ß-glucan, including *C. albicans*, *C. tropicalis* and *C. krusei*, varied DC immune responses through changes of dectin-1-ß-glucan interaction. In addition, the different dependence on dectin-1/Syk axis signaling pathway were displayed among these *Candida* ß-glucan. Interestingly, there was not only the induction of host protective Th1 and Th17 cell responses, but the regulatory T cell response were also promoted in relevant to those distinct *Candida* ß-glucan-stimulated DC functions. Hence, different ß-glucan component on yeast cell wall possibly plays a root factor of immunological mechanisms and distinct pathogenesis among infections of *Candida albicans* and non-*albicans* spp. These findings of this work initially illustrated a better understanding of an interrelationship between NACs and host immunity. The cell wall components may involve in the outcome of host immunity against NACs infection through interaction with antigen-presenting cells such as DCs. It is necessary to deeply explore the interaction mechanisms of DCs and NACs-derived ß-glucan as well as the role of immunomodulation effects of ß-glucan in NACs infections. Moreover, this is a promise of opening new approaches in therapeutic aspects for candidiasis patients.

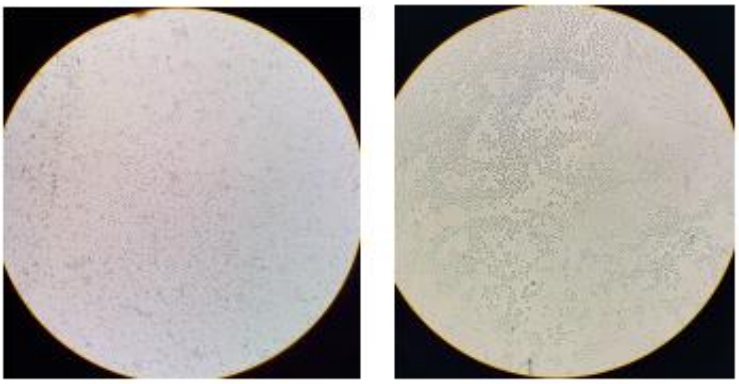
APPENDIX

Yeast form of *Candida* spp.

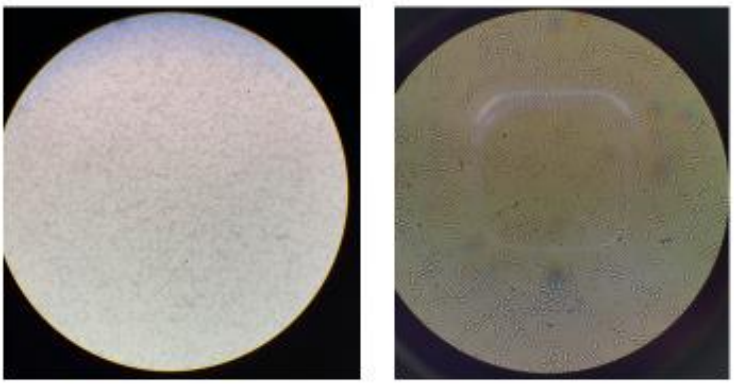
C. albicans SC5314



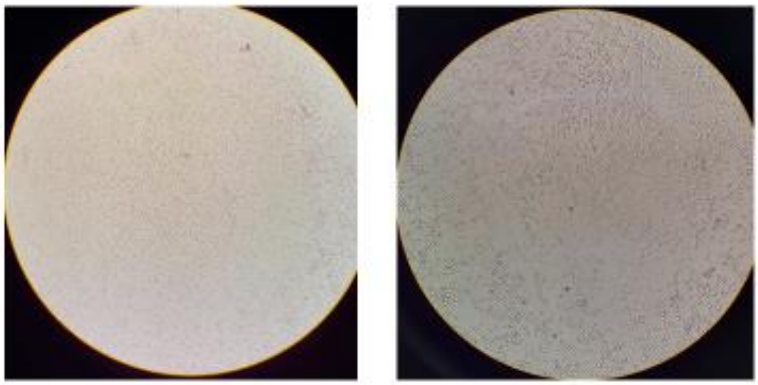
C. tropicalis ATCC750



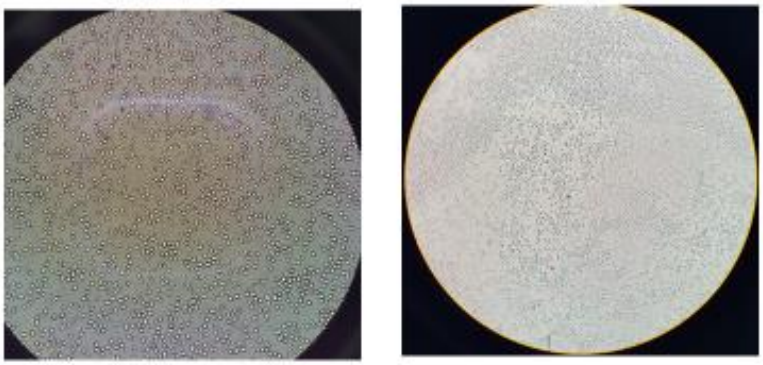
C. krusei ATCC6528



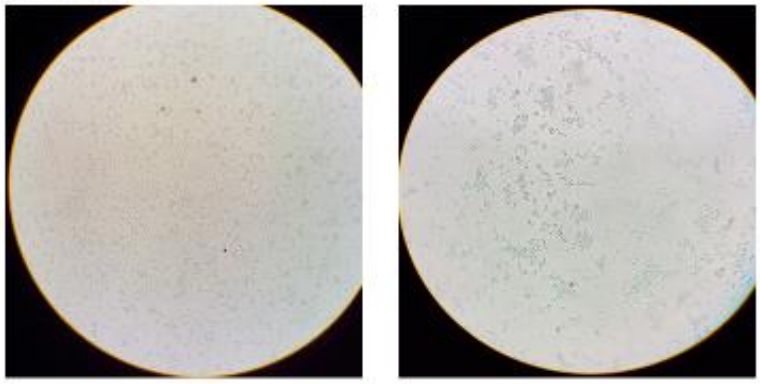
C. parapsilosis ATCC90018



C. dubliniensis NCPF 3490



C. glabrata ATCC2001



REFERENCES

Adams, E. L., Rice, P. J., Graves, B., Ensley, H. E., Yu, H., Brown, G. D., Gordon, S., Monteiro, M. A., Papp-Szabo, E., Lowman, D. W., Power, T. D., Wempe, M. F., & Williams, D. L. (2008). Differential high-affinity interaction of Dectin-1 with natural or synthetic glucans is dependent upon primary structure and is influenced by polymer chain length and side-chain branching [Article]. *Journal of Pharmacology and Experimental Therapeutics, 325*(1), 115-123. https://doi.org/10.1124/jpet.107.133124

Alvarez, D., Vollmann, E. H., & von Andrian, U. H. (2008). Mechanisms and consequences of dendritic cell migration. *Immunity, 29*(3), 325-342.

Anderson, A. E., Sayers, B. L., Haniffa, M. A., Swan, D. J., Diboll, J., Wang, X.-N., Isaacs, J. D., & Hilkens, C. M. U. (2008). Differential regulation of naïve and memory CD4+ T cells by alternatively activated dendritic cells. *Journal of Leukocyte Biology, 84*(1), 124-133. https://doi.org/https://doi.org/10.1189/jlb.1107744

Arana, D. M., Prieto, D., Roman, E., Nombela, C., Alonso-Monge, R., & Pla, J. (2009, May). The role of the cell wall in fungal pathogenesis. *Microb Biotechnol, 2*(3), 308-320. https://doi.org/10.1111/j.1751-7915.2008.00070.x

Arendrup, M., Horn, T., & Frimodt-Moller, N. (2002, Oct). In vivo pathogenicity of eight medically relevant Candida species in an animal model. *Infection, 30*(5), 286-291. https://doi.org/10.1007/s15010-002-2131-0

Azim, A., Ahmed, A., Baronia, A. K., Marak, R. S., & Muzzafar, N. (2017). Intra-abdominal candidiasis. *NEPHROLOGY*.

Bain, J. M., Louw, J., Lewis, L. E., Okai, B., Walls, C. A., Ballou, E. R., Walker, L. A., Reid, D., Munro, C. A., & Brown, A. J. (2014). Candida albicans hypha formation and mannan masking of β-glucan inhibit macrophage phagosome maturation. *MBio, 5*(6).

Banerjee, M., Lazzell, A. L., Romo, J. A., Lopez-Ribot, J. L., & Kadosh, D. (2019). Filamentation Is Associated with Reduced Pathogenicity of Multiple Non-albicans Candida Species. *mSphere, 4*(5), e00656-00619. https://doi.org/10.1128/mSphere.00656-19

Bashir, K. M. I., & Choi, J. S. (2017, Sep 5). Clinical and Physiological Perspectives of beta-Glucans: The Past, Present, and Future. *International journal of molecular sciences, 18*(9), 1906. https://doi.org/10.3390/ijms18091906

Batbayar, S., Lee, D. H., & Kim, H. W. (2012). Immunomodulation of fungal β-glucan in host defense signaling by dectin-1. *Biomolecules & therapeutics, 20*(5), 433.

Bazan, S. B., Walch-Rückheim, B., Schmitt, M. J., & Breinig, F. (2018, Feb). Maturation and cytokine pattern of human dendritic cells in response to different yeasts. *Med Microbiol Immunol, 207*(1), 75-81. https://doi.org/10.1007/s00430-017-0528-8

Behrens, E. M., Sriram, U., Shivers, D. K., Gallucci, M., Ma, Z., Finkel, T. H., & Gallucci, S. (2007). Complement Receptor 3 Ligation of Dendritic Cells Suppresses Their Stimulatory Capacity. *The Journal of Immunology, 178*(10), 6268. https://doi.org/10.4049/jimmunol.178.10.6268

Benjamin N Gantner, R. M. S. a. D. M. U. (2005). Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments. *The EMBO Journal 24*, 1277–1286.

Bouklas, T., Alonso-Crisóstomo, L., Székely, T., Jr., Diago-Navarro, E., Orner, E. P., Smith, K., Munshi, M. A., Del Poeta, M., Balázsi, G., & Fries, B. C. (2017). Generational distribution of a *Candida glabrata* population: Resilient old cells prevail, while younger cells dominate in the vulnerable host. *PLOS Pathogens, 13*(5), e1006355-e1006355. https://doi.org/10.1371/journal.ppat.1006355

Brockmann, L., Gagliani, N., Steglich, B., Giannou, A. D., Kempski, J., Pelczar, P., Geffken, M., Mfarrej, B., Huber, F., & Herkel, J. (2017). IL-10 receptor signaling is essential for TR1 cell function in vivo. *The Journal of Immunology, 198*(3), 1130-1141.

Brown, G. D., & Gordon, S. (2001). Immune recognition: a new receptor for ß-glucans. *Nature, 413*(6851), 36.

Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L., Wong, S. Y., & Gordon, S. (2002). Dectin-1 is a major β-glucan receptor on macrophages. *The Journal of experimental medicine, 196*(3), 407-412.

Brunke, S., & Hube, B. (2013). Two unlike cousins: C andida albicans and C. glabrata infection strategies. *Cellular Microbiology, 15*(5), 701-708.

Brunke, S., & Hube, B. (2013, May). Two unlike cousins: Candida albicans and C. glabrata infection strategies. *Cellular Microbiology, 15*(5), 701-708. https://doi.org/10.1111/cmi.12091

Cambi, A., Netea, M. G., Mora-Montes, H. M., Gow, N. A., Hato, S. V., Lowman, D. W., Kullberg, B. J., Torensma, R., Williams, D. L., & Figdor, C. G. (2008, Jul 18). Dendritic cell interaction with Candida albicans critically depends on N-linked mannan. *Journal of Biological Chemistry, 283*(29), 20590-20599. https://doi.org/10.1074/jbc.M709334200

Camilli, G., Tabouret, G., & Quintin, J. (2018). The Complexity of Fungal beta-Glucan in Health and Disease: Effects on the Mononuclear Phagocyte System. *Front Immunol, 9*, 673. https://doi.org/10.3389/fimmu.2018.00673

Cardoso, L. S., Araujo, M. I., Góes, A. M., Pacífico, L. G., Oliveira, R. R., & Oliveira, S. C. (2007). Polymyxin B as inhibitor of LPS contamination of Schistosoma mansoni recombinant proteins in human cytokine analysis. *Microbial cell factories, 6*(1), 1-6.

Chen, P. Y., Chuang, Y. C., Wu, U. I., Sun, H. Y., Wang, J. T., Sheng, W. H., Lo, H. J., Wang, H. Y., Chen, Y. C., & Chang, S. C. (2019, Sep). Clonality of Fluconazole-Nonsusceptible *Candida tropicalis* in Bloodstream Infections, Taiwan, 2011-2017. *Emerg Infect Dis, 25*(9), 1660-1667. https://doi.org/10.3201/eid2509.190520

Chen, S. M., Shen, H., Zhang, T., Huang, X., Liu, X. Q., Guo, S. Y., Zhao, J. J., Wang, C. F., Yan, L., Xu, G. T., Jiang, Y. Y., & An, M. M. (2017). Dectin-1 plays an important role in host defense against systemic *Candida glabrata* infection. *Virulence, 8*(8), 1643-1656. https://doi.org/10.1080/21505594.2017.1346756

Chen, S. M., Zou, Z., Qiu, X. R., Hou, W. T., Zhang, Y., Fang, W., Chen, Y. L., Wang, Y. D., Jiang, Y. Y., Shen, H., & An, M. M. (2019). The critical role of Dectin-1 in host controlling systemic *Candida krusei* infection. *Am J Transl Res, 11*(2), 721-732. https://www.ncbi.nlm.nih.gov/pubmed/30899374

Chen, X., & Chang, L.-J. (2005). Mycoplasma-mediated alterations of in vitro generation and functions of human dendritic cells. *Journal of biomedical science, 12*(1), 31-46.

Chrisikos, T. T., Zhou, Y., Slone, N., Babcock, R., Watowich, S. S., & Li, H. S. (2019). Molecular regulation of dendritic cell development and function in homeostasis, inflammation, and cancer. *Molecular Immunology, 110*, 24-39.

Clement, L. T., Tilden, A. B., & Dunlap, N. E. (1985). Analysis of the monocyte Fc receptors and antibody-mediated cellular interactions required for the induction of T cell proliferation by anti-T3 antibodies. *The Journal of Immunology, 135*(1), 165-171. https://www.jimmunol.org/content/jimmunol/135/1/165.full.pdf

Coleman, D. C., Sullivan, D. J., Bennett, D. E., Moran, G. P., Barry, H. J., & Shanley, D. B. (1997). Candidiasis: the emergence of a novel species, *Candida dubliniensis*. *AIDS, 11*(5), 557-567.

Comi, M., Amodio, G., & Gregori, S. (2018, 2018-April-06). Interleukin-10-Producing DC-10 Is a Unique Tool to Promote Tolerance Via Antigen-Specific T Regulatory Type 1 Cells [Mini Review]. *Frontiers in Immunology, 9*(682). https://doi.org/10.3389/fimmu.2018.00682

Conti, H. R., & Gaffen, S. L. (2015). IL-17-Mediated Immunity to the Opportunistic Fungal Pathogen Candida albicans. *J Immunol, 195*(3), 780-788. https://doi.org/10.4049/jimmunol.1500909

Costa, C. R., Passos, X. S., e Souza, L. K., Lucena Pde, A., Fernandes Ode, F., & Silva Mdo, R. (2010, May-Jun). Differences in exoenzyme production and adherence ability of Candida spp. isolates from catheter, blood and oral cavity. *Rev Inst Med Trop Sao Paulo, 52*(3), 139-143. https://doi.org/10.1590/s0036-46652010000300005

Csank, C., & Haynes, K. (2000, 2000/08/01/). *Candida glabrata* displays pseudohyphal growth. *FEMS Microbiology Letters, 189*(1), 115-120. https://doi.org/https://doi.org/10.1016/S0378-1097(00)00241-X

d'Ostiani, C. F., Del Sero, G., Bacci, A., Montagnoli, C., Spreca, A., Mencacci, A., Ricciardi-Castagnoli, P., & Romani, L. J. J. o. E. M. (2000). Dendritic cells discriminate between yeasts and hyphae of the fungus Candida albicans: implications for initiation of T helper cell immunity in vitro and in vivo. *191*(10), 1661-1674.

Dalia Akramienė, A. K., Janina Didžiapetrienė, and Egidijus Kėvelaitis. (2007). Effects of b-glucans on the immune system. *Medicina (Kaunas, Lithuania), 43*, 597-606.

David L. Williams S., R. B. M., Ernest L. Jones, Henry A. Pretus, Harry E. Ensley, I. William Browder, and Nicholas R. Di Luzio. (1991). A method for the solubilization of a (1- 3)-b-D-glucan isolated from Saccharomyces cerevisiaea. *Carbohydrate Research, 219*, 203-213.

Davis, S. E., Hopke, A., Minkin, S. C., Montedonico, A. E., Wheeler, R. T., & Reynolds, T. B. (2014). Masking of β-(1-3)-glucan in the cell wall of Candida albicans from detection by innate immune cells depends on phosphatidylserine. *Infection and immunity, 82*(10), 4405-4413.

De Groot, P. W., Kraneveld, E. A., Yin, Q. Y., Dekker, H. L., Groß, U., Crielaard, W., de Koster, C. G., Bader, O., Klis, F. M., & Weig, M. (2008). The cell wall of the human pathogen *Candida glabrata*: differential incorporation of novel adhesin-like wall proteins. *Eukaryotic cell, 7*(11), 1951-1964.

Deorukhkar, S. C., Saini, S., & Mathew, S. (2014). Virulence Factors Contributing to Pathogenicity of *Candida tropicalis* and Its Antifungal Susceptibility Profile. *International Journal of Microbiology, 2014*, 456878-456878. https://doi.org/10.1155/2014/456878

Duan, Z., Chen, Q., Zeng, R., Du, L., Liu, C., Chen, X., & Li, M. (2018, Dec). *Candida tropicalis* induces pro-inflammatory cytokine production, NF-kappaB and MAPKs pathways regulation, and dectin-1 activation. *Can J Microbiol, 64*(12), 937-944. https://doi.org/10.1139/cjm-2017-0559

Dudek, A. M., Martin, S., Garg, A. D., & Agostinis, P. (2013). Immature, semi-mature, and fully mature dendritic cells: toward a DC-cancer cells interface that augments anticancer immunity. *Frontiers in Immunology, 4*, 438.

Durai, V., & Murphy, Kenneth M. (2016, 2016/10/18/). Functions of Murine Dendritic Cells. *Immunity, 45*(4), 719-736. https://doi.org/https://doi.org/10.1016/j.immuni.2016.10.010

Ehirchiou, D., Xiong, Y., Xu, G., Chen, W., Shi, Y., & Zhang, L. (2007). CD11b facilitates the development of peripheral tolerance by suppressing Th17 differentiation. *The Journal of experimental medicine, 204*(7), 1519-1524.

Ehlers, M. R. W. (2000, 2000/03/01/). CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes and Infection, 2*(3), 289-294. https://doi.org/https://doi.org/10.1016/S1286-4579(00)00299-9

Eisenbarth, S. C. (2019, Feb). Dendritic cell subsets in T cell programming: location dictates function. *Nat Rev Immunol, 19*(2), 89-103. https://doi.org/10.1038/s41577-018-0088-1

El-Kirat-Chatel, S., Beaussart, A., Derclaye, S., Alsteens, D., Kucharíková, S., Van Dijck, P., & Dufrêne, Y. F. (2015, Feb 24). Force nanoscopy of hydrophobic interactions in the fungal pathogen *Candida glabrata*. *ACS Nano, 9*(2), 1648-1655. https://doi.org/10.1021/nn506370f

Elder, M. J., Webster, S. J., Chee, R., Williams, D. L., Hill Gaston, J. S., & Goodall, J. C. (2017). β-Glucan Size Controls Dectin-1-Mediated Immune Responses in Human Dendritic Cells by Regulating IL-1β Production. *Front Immunol, 8*, 791. https://doi.org/10.3389/fimmu.2017.00791

Engler, J. B., Kursawe, N., Solano, M. E., Patas, K., Wehrmann, S., Heckmann, N., Lühder, F., Reichardt, H. M., Arck, P. C., Gold, S. M., & Friese, M. A. (2017). Glucocorticoid receptor in T cells mediates protection from autoimmunity in pregnancy. *Proceedings of the National Academy of Sciences, 114*(2), E181-E190. https://doi.org/10.1073/pnas.1617115114

Esteban, A., Popp, M. W., Vyas, V. K., Strijbis, K., Ploegh, H. L., & Fink, G. R. (2011). Fungal recognition is mediated by the association of dectin-1 and galectin-3 in macrophages. *Proceedings of the National Academy of Sciences, 108*(34), 14270-14275. https://doi.org/10.1073/pnas.1111415108

Fatima, N., Upadhyay, T., Sharma, D., & Sharma, R. (2017, Jan 1). Particulate beta-glucan induces early and late phagosomal maturation in murine macrophages. *Front Biosci (Elite Ed), 9*, 129-140. https://doi.org/10.2741/e791

Feldman, M. B., Vyas, J. M., & Mansour, M. K. (2019, May). It takes a village: Phagocytes play a central role in fungal immunity. *Semin Cell Dev Biol, 89*, 16-23. https://doi.org/10.1016/j.semcdb.2018.04.008

Ferwerda, G., Meyer-Wentrup, F., Kullberg, B.-J., Netea, M. G., & Adema, G. J. (2008). Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. *Cellular Microbiology, 10*(10), 2058-2066. https://doi.org/10.1111/j.1462-5822.2008.01188.x

Fidel, P. L., Jr., Vazquez, J. A., & Sobel, J. D. (1999, Jan). *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. *Clinical Microbiology Reviews, 12*(1), 80-96.

Free, S. J. (2013). Fungal cell wall organization and biosynthesis. In *Advances in genetics* (Vol. 81, pp. 33-82). Elsevier.

Galocha, M., Pais, P., Cavalheiro, M., Pereira, D., Viana, R., & Teixeira, M. C. (2019, May 11). Divergent Approaches to Virulence in C. albicans and C. glabrata: Two Sides of the Same Coin. *International Journal of Molecular Sciences, 20*(9). https://doi.org/10.3390/ijms20092345

Ganesan, S., Rathinam, V. A. K., Bossaller, L., Army, K., Kaiser, W. J., Mocarski, E. S., Dillon, C. P., Green, D. R., Mayadas, T. N., Levitz, S. M., Hise, A. G., Silverman, N., & Fitzgerald, K. A. (2014, Sep 1). Caspase-8 modulates dectin-1 and complement receptor 3-driven IL-1beta production in response to beta-glucans and the fungal pathogen, Candida albicans. *Journal of Immunology, 193*(5), 2519-2530. https://doi.org/10.4049/jimmunol.1400276

García-Rodas, R., González-Camacho, F., Rodríguez-Tudela, J. L., Cuenca-Estrella, M., & Zaragoza, O. (2011). The interaction between *Candida krusei* and murine macrophages results in multiple outcomes, including

intracellular survival and escape from killing. *Infection and immunity, 79*(6), 2136-2144. https://doi.org/10.1128/IAI.00044-11

Giacobbe, D., Esteves, P., Bruzzi, P., Mikulska, M., Furfaro, E., Mesini, A., Tatarelli, P., Grignolo, S., Viscoli, C., & Colombo, A. (2015). Initial serum (1, 3)-ß-d-glucan as a predictor of mortality in proven candidaemia: findings from a retrospective study in two teaching hospitals in Italy and Brazil. *Clinical Microbiology and Infection, 21*(10), 954. e959-954. e917.

Gilbert, A. S., Wheeler, R. T., & May, R. C. (2015). Fungal pathogens: survival and replication within macrophages. *Cold Spring Harbor Perspectives in Medicine, 5*(7), a019661.

Glöckner, A., & Cornely, O. A. (2015, Aug). *Candida glabrata*--unique features and challenges in the clinical management of invasive infections. *Mycoses, 58*(8), 445-450. https://doi.org/10.1111/myc.12348

Godfrey Chi-Fung Chan, Wing Keung Chan, & Sze., D. M.-Y. (2009). The effects of β-glucan on human immune and cancer cells. *J Hematol & Oncol, 2*(25), 1-11.

Gómez-Gaviria, M., & Mora-Montes, H. M. (2020). Current Aspects in the Biology, Pathogeny, and Treatment of *Candida krusei*, a Neglected Fungal Pathogen. *Infection and Drug Resistance, 13*, 1673-1689. https://doi.org/10.2147/IDR.S247944

Gong, J., Xiao, M., Wang, H., Kudinha, T., Wang, Y., Zhao, F., Wu, W., He, L., Xu, Y.-C., & Zhang, J. (2018, 2018-November-20). Genetic Differentiation, Diversity, and Drug Susceptibility of *Candida krusei* [Original Research]. *Frontiers in Microbiology, 9*(2717). https://doi.org/10.3389/fmicb.2018.02717

Goodridge, H. S., Reyes, C. N., Becker, C. A., Katsumoto, T. R., Ma, J., Wolf, A. J., Bose, N., Chan, A. S., Magee, A. S., & Danielson, M. E. (2011). Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. *Nature, 472*(7344), 471.

Goodridge, H. S., Simmons, R. M., & Underhill, D. M. (2007, Mar 1). Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. *J Immunol, 178*(5), 3107-3115. https://doi.org/10.4049/jimmunol.178.5.3107

Goodridge, H. S., Wolf, A. J., & Underhill, D. M. (2009). Beta-glucan recognition by the innate immune system. *Immunological Reviews, 230*(1), 38-50. https://doi.org/10.1111/j.1600-065X.2009.00793.x

Gow, N. A., & Hube, B. (2012). Importance of the Candida albicans cell wall during commensalism and infection. *Current opinion in microbiology, 15*(4), 406-412.

Gow, N. A., Netea, M. G., Munro, C. A., Ferwerda, G., Bates, S., Mora-Montes, H. M., Walker, L., Jansen, T., Jacobs, L., Tsoni, V., Brown, G. D., Odds, F. C., Van der Meer, J. W., Brown, A. J., & Kullberg, B. J. (2007). Immune recognition of Candida albicans beta-glucan by dectin-1. *J Infect Dis, 196*(10), 1565-1571. https://doi.org/10.1086/523110

Goyal, S., Castrillón-Betancur, J. C., Klaile, E., & Slevogt, H. (2018). The Interaction of Human Pathogenic Fungi With C-Type Lectin Receptors [Review]. *Frontiers in Immunology, 9*(1261). https://doi.org/10.3389/fimmu.2018.01261

Graus, M. S., Wester, M. J., Lowman, D. W., Williams, D. L., Kruppa, M. D., Martinez, C. M., Young, J. M., Pappas, H. C., Lidke, K. A., & Neumann, A. K. (2018). Mannan Molecular Substructures Control Nanoscale Glucan Exposure in Candida. *Cell Rep, 24*(9), 2432-2442 e2435. https://doi.org/10.1016/j.celrep.2018.07.088

Griffiths, K. L., Tan, J. K., & O'Neill, H. C. (2014). Characterization of the effect of LPS on dendritic cell subset discrimination in spleen. *Journal of cellular and molecular medicine, 18*(9), 1908-1912.

Gringhuis, S. I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S. C., & Geijtenbeek, T. B. (2009, Feb). Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nature Immunology, 10*(2), 203-213. https://doi.org/10.1038/ni.1692

Gross, O., Gewies, A., Finger, K., Schafer, M., Sparwasser, T., Peschel, C., Forster, I., & Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature, 442*(7103), 651-656. https://doi.org/10.1038/nature04926

Gupta, A., Gupta, A., & Varma, A. (2015). *Candida glabrata* candidemia: An emerging threat in critically ill patients. *Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine, 19*(3), 151-154. https://doi.org/10.4103/0972-5229.152757

Hachem, R., Hanna, H., Kontoyiannis, D., Jiang, Y., & Raad, I. (2008). The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. *Cancer: Interdisciplinary International Journal of the American Cancer Society, 112*(11), 2493-2499.

Han, B., Baruah, K., Cox, E., Vanrompay, D., & Bossier, P. (2020). Structure-Functional Activity Relationship of β-Glucans From the Perspective of Immunomodulation: A Mini-Review. *Frontiers in Immunology, 11*, 658-658. https://doi.org/10.3389/fimmu.2020.00658

Heinsbroek, S. E. M., Taylor, P. R., Martinez, F. O., Martinez-Pomares, L., Brown, G. D., & Gordon, S. (2008). Stage-Specific Sampling by Pattern Recognition Receptors during Candida albicans Phagocytosis. *PLOS Pathogens, 4*(11), e1000218. https://doi.org/10.1371/journal.ppat.1000218

Hirayama, T., Miyazaki, T., Ito, Y., Wakayama, M., Shibuya, K., Yamashita, K., Takazono, T., Saijo, T., Shimamura, S., Yamamoto, K., Imamura, Y., Izumikawa, K., Yanagihara, K., Kohno, S., & Mukae, H. (2020, 2020/03/02). Virulence assessment of six major pathogenic Candida species in the mouse model of invasive candidiasis caused by fungal translocation. *Scientific Reports, 10*(1), 3814. https://doi.org/10.1038/s41598-020-60792-y

Hopke, A., Nicke, N., Hidu, E. E., Degani, G., Popolo, L., & Wheeler, R. T. (2016, May). Neutrophil Attack Triggers Extracellular Trap-Dependent Candida Cell Wall Remodeling and Altered Immune Recognition. *PLoS Pathog, 12*(5), e1005644. https://doi.org/10.1371/journal.ppat.1005644

Hsu, P., Santner-Nanan, B., Hu, M., Skarratt, K., Lee, C. H., Stormon, M., Wong, M., Fuller, S. J., & Nanan, R. (2015). IL-10 Potentiates Differentiation of Human Induced Regulatory T Cells via STAT3 and Foxo1. *The Journal of Immunology, 195*(8), 3665-3674. https://doi.org/10.4049/jimmunol.1402898

Iberg, C. A., Jones, A., & Hawiger, D. (2017). Dendritic Cells As Inducers of Peripheral Tolerance. *Trends in immunology, 38*(11), 793-804. https://doi.org/10.1016/j.it.2017.07.007

Inaba, K., Swiggard, W. J., Steinman, R. M., Romani, N., Schuler, G., & Brinster, C. (2009, Aug). Isolation of dendritic cells. *Curr Protoc Immunol, Chapter 3*(1), Unit 3 7. https://doi.org/10.1002/0471142735.im0307s86

Inoue, M., & Shinohara, M. L. (2014, Feb). Clustering of pattern recognition receptors for fungal detection. *PLoS Pathog, 10*(2), e1003873. https://doi.org/10.1371/journal.ppat.1003873

J. C. O. Sardi, L. S., T. Bernardi, A. M. Fusco-Almeida and M. J. S. Mendes Giannini. (2013). Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology, 62*, 10-24.

Jabra-Rizk, M. A., Baqui, A. A., Kelley, J. I., Falkler, W. A., Jr., Merz, W. G., & Meiller, T. F. (1999). Identification of *Candida dubliniensis* in a prospective study of patients in the United States. *Journal of Clinical Microbiology, 37*(2), 321-326. https://pubmed.ncbi.nlm.nih.gov/9889211

Jackson, A. P., Gamble, J. A., Yeomans, T., Moran, G. P., Saunders, D., Harris, D., Aslett, M., Barrell, J. F., Butler, G., Citiulo, F., Coleman, D. C., de Groot, P. W. J., Goodwin, T. J., Quail, M. A., McQuillan, J., Munro, C. A., Pain, A., Poulter, R. T., Rajandream, M.-A., Renauld, H., Spiering, M. J., Tivey, A., Gow, N. A. R., Barrell, B., Sullivan, D. J., & Berriman, M. (2009). Comparative genomics of the fungal pathogens *Candida dubliniensis* and Candida albicans. *Genome Research, 19*(12), 2231-2244. https://doi.org/10.1101/gr.097501.109

Jacobsen, I. D., Brunke, S., Seider, K., Schwarzmüller, T., Firon, A., d'Enfért, C., Kuchler, K., & Hube, B. (2010). *Candida glabrata* Persistence in Mice Does Not Depend on Host Immunosuppression and Is Unaffected by Fungal Amino Acid Auxotrophy. *Infection and Immunity, 78*(3), 1066-1077. https://doi.org/10.1128/iai.01244-09

Jamiu, A. T., Albertyn, J., Sebolai, O. M., & Pohl, C. H. (2020). Update on *Candida krusei*, a potential multidrug-resistant pathogen. *Medical Mycology*. https://doi.org/10.1093/mmy/myaa031

Jankovic, D., Kugler, D. G., & Sher, A. (2010, 2010/05/01). IL-10 production by CD4+ effector T cells: a mechanism for self-regulation. *Mucosal Immunology, 3*(3), 239-246. https://doi.org/10.1038/mi.2010.8

Joo, H., Upchurch, K., Zhang, W., Ni, L., Li, D., Xue, Y., Li, X.-H., Hori, T., Zurawski, S., Liu, Y.-J., Zurawski, G., & Oh, S. (2015). Opposing Roles of Dectin-1 Expressed on Human Plasmacytoid Dendritic Cells and Myeloid Dendritic Cells in Th2 Polarization. *J Immunol, 195*(4), 1723-1731. https://doi.org/10.4049/jimmunol.1402276

Józefowski, S., Yang, Z., Marcinkiewicz, J., & Kobzik, L. (2012). Scavenger receptors and ß-glucan receptors participate in the recognition of yeasts by murine macrophages. *Inflammation Research, 61*(2), 113-126.

Kanjan, P., Sahasrabudhe, N. M., de Haan, B. J., & de Vos, P. (2017). Immune effects of β-glucan are determined by combined effects on Dectin-1, TLR2, 4 and 5. *Journal of Functional Foods, 37*, 433-440. https://doi.org/10.1016/j.jff.2017.07.061

Kashem, S. W., Igyarto, B. Z., Gerami-Nejad, M., Kumamoto, Y., Mohammed, J. A., Jarrett, E., Drummond, R. A., Zurawski, S. M., Zurawski, G., Berman, J., Iwasaki, A., Brown, G. D., & Kaplan, D. H. (2015, Feb 17). Candida albicans morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity, 42*(2), 356-366. https://doi.org/10.1016/j.immuni.2015.01.008

Kasper, L., Seider, K., & Hube, B. (2015). Intracellular survival of *Candida glabrata* in macrophages: immune evasion and persistence. *FEMS Yeast Res, 15*(5), fov042. https://doi.org/10.1093/femsyr/fov042

Khatib, R., Johnson, L. B., Fakih, M. G., Riederer, K., & Briski, L. (2016). Current trends in candidemia and species distribution among adults: *Candida glabrata* surpasses C. albicans in diabetic patients and abdominal sources. *Mycoses, 59*(12), 781-786. https://doi.org/10.1111/myc.12531

Kikuchi, T., Ohno, N., & Ohno, T. (2002, Sep). Maturation of dendritic cells induced by Candida beta-D-glucan. *Int Immunopharmacol, 2*(10), 1503-1508. https://doi.org/10.1016/s1567-5769(02)00084-x

Kim, G. Y., Jeon, J. S., & Kim, J. K. (2016, Jun). Isolation Frequency Characteristics of Candida Species from Clinical Specimens. *Mycobiology, 44*(2), 99-104. https://doi.org/10.5941/MYCO.2016.44.2.99

Kim, H. S., Park, K. H., Lee, H. K., Kim, J. S., Kim, Y. G., Lee, J. H., Kim, K. H., Yun, J., Hwang, B. Y., & Hong, J. T. (2016). Curdlan activates dendritic cells through dectin-1 and toll-like receptor 4 signaling. *International immunopharmacology, 39*, 71-78.

Kingeter, L. M., & Lin, X. (2012). C-type lectin receptor-induced NF-**K**B activation in innate immune and inflammatory responses. *Cellular & Molecular Immunology, 9*(2), 105-112.

Korolenko, T. A., Bgatova, N. P., & Vetvicka, V. (2019). Glucan and Mannan-Two Peas in a Pod. *International journal of molecular sciences, 20*(13), 3189. https://doi.org/10.3390/ijms20133189

Kotthoff, P., Heine, A., Held, S. A. E., & Brossart, P. (2017). Dexamethasone induced inhibition of Dectin-1 activation of antigen presenting cells is mediated via STAT-3 and NF-**K**B signaling pathways. *Scientific Reports, 7*(1), 1-11.

Kumar, K., Askari, F., Sahu, M. S., & Kaur, R. (2019). *Candida glabrata*: A lot more than meets the eye [Review]. *Microorganisms, 7*(2), Article 39. https://doi.org/10.3390/microorganisms7020039

Lackey, E., Vipulanandan, G., Childers, D. S., & Kadosh, D. (2013, Oct). Comparative evolution of morphological regulatory functions in Candida species. *Eukaryotic cell, 12*(10), 1356-1368. https://doi.org/10.1128/ec.00164-13

Lamoth, F., Lockhart, S. R., Berkow, E. L., & Calandra, T. (2018, Jan 1). Changes in the epidemiological landscape of invasive candidiasis. *Journal of Antimicrobial Chemotherapy, 73*(suppl_1), i4-i13. https://doi.org/10.1093/jac/dkx444

Latge, J. P. (2010). Tasting the fungal cell wall. *Cellular Microbiology, 12*(7), 863-872. https://doi.org/10.1111/j.1462-5822.2010.01474.x

Legentil, L., Paris, F., Ballet, C., Trouvelot, S., Daire, X., Vetvicka, V., & Ferrières, V. (2015). Molecular interactions of β-(1⟶ 3)-glucans with their receptors. *Molecules, 20*(6), 9745-9766.

LeibundGut-Landmann, S., Groß, O., Robinson, M. J., Osorio, F., Slack, E. C., Tsoni, S. V., Schweighoffer, E., Tybulewicz, V., Brown, G. D., & Ruland, J. J. N. i. (2007). Syk-and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol, 8*(6), 630-638.

Leibundgut-Landmann, S., Osorio, F., Brown, G. D., & Reis e Sousa, C. (2008, Dec 15). Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses. *Blood, 112*(13), 4971-4980. https://doi.org/10.1182/blood-2008-05-158469

Leroy, O., Gangneux, J.-P., Montravers, P., Mira, J.-P., Gouin, F., Sollet, J.-P., Carlet, J., Reynes, J., Rosenheim, M., Regnier, B., Lortholary, O., & for the AmarCand Study, G. (2009). Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: A multicenter, prospective, observational study in France (2005–2006). *Critical Care Medicine, 37*(5). https://journals.lww.com/ccmjournal/Fulltext/2009/05000/Epidemiology,_management,_and_risk_factors_for.10.aspx

Leung, S., Liu, X., Fang, L., Chen, X., Guo, T., & Zhang, J. (2010, May). The cytokine milieu in the interplay of pathogenic Th1/Th17 cells and regulatory T cells in autoimmune disease. *Cellular & Molecular Immunology, 7*(3), 182-189. https://doi.org/10.1038/cmi.2010.22

Lewis, L. E., Bain, J. M., Lowes, C., Gow, N. A. R., & Erwig, L.-P. (2012, 2012/09/01/). Candida albicans infection inhibits macrophage cell division and proliferation. *Fungal Genetics and Biology, 49*(9), 679-680. https://doi.org/https://doi.org/10.1016/j.fgb.2012.05.007

Li, D., Bai, C., Zhang, Q., Li, Z., Shao, D., & Li, X. (2019). β-1, 3-Glucan/CR3/SYK pathway-dependent LC3B-II accumulation enhanced the fungicidal activity in human neutrophils. *Journal of Microbiology, 57*(4), 263-270.

Li, Y., & Kurlander, R. J. (2010). Comparison of anti-CD3 and anti-CD28-coated beads with soluble anti-CD3 for expanding human T cells: differing impact on CD8 T cell phenotype and responsiveness to restimulation. *Journal of Translational Medicine, 8*(1), 1-15.

Li, Y., & Kurlander, R. J. (2010, 2010/10/26). Comparison of anti-CD3 and anti-CD28-coated beads with soluble anti-CD3 for expanding human T cells: Differing impact on CD8 T cell phenotype and responsiveness to restimulation. *Journal of Translational Medicine, 8*(1), 104. https://doi.org/10.1186/1479-5876-8-104

Lowman, D. W., Ferguson, D. A., & Williams, D. L. (2003). Structural characterization of (1-->3)-beta-D-glucans isolated from blastospore and hyphal forms of Candida albicans. *Carbohydrate Research, 338*(14), 1491-1496. https://doi.org/10.1016/s0008-6215(03)00169-1

Lowman, D. W., Greene, R. R., Bearden, D. W., Kruppa, M. D., Pottier, M., Monteiro, M. A., Soldatov, D. V., Ensley, H. E., Cheng, S. C., Netea, M. G., & Williams, D. L. (2014, Feb 7). Novel structural features in Candida albicans hyphal glucan provide a basis for differential innate immune recognition of hyphae versus yeast. *Journal of Biological Chemistry, 289*(6), 3432-3443. https://doi.org/10.1074/jbc.M113.529131

Lowman, D. W., West, L. J., Bearden, D. W., Wempe, M. F., Power, T. D., Ensley, H. E., Haynes, K., Williams, D. L., & Kruppa, M. D. (2011). New insights into the structure of (1-->3,1-->6)-beta-D-glucan side chains in the *Candida glabrata* cell wall. *PLoS One, 6*(11), e27614. https://doi.org/10.1371/journal.pone.0027614

Lukácsi, S., Gerecsei, T., Balázs, K., Francz, B., Szabó, B., Erdei, A., & Bajtay, Z. (2020). The differential role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in the adherence, migration and podosome formation of human macrophages and dendritic cells under inflammatory conditions. *PLoS One, 15*(5), e0232432. https://doi.org/10.1371/journal.pone.0232432

Lutz, M. (2012, 2012-May-18). Therapeutic Potential of Semi-Mature Dendritic Cells for Tolerance Induction [Review]. *Frontiers in Immunology, 3*(123). https://doi.org/10.3389/fimmu.2012.00123

Matveev, A. L., Krylov, V. B., Khlusevich, Y. A., Baykov, I. K., Yashunsky, D. V., Emelyanova, L. A., Tsvetkov, Y. E., Karelin, A. A., Bardashova, A. V., & Wong, S. S. (2019). Novel mouse monoclonal antibodies specifically recognizing β-(1⟶ 3)-D-glucan antigen. *PLoS One, 14*(4), e0215535.

Mayer, F. L., Wilson, D., & Hube, B. (2013). Candida albicans pathogenicity mechanisms. *Virulence, 4*(2), 119-128. https://doi.org/10.4161/viru.22913

Melanie Polke, B. H. a. I. D. J. (2015). Candida Survival Strategies. In *Advances in Applied Microbiology* (Vol. 91, pp. 139-235).

Merseguel, K. B., Nishikaku, A. S., Rodrigues, A. M., Padovan, A. C., e Ferreira, R. C., de Azevedo Melo, A. S., Briones, M. R. d. S., & Colombo, A. L. (2015). Genetic diversity of medically important and emerging Candida species

causing invasive infection. *BMC Infectious Diseases, 15*, 57-57. https://doi.org/10.1186/s12879-015-0793-3

Mesa-Arango, A. C., Rueda, C., Román, E., Quintin, J., Terrón, M. C., Luque, D., Netea, M. G., Pla, J., & Zaragoza, O. (2016). Cell wall changes in amphotericin B-resistant strains from *Candida tropicalis* and relationship with the immune responses elicited by the host. *Antimicrobial Agents and Chemotherapy, 60*(4), 2326-2335.

Mihai G. Netea, L. A. B. J., Jos W. M. van der Meer, Bart-Jan Kullberg and Frank L. van de Veerdonk. (2015). Immune defence against Candida fungal infections. *Nature Reviews: Immunology, 15*, 630-642.

Mildner, A., & Jung, S. (2014). Development and function of dendritic cell subsets. *Immunity, 40*(5), 642-656.

Miranda-Cadena, K., Marcos-Arias, C., Mateo, E., Aguirre, J. M., Quindós, G., & Eraso, E. (2018). Prevalence and antifungal susceptibility profiles of *Candida glabrata*, *Candida parapsilosis* and their close-related species in oral candidiasis. *Arch Oral Biol, 95*, 100-107. https://doi.org/10.1016/j.archoralbio.2018.07.017

Miura, N. N., Adachi, Y., Yadomae, T., Tamura, H., Tanaka, S., & Ohno, N. (2003). Structure and Biological Activities of ß-Glucans from Yeast and Mycelial Forms of Candida albicans. *Microbiology and immunology, 47*(3), 173-182.

Mokaddas, E., Khan, Z. U., Ahmad, S., Nampoory, M. R. N., & Burhamah, M. (2011). Value of (1-3)-ß-d-glucan, Candida mannan and Candida DNA detection in the diagnosis of candidaemia. *Clinical Microbiology and Infection, 17*(10), 1549-1553. https://doi.org/10.1111/j.1469-0691.2011.03608.x

Montravers, P., Dupont, H., Gauzit, R., Veber, B., Auboyer, C., Blin, P., Hennequin, C., & Martin, C. (2006, Mar). Candida as a risk factor for mortality in peritonitis. *Crit Care Med, 34*(3), 646-652. https://doi.org/10.1097/01.Ccm.0000201889.39443.D2

Mora-Montes, H. M., Netea, M. G., Ferwerda, G., Lenardon, M. D., Brown, G. D., Mistry, A. R., Kullberg, B. J., O'Callaghan, C. A., Sheth, C. C., & Odds, F. C. (2011). Recognition and blocking of innate immunity cells by Candida albicans chitin. *Infection and immunity, 79*(5), 1961-1970.

Moran, G. P., Coleman, D. C., & Sullivan, D. J. (2012). Candida albicans versus *Candida dubliniensis*: Why Is C. albicans More Pathogenic? *International Journal of Microbiology, 2012*, 205921-205921. https://doi.org/10.1155/2012/205921

Morrison, V. L., James, M. J., Grzes, K., Cook, P., Glass, D. G., Savinko, T., Lek, H. S., Gawden-Bone, C., Watts, C., Millington, O. R., MacDonald, A. S., & Fagerholm, S. C. (2014). Loss of beta2-integrin-mediated cytoskeletal linkage reprogrammes dendritic cells to a mature migratory phenotype. *Nature communications, 5*, 5359-5359. https://doi.org/10.1038/ncomms6359

Nadeem, S. G., Shafiq, A., Hakim, S. T., Anjum, Y., & Kazm, S. U. (2013). Effect of growth media, pH and temperature on yeast to hyphal transition in Candida albicans.

Nakagawa, Y., Ohno, N., & Murai, T. (2003, Feb 15). Suppression by Candida albicans beta-glucan of cytokine release from activated human monocytes and from T cells in the presence of monocytes. *Journal of Infectious Diseases, 187*(4), 710-713. https://doi.org/10.1086/368334

Navarro-Arias, M. J., Hernandez-Chavez, M. J., Garcia-Carnero, L. C., Amezcua-Hernandez, D. G., Lozoya-Perez, N. E., Estrada-Mata, E., Martinez-Duncker, I., Franco, B., & Mora-Montes, H. M. (2019). Differential recognition of *Candida tropicalis*, Candida guilliermondii, *Candida krusei*, and Candida auris by human innate immune cells. *Infect Drug Resist, 12*, 783-794. https://doi.org/10.2147/IDR.S197531

Neil AR Gow, A. J. B. a. F. C. O. (2002). Fungal morphogenesis and host invasion. *Current Opinion in Microbiology* 366-371.

Neumann, C., Scheffold, A., & Rutz, S. (2019). Functions and regulation of T cell-derived interleukin-10. Seminars in immunology,

Newman, S. L., & Holly, A. (2001). Candida albicans is phagocytosed, killed, and processed for antigen presentation by human dendritic cells. *Infection and Immunity, 69*(11), 6813-6822.

Ng, T. H. S., Britton, G., Hill, E., Verhagen, J., Burton, B., & Wraith, D. (2013, 2013-May-31). Regulation of Adaptive Immunity; The Role of Interleukin-10 [Review]. *Frontiers in Immunology, 4*(129). https://doi.org/10.3389/fimmu.2013.00129

Nguyen, T. N. Y., Matangkasombut, O., & Ritprajak, P. (2018). Differential dendritic cell responses to cell wall mannan of Candida albicans, *Candida parapsilosis*, and *Candida dubliniensis*. *J Oral Sci, 60*(4), 557-566. https://doi.org/10.2334/josnusd.17-0426

Noss, I., Doekes, G., Thorne, P. S., Heederik, D. J., & Wouters, I. M. (2013). Comparison of the potency of a variety of ß-glucans to induce cytokine production in human whole blood. *Innate immunity, 19*(1), 10-19.

O'Connor, L., Caplice, N., Coleman, D. C., Sullivan, D. J., & Moran, G. P. (2010). Differential filamentation of Candida albicans and *Candida dubliniensis* Is governed by nutrient regulation of UME6 expression. *Eukaryotic cell, 9*(9), 1383-1397. https://doi.org/10.1128/EC.00042-10

Odds, F. C. (1994). Pathogenesis of Candida infections. *Journal of the American Academy of Dermatology, 31*(3), S2-S5.

Öncü, B., Belet, N., Emecen, A. N., & Birinci, A. (2019). Health care-associated invasive Candida infections in children. *Medical Mycology, 57*(8), 929-936. https://doi.org/10.1093/mmy/myz005

Ortega-Riveros, M., De-la-Pinta, I., Marcos-Arias, C., Ezpeleta, G., Quindós, G., & Eraso, E. (2017, Oct). Usefulness of the Non-conventional Caenorhabditis elegans Model to Assess Candida Virulence. *Mycopathologia, 182*(9-10), 785-795. https://doi.org/10.1007/s11046-017-0142-8

Ostrop, J., & Lang, R. (2017). Contact, Collaboration, and Conflict: Signal Integration of Syk-Coupled C-Type Lectin Receptors. *Journal of Immunology, 198*(4), 1403-1414. https://doi.org/10.4049/jimmunol.1601665

Pais, P., Galocha, M., Viana, R., Cavalheiro, M., Pereira, D., & Teixeira, M. C. (2019). Microevolution of the pathogenic yeasts Candida albicans and *Candida glabrata* during antifungal therapy and host infection. *Microbial cell (Graz, Austria), 6*(3), 142-159. https://doi.org/10.15698/mic2019.03.670

Palma, A. S., Feizi, T., Zhang, Y., Stoll, M. S., Lawson, A. M., Díaz-Rodríguez, E., Campanero-Rhodes, M. A., Costa, J., Gordon, S., & Brown, G. D. (2006). Ligands for the β-glucan receptor, Dectin-1, assigned using "designer" microarrays of oligosaccharide probes (neoglycolipids) generated from glucan polysaccharides. *Journal of Biological Chemistry, 281*(9), 5771-5779.

Papon, N., Courdavault, V., Clastre, M., & Bennett, R. J. (2013). Emerging and emerged pathogenic Candida species: beyond the Candida albicans paradigm. *PLoS Pathogens, 9*(9), e1003550-e1003550. https://doi.org/10.1371/journal.ppat.1003550

Pappas, P. G., Lionakis, M. S., Arendrup, M. C., Ostrosky-Zeichner, L., & Kullberg, B. J. (2018). Invasive candidiasis. *Nature Reviews Disease Primers, 4*(1), 1-20.

Pfaller, M. A., Diekema, D. J., Gibbs, D. L., Newell, V. A., Nagy, E., Dobiasova, S., Rinaldi, M., Barton, R., Veselov, A., & Global Antifungal Surveillance, G. (2008, Feb). *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. *J Clin Microbiol, 46*(2), 515-521. https://doi.org/10.1128/JCM.01915-07

Plato, A., Willment, J. A., & Brown, G. D. (2013). C-type lectin-like receptors of the dectin-1 cluster: ligands and signaling pathways. *International Reviews of Immunology, 32*(2), 134-156.

Pletinckx, K., Döhler, A., Pavlovic, V., & Lutz, M. B. (2011). Role of dendritic cell maturity/costimulation for generation, homeostasis, and suppressive activity of regulatory T cells. *Frontiers in Immunology, 2*, 39.

Podgrabinska, S., Kamalu, O., Mayer, L., Shimaoka, M., Snoeck, H., Randolph, G. J., & Skobe, M. (2009). Inflamed Lymphatic Endothelium Suppresses Dendritic Cell Maturation and Function via Mac-1/ICAM-1-Dependent Mechanism. *The Journal of Immunology, 183*(3), 1767. https://doi.org/10.4049/jimmunol.0802167

Probst, H. C., Muth, S., & Schild, H. (2014). Regulation of the tolerogenic function of steady-state DCs. *European journal of immunology, 44*(4), 927-933.

Qi, C., Cai, Y., Gunn, L., Ding, C., Li, B., Kloecker, G., Qian, K., Vasilakos, J., Saijo, S., & Iwakura, Y. (2011). Differential pathways regulating innate and adaptive anti-tumor immune responses by particulate and soluble yeast-derived β-glucans. *Blood*, blood-2011-2002-339812.

Qin, Y., Zhang, L., Xu, Z., Zhang, J., Jiang, Y. Y., Cao, Y., & Yan, T. (2016, Jul 3). Innate immune cell response upon Candida albicans infection. *Virulence, 7*(5), 512-526. https://doi.org/10.1080/21505594.2016.1138201

Ramage, G., Saville, S. P., Thomas, D. P., & López-Ribot, J. L. (2005). Candida biofilms: an update. *Eukaryotic cell, 4*(4), 633-638. https://doi.org/10.1128/EC.4.4.633-638.2005

Ramirez-Ortiz, Z. G., & Means, T. K. (2012). The role of dendritic cells in the innate recognition of pathogenic fungi (A. fumigatus, C. neoformans and C. albicans). *Virulence, 3*(7), 635-646. https://doi.org/10.4161/viru.22295

Reid, D. M., Gow, N. A. R., & Brown, G. D. (2009). Pattern recognition: recent insights from Dectin-1. *Current opinion in immunology, 21*(1), 30-37. https://doi.org/10.1016/j.coi.2009.01.003

Richardson, J. P., Moyes, D. L., Ho, J., & Naglik, J. R. (2019, May). Candida innate immunity at the mucosa. *Semin Cell Dev Biol, 89*, 58-70. https://doi.org/10.1016/j.semcdb.2018.02.026

Ritprajak, P., Kaewraemruaen, C., & Hirankarn, N. (2019, Oct 21). Current Paradigms of Tolerogenic Dendritic Cells and Clinical Implications for Systemic Lupus Erythematosus. *Cells, 8*(10). https://doi.org/10.3390/cells8101291

Rizzetto, L., Kuka, M., De Filippo, C., Cambi, A., Netea, M. G., Beltrame, L., Napolitani, G., Torcia, M. G., D'Oro, U., & Cavalieri, D. (2010, Apr 15). Differential IL-17 production and mannan recognition contribute to fungal pathogenicity and commensalism. *J Immunol, 184*(8), 4258-4268. https://doi.org/10.4049/jimmunol.0902972

Rogers, N. C., Slack, E. C., Edwards, A. D., Nolte, M. A., Schulz, O., Schweighoffer, E., Williams, D. L., Gordon, S., Tybulewicz, V. L., Brown, G. D., & Reis e Sousa, C. (2005, Apr). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity, 22*(4), 507-517. https://doi.org/10.1016/j.immuni.2005.03.004

Ross, G. D., & Vĕtvicka, V. (1993). CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificities and functions. *Clinical and Experimental Immunology, 92*(2), 181-184. https://doi.org/10.1111/j.1365-2249.1993.tb03377.x

Roussey, J. A., Olszewski, M. A., & Osterholzer, J. J. (2016). Immunoregulation in Fungal Diseases. *Microorganisms, 4*(4), 47. https://doi.org/10.3390/microorganisms4040047

Rubin-Bejerano, I., Abeijon, C., Magnelli, P., Grisafi, P., & Fink, G. R. (2007). Phagocytosis by human neutrophils is stimulated by a unique fungal cell wall component. *Cell Host & Microbe, 2*(1), 55-67. https://doi.org/10.1016/j.chom.2007.06.002

Ruiz-Herrera, J., Elorza, M. V., Valentin, E., & Sentandreu, R. (2006). Molecular organization of the cell wall of *Candida* albicans and its relation to pathogenicity. *FEMS Yeast Res, 6*(1), 14-29. https://doi.org/10.1111/j.1567-1364.2005.00017.x

Saijo, S., Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., Fujikado, N., Kusaka, T., Kubo, S., Chung, S.-h., Komatsu, R., Miura, N., Adachi, Y., Ohno, N., Shibuya, K., Yamamoto, N., Kawakami, K., Yamasaki, S., Saito, T., Akira, S., & Iwakura, Y. (2010, 2010/05/28/). Dectin-2 Recognition of α-Mannans and Induction of Th17 Cell

Differentiation Is Essential for Host Defense against *Candida* albicans. *Immunity, 32*(5), 681-691. https://doi.org/https://doi.org/10.1016/j.immuni.2010.05.001

Saijo, S., & Iwakura, Y. (2011, Aug). Dectin-1 and Dectin-2 in innate immunity against fungi. *Int Immunol, 23*(8), 467-472. https://doi.org/10.1093/intimm/dxr046

Salazar, F., & Brown, G. D. (2018). Antifungal Innate Immunity: A Perspective from the Last 10 Years. *J Innate Immun, 10*(5-6), 373-397. https://doi.org/10.1159/000488539

Samaranayake, Y., Wu, P., Samaranayake, L., & Ho, P. (1998). The relative pathogenicity of *Candida krusei* and C. albicans in the rat oral mucosa. *Journal of Medical Microbiology, 47*(12), 1047-1057.

Samaranayake, Y., Wu, P., Samaranayake, L., So, M., & Yuen, K. (1994). Adhesion and colonisation of *Candida krusei* on host surfaces. *Journal of Medical Microbiology, 41*(4), 250-258.

Saravia, J., Chapman, N. M., & Chi, H. (2019, 2019/07/01). Helper T cell differentiation. *Cellular & Molecular Immunology, 16*(7), 634-643. https://doi.org/10.1038/s41423-019-0220-6

Sato, T., Iwabuchi, K., Nagaoka, I., Adachi, Y., Ohno, N., Tamura, H., Seyama, K., Fukuchi, Y., Nakayama, H., & Yoshizaki, F. (2006). Induction of human neutrophil chemotaxis by *Candida albicans*-derived β-1, 6-long glycoside side-chain-branched β-glucan. *Journal of Leukocyte Biology, 80*(1), 204-211.

Savastano, C., de Oliveira Silva, E., Gonçalves, L. L., Nery, J. M., Silva, N. C., & Dias, A. L. (2016). *Candida glabrata* among Candida spp. from environmental health practitioners of a Brazilian Hospital. *Brazilian Journal of Microbiology, 47*(2), 367-372. https://doi.org/10.1016/j.bjm.2015.05.001

Schittenhelm, L., Hilkens, C. M., & Morrison, V. L. (2017). β2 integrins as regulators of dendritic cell, monocyte, and macrophage function. *Frontiers in Immunology, 8*, 1866.

Schmidt, S. V., Nino-Castro, A. C., & Schultze, J. L. (2012). Regulatory dendritic cells: there is more than just immune activation. *Frontiers in Immunology, 3*, 274.

Seider, K., Brunke, S., Schild, L., Jablonowski, N., Wilson, D., Majer, O., Barz, D., Haas, A., Kuchler, K., Schaller, M., & Hube, B. (2011). The facultative intracellular pathogen *Candida glabrata* subverts macrophage cytokine production and phagolysosome maturation [Article]. *Journal of Immunology, 187*(6), 3072-3086. https://doi.org/10.4049/jimmunol.1003730

Sem, X., & , G. T. T. L., Alrina S. M. Tan, Gloria Tso, Marina Yurieva, Webber W. P. Liao, Josephine Lum, Kandhadayar G. Srinivasan, Michael Poidinger, Francesca Zolezzi and Norman Pavelka. (2016). β-glucan Exposure on the Fungal Cell Wall Tightly Correlates with Competitive Fitness of Candida Species in the Mouse Gastrointestinal Tract. *Frontiers in Cellular and Infection Microbiology, 6*(186), 1-12.

Serrano, I., Luque, A., & Aran, J. M. (2018). Exploring the immunomodulatory moonlighting activities of acute phase proteins for tolerogenic dendritic cell generation. *Frontiers in Immunology, 9*, 892.

Sherman, F. (2002). Getting started with yeast. In *Methods in enzymology* (Vol. 350, pp. 3-41). Elsevier.

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W., & Azeredo, J. (2012, Mar). *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews, 36*(2), 288-305. https://doi.org/10.1111/j.1574-6976.2011.00278.x

Sims, C. R., Jaijakul, S., Mohr, J., Rodriguez, J., Finkelman, M., & Ostrosky-Zeichner, L. (2012). Correlation of clinical outcomes with β-glucan levels in patients with invasive candidiasis. *Journal of Clinical Microbiology, 50*(6), 2104-2106.

Singh-Jasuja, H., Thiolat, A., Ribon, M., Boissier, M.-C., Bessis, N., Rammensee, H.-G., & Decker, P. (2013). The mouse dendritic cell marker CD11c is down-regulated upon cell activation through Toll-like receptor triggering. *Immunobiology, 218*(1), 28-39.

Singh, D. K., Németh, T., Papp, A., Tóth, R., Lukácsi, S., Heidingsfeld, O., Dostal, J., Vágvölgyi, C., Bajtay, Z., Józsi, M., & Gácser, A. (2019, Aug 21). Functional Characterization of Secreted Aspartyl Proteases in *Candida parapsilosis*. *mSphere, 4*(4). https://doi.org/10.1128/mSphere.00484-19

Sletmoen, M., & Stokke, B. T. (2008). Higher order structure of (1,3)-β-D-glucans and its influence on their biological activities and complexation abilities. *Biopolymers, 89*(4), 310-321. https://doi.org/10.1002/bip.20920

Smeekens, S. P., Gresnigt, M. S., Becker, K. L., Cheng, S. C., Netea, S. A., Jacobs, L., Jansen, T., van de Veerdonk, F. L., Williams, D. L., Joosten, L. A., Dinarello, C. A., & Netea, M. G. (2015). An anti-inflammatory property of Candida albicans beta-glucan: Induction of high levels of interleukin-1 receptor antagonist via a Dectin-1/CR3 independent mechanism. *Cytokine, 71*(2), 215-222. https://doi.org/10.1016/j.cyto.2014.10.013

Smeekens, S. P., van de Veerdonk, F. L., van der Meer, J. W. M., Kullberg, B. J., Joosten, L. A. B., & Netea, M. G. (2010). The Candida Th17 response is dependent on mannan- and β-glucan-induced prostaglandin E2. *International Immunology, 22*(11), 889-895. https://doi.org/10.1093/intimm/dxq442

Snarr, B. D., Qureshi, S. T., & Sheppard, D. C. (2017, Aug 28). Immune Recognition of Fungal Polysaccharides. *J Fungi (Basel), 3*(3), 47. https://doi.org/10.3390/jof3030047

Solano-Gálvez, S. G., Tovar-Torres, S. M., Tron-Gómez, M. S., Weiser-Smeke, A. E., Álvarez-Hernández, D. A., Franyuti-Kelly, G. A., Tapia-Moreno, M., Ibarra, A., Gutiérrez-Kobeh, L., & Vázquez-López, R. (2018). Human dendritic cells: ontogeny and their subsets in health and disease. *Medical Sciences, 6*(4), 88.

Sonja I Gringhuis, T. M. K., Brigitte A Wevers, Bart Theelen, Michiel van der Vlist, Teun Boekhout & Teunis B H Geijtenbeek. (2012). Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. *Nature Immunology, 13*(3), 246-255.

Spolski, R., Li, P., & Leonard, W. J. (2018). Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nature Reviews Immunology, 18*(10), 648-659.

Steele, C., Rapaka, R. R., Metz, A., Pop, S. M., Williams, D. L., Gordon, S., Kolls, J. K., & Brown, G. D. (2005, Dec). The beta-glucan receptor dectin-1 recognizes specific morphologies of Aspergillus fumigatus. *PLoS Pathog, 1*(4), e42. https://doi.org/10.1371/journal.ppat.0010042

Sukumaran, V., Lowman, D. W., Sajeevan, T. P., & Philip, R. (2010). Marine yeast glucans confer better protection than that of baker's yeast in Penaeus monodon against white spot syndrome virus infection. *Aquaculture Research, 41*(12), 1799-1805. https://doi.org/https://doi.org/10.1111/j.1365-2109.2010.02520.x

Sullivan, D., & Coleman, D. (1998). *Candida dubliniensis*: Characteristics and Identification. *Journal of clinical microbiology, 36*(2), 329. https://doi.org/10.1128/JCM.36.2.329-334.1998

Svobodová, E., Staib, P., Losse, J., Hennicke, F., Barz, D., & Józsi, M. J. T. J. o. I. (2012). Differential interaction of the two related fungal species *Candida albicans* and *Candida dubliniensis* with human neutrophils. *189*(5), 2502-2511.

Takahara, K., Tokieda, S., Nagaoka, K., Takeda, T., Kimura, Y., & Inaba, K. (2011). C-type lectin SIGNR1 enhances cellular oxidative burst response against C. albicans in cooperation with Dectin-1. *European journal of immunology, 41*(5), 1435-1444. https://doi.org/10.1002/eji.200940188

Takano, T., Motozono, C., Imai, T., Sonoda, K.-H., Nakanishi, Y., & Yamasaki, S. (2017). Dectin-1 intracellular domain determines species-specific ligand spectrum by modulating receptor sensitivity. *Journal of Biological Chemistry, 292*(41), 16933-16941.

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell, 140*(6), 805-820.

Tang, J., Lin, G., Langdon, W. Y., Tao, L., & Zhang, J. (2018). Regulation of C-Type Lectin Receptor-Mediated Antifungal Immunity. *Front Immunol, 9*, 123. https://doi.org/10.3389/fimmu.2018.00123

Taylor, P. R., Brown, G. D., Reid, D. M., Willment, J. A., Martinez-Pomares, L., Gordon, S., & Wong, S. Y. (2002, Oct 1). The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *Journal of Immunology, 169*(7), 3876-3882. https://doi.org/10.4049/jimmunol.169.7.3876

Taylor, P. R., Tsoni, S. V., Willment, J. A., Dennehy, K. M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., & Brown, G. D. (2007). Dectin-1 is required for beta-glucan recognition and control of fungal infection [Article]. *Nat Immunol, 8*(1), 31-38. https://doi.org/10.1038/ni1408

Thompson, A., Griffiths, J. S., Walker, L., da Fonseca, D. M., Lee, K. K., Taylor, P. R., Gow, N. A. R., & Orr, S. J. (2019). Dependence on Dectin-1 Varies With Multiple *Candida* Species. *Frontiers in Microbiology, 10*, 1800. https://doi.org/10.3389/fmicb.2019.01800

Thornton, B. P., Vetvicka, V., Pitman, M., Goldman, R. C., & Ross, G. D. (1996, Feb 1). Analysis of the sugar specificity and molecular location of the beta-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). *J Immunol, 156*(3), 1235-1246.

Thu Ngoc Yen Nguyen, P. P., Panachai Wongsrisupphakul, Noppadol SaArd-Iam, Rangsini Mahanonda, Oranart Matangkasombut, Min-Kyung Choo & Patcharee Ritprajak. (2018). Cell wall mannan of *Candida krusei* mediates dendritic cell apoptosis and orchestrates Th17 polarization via TLR-2/MyD88-dependent pathway. *Scientific Reports*. https://doi.org/10.1038/s41598-018-35101-3

Thwe, P. M., Fritz, D. I., Snyder, J. P., Smith, P. R., Curtis, K. D., O'Donnell, A., Galasso, N. A., Sepaniac, L. A., Adamik, B. J., Hoyt, L. R., Rodriguez, P. D., Hogan, T. C., Schmidt, A. F., Poynter, M. E., & Amiel, E. (2019, Dec). Syk-dependent glycolytic reprogramming in dendritic cells regulates IL-1β production to β-glucan ligands in a TLR-independent manner. *J Leukoc Biol, 106*(6), 1325-1335. https://doi.org/10.1002/jlb.3a0819-207rr

Tian, Y., Zhuang, Y., Chen, Z., Mao, Y., Zhang, J., Lu, R., & Guo, L. (2020, 2020/10/01/). A gain-of-function mutation in PDR1 of *Candida glabrata* decreases EPA1 expression and attenuates adherence to epithelial cells through enhancing recruitment of the Mediator subunit Gal11A. *Microbiological Research, 239*, 126519. https://doi.org/https://doi.org/10.1016/j.micres.2020.126519

Timmermans, B., De Las Peñas, A., Castaño, I., & Van Dijck, P. (2018, May 20). Adhesins in *Candida glabrata*. *J Fungi (Basel), 4*(2). https://doi.org/10.3390/jof4020060

Tóth, R., Nosek, J., Mora-Montes, H. M., Gabaldon, T., Bliss, J. M., Nosanchuk, J. D., Turner, S. A., Butler, G., Vágvölgyi, C., & Gácser, A. (2019, Mar 20). *Candida parapsilosis*: from Genes to the Bedside. *Clinical Microbiology Reviews, 32*(2). https://doi.org/10.1128/cmr.00111-18

Tóth, R., Tóth, A., Papp, C., Jankovics, F., Vágvölgyi, C., Alonso, M. F., Bain, J. M., Erwig, L.-P., & Gácser, A. J. F. i. m. (2014). Kinetic studies of *Candida parapsilosis* phagocytosis by macrophages and detection of intracellular survival mechanisms. *5*, 633.

Tsang, C. S. P., Chu, F. C. S., Leung, W. K., Jin, L. J., Samaranayake, L. P., & Siu, S. C. (2007, Oct). Phospholipase, proteinase and haemolytic activities of *Candida albicans* isolated from oral cavities of patients with type 2 diabetes mellitus. *Journal of Medical Microbiology, 56*(Pt 10), 1393-1398. https://doi.org/10.1099/jmm.0.47303-0

Turner, S. A., & Butler, G. (2014). The *Candida* pathogenic species complex. *Cold Spring Harbor perspectives in medicine, 4*(9), a019778-a019778. https://doi.org/10.1101/cshperspect.a019778

Vale-Silva, L. A., Moeckli, B., Torelli, R., Posteraro, B., Sanguinetti, M., & Sanglard, D. (2016, Mar-Apr). Upregulation of the Adhesin Gene EPA1 Mediated by PDR1 in *Candida glabrata* Leads to Enhanced Host Colonization. *mSphere, 1*(2). https://doi.org/10.1128/mSphere.00065-15

van Vliet, S. J., den Dunnen, J., Gringhuis, S. I., Geijtenbeek, T. B., & van Kooyk, Y. (2007, Aug). Innate signaling and regulation of Dendritic cell immunity. *Current opinion in immunology, 19*(4), 435-440. https://doi.org/10.1016/j.coi.2007.05.006

Varga, G., Balkow, S., Wild, M. K., Stadtbaeumer, A., Krummen, M., Rothoeft, T., Higuchi, T., Beissert, S., Wethmar, K., Scharffetter-Kochanek, K., Vestweber, D., & Grabbe, S. (2006). Active MAC-1 (CD11b/CD18) on DCs inhibits full T-cell activation. *Blood, 109*(2), 661-669. https://doi.org/10.1182/blood-2005-12-023044

Wakkach, A., Fournier, N., Brun, V., Breittmayer, J.-P., Cottrez, F., & Groux, H. (2003). Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity, 18*(5), 605-617.

Walachowski, S., Tabouret, G., & Foucras, G. (2016). Triggering dectin-1-pathway alone is not sufficient to induce cytokine production by murine macrophages. *PLoS One, 11*(2), e0148464.

Walsh, T. J., Katragkou, A., Chen, T., Salvatore, C. M., & Roilides, E. (2019, Jan 24). Invasive Candidiasis in Infants and Children: Recent Advances in Epidemiology, Diagnosis, and Treatment. *J Fungi (Basel), 5*(1), 1-9. https://doi.org/10.3390/jof5010011

Wang, D., Gao, S., Chen, J., Zhao, Y., Jiang, Y., Chu, X., Wang, X., Liu, N., Qin, T., Yi, Q., Yue, Y., & Wang, S. (2018, Jun). Dectin-1 stimulates IL-33 expression in dendritic cells via upregulation of IRF4. *Lab Invest, 98*(6), 708-714. https://doi.org/10.1038/s41374-018-0047-2

Wang, S., Gao, X., Shen, G., Wang, W., Li, J., Zhao, J., Wei, Y.-Q., & Edwards, C. K. (2016). Interleukin-10 deficiency impairs regulatory T cell-derived neuropilin-1 functions and promotes Th1 and Th17 immunity. *Scientific Reports, 6*(1), 1-16.

Wang, Y., Shi, C., Liu, J. Y., Li, W. J., Zhao, Y., & Xiang, M. J. (2016, Oct). Multilocus sequence typing of *Candida tropicalis* shows clonal cluster enrichment in azole-resistant isolates from patients in Shanghai, China. *Infect Genet Evol, 44*, 418-424. https://doi.org/10.1016/j.meegid.2016.07.026

Whibley, N., & Gaffen, S. L. (2015, 2015/11/01/). Beyond *Candida albicans*: Mechanisms of immunity to non-*albicans Candida* species. *Cytokine, 76*(1), 42-52. https://doi.org/https://doi.org/10.1016/j.cyto.2015.07.025

Whitney, P. G., Bär, E., Osorio, F., Rogers, N. C., Schraml, B. U., Deddouche, S., LeibundGut-Landmann, S., & Reis e Sousa, C. (2014). Syk signaling in dendritic cells orchestrates innate resistance to systemic fungal infection. *PLOS Pathogens, 10*(7), e1004276-e1004276. https://doi.org/10.1371/journal.ppat.1004276

Willment, J. A., Lin, H.-H., Reid, D. M., Taylor, P. R., Williams, D. L., Wong, S. Y., Gordon, S., & Brown, G. D. (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. *The Journal of Immunology, 171*(9), 4569-4573.

Wu, P. F., Liu, W. L., Hsieh, M. H., Hii, I. M., Lee, Y. L., Lin, Y. T., Ho, M. W., Liu, C. E., Chen, Y. H., & Wang, F. D. (2017, Oct 11). Epidemiology and antifungal susceptibility of candidemia isolates of non-*albicans Candida* species from cancer patients. *Emerg Microbes Infect, 6*(10), e87. https://doi.org/10.1038/emi.2017.74

Wüthrich, M., George S. Deepe, J., & Klein, B. (2012). Adaptive Immunity to Fungi. *Annual Review of Immunology, 30*(1), 115-148. https://doi.org/10.1146/annurev-immunol-020711-074958

Xia, Y., Vetvicka, V., Yan, J., Hanikýrová, M., Mayadas, T., & Ross, G. D. (1999, Feb 15). The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J Immunol, 162*(4), 2281-2290.

Xiao, Z., Zhou, W., & Zhang, Y. (2020). Fungal polysaccharides. *Advances in Pharmacology, 87*, 277-299. https://doi.org/10.1016/bs.apha.2019.08.003

Yadav, B., Mora-Montes, H. M., Wagener, J., Cunningham, I., West, L., Haynes, K., Brown, A. J. P., & Gow, N. A. R. (2020, 2020/12/01/). Differences in fungal immune recognition by monocytes and macrophages: N-mannan can be a shield or activator of immune recognition. *The Cell Surface, 6*, 100042. https://doi.org/https://doi.org/10.1016/j.tcsw.2020.100042

Yamanaka, D., Takatsu, K., Kimura, M., Swamydas, M., Ohnishi, H., Umeyama, T., Oyama, F., Lionakis, M. S., & Ohno, N. (2020). Development of a novel ß-1, 6-glucan–specific detection system using functionally-modified recombinant endo-ß-1, 6-glucanase. *Journal of Biological Chemistry, 295*(16), 5362-5376.

Yu, S., Li, W., Liu, X., Che, J., Wu, Y., & Lu, J. (2016). Distinct Expression Levels of ALS, LIP, and SAP Genes in *Candida tropicalis* with Diverse Virulent Activities. *Frontiers in Microbiology, 7*, 1175-1175. https://doi.org/10.3389/fmicb.2016.01175

Zhao, X., Guo, Y., Jiang, C., Chang, Q., Zhang, S., Luo, T., Zhang, B., Jia, X., Hung, M.-C., Dong, C., & Lin, X. (2017, 2017/03/01). JNK1 negatively controls antifungal innate immunity by suppressing CD23 expression. *Nature Medicine, 23*(3), 337-346. https://doi.org/10.1038/nm.4260

Zhou, L., Chong, M. M., & Littman, D. R. (2009). Plasticity of CD4+ T cell lineage differentiation. *Immunity, 30*(5), 646-655.

Zimmerman, J. W., Lindermuth, J., Fish, P. A., Palace, G. P., Stevenson, T. T., & DeMong, D. E. (1998). A novel carbohydrate-glycosphingolipid interaction between a ß-(1–3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. *Journal of Biological Chemistry, 273*(34), 22014-22020.

VITA

NAME	TRUC THI HUONG DINH
DATE OF BIRTH	10 Dec 1983
PLACE OF BIRTH	Soc Trang province, Vietnam country
INSTITUTIONS ATTENDED	1. Bachelor's degree in Medical doctor at Can Tho Univesity of Medicine and Pharmacy, Can Tho province, Vietnam country. 2. Master's degree in Functional Medicine at Hue University of Medicine and Pharmacy, Thua Thien Hue province, Vietnam country. 3. All her researches were mainly supervised by supports of Assist. Prof. Patcharee Ritprajak and performed at Oral Biology Research Center, Faculty of Dentistry, Chulalongkorn University.
HOME ADDRESS	No. 85 - Cong Binh street - Tra An ward - Binh Thuy district - Can Tho city - Vietnam country