

AN IL-4-DEPENDENT MACROPHAGE-INKT CELL CIRCUIT RESOLVES
STERILE INFLAMMATION AND IS DEFECTIVE IN MICE WITH CHRONIC
GRANULOMATOUS DISEASE

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DEDICATION

I dedicate this dissertation to my mother, Miaoxi Zeng, who inspired me to be a caring, giving and active person.

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ABSTRACT

Melody Yue Zeng

AN IL-4-DEPENDENT MACROPHAGE-INKT CELL CIRCUIT RESOLVES STERILE INFLAMMATION AND IS DEFECTIVE IN MICE WITH CHRONIC GRANULOMATOUS DISEASE

The immune system initiates tissue repair following injury. In response to sterile tissue injury, neutrophils infiltrate the tissue to remove tissue debris and subsequently undergo apoptosis. Proper clearance of apoptotic neutrophils in the tissue by recruited macrophages, in a process termed efferocytosis, is critical to facilitate the resolution of inflammation and tissue repair. However, the events leading to suppression of sterile inflammation following efferocytosis, and the contribution of other innate cell types are not clearly defined in an in vivo setting. Using a sterile mouse peritonitis model, we identified IL-4 production from efferocytosing macrophages in the peritoneum that activate invariant NKT cells to produce cytokines including IL-4 and IL-13. Importantly, IL-4 from macrophages functions in autocrine and paracrine circuits to promote alternative activation of peritoneal exudate macrophages and augment type-2 cytokine production from NKT cells to suppress inflammation. The increased peritonitis in mice deficient in IL-4, NKT cells, or IL-4Ra expression on myeloid cells suggested that each is a key component for resolution of sterile inflammation. The phagocyte NADPH oxidase, a multi-subunit enzyme complex we demonstrated to require a physical interaction between the Rac GTPase and the oxidase subunit gp91^{phox} for generation of reactive oxygen species (ROS), is required for production of ROS within macrophage phagosomes containing ingested apoptotic cells. In mice with X-linked chronic

granulomatous disease (X-CGD) that lack gp91^{phox}, efferocytosing macrophages were unable to produce ROS and were defective in activating iNKT during sterile peritonitis, resulting in enhanced and prolonged inflammation. Thus, efferocytosis-induced IL-4 production and activation of IL-4-producing iNKT cells by macrophages are immunomodulatory events in an innate immune circuit required to resolve sterile inflammation and promote tissue repair.

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TABLE OF CONTENTS

LIST OF FIGURES	xvii
ABBREVIATIONS	xxii
CHAPTER ONE: INTRODUCTION	1
I. The immune system	1
A. Innate immunity.....	2
B. Adaptive immunity	6
II. Macrophage biology	10
A. The origins and development of macrophages.....	10
B. Monocyte subsets and monocyte recruitment to the tissue	11
C. Macrophage functions	13
1. Phagocytosis.....	13
2. Antigen presentation.....	15
D. Macrophage activation phenotypes	15
1. Classically activated (M1) macrophages.....	15
2. Alternative activated (M2) macrophages	17
E. Other effects of IL-4 on macrophages	19
F. Macrophages in human diseases	20
1. Type 2 diabetes.....	20
2. Cancer.....	20
3. Rheumatoid arthritis	21
4. Inflammatory bowel disease.....	21

III. Neutrophil biology	22
A. Neutrophil mobilization and recruitment	23
B. Anti-microbial function of neutrophils	24
IV. Natural killer T cell biology	25
V. Efferocytosis	28
VI. Sterile inflammation	30
VII. The phagocyte NADPH oxidase and Chronic Granulomatous Disease	34
A. The phagocyte NADPH oxidase	34
B. Regulation of the phagocyte NADPH Oxidase by Rac GTPase	38
C. Chronic Granulomatous Disease	42
D. Dysregulated inflammation in CGD patients	43
E. Dysregulated inflammation in CGD mice	44
VIII. Research goals	46
CHAPTER TWO: MATERIALS AND METHODS	48
I. Antibodies and reagents	48
II. Mice	49
III. Cell lines	49
A. Generation of PLB-985 cell lines	49
B. Generation of COS7 cell lines	50
IV. NADPH oxidase activity	50
A. Isoluminol or luminol chemiluminescence assay for superoxide generation by neutrophils	50

B. Luminol chemiluminescence assay for superoxide generation	
by macrophages	51
C. Nitroblue tetrazolium assay	52
D. Translocation of oxidase cytosolic components to the plasma membrane.....	52
E. INT reduction in permeabilized PLB-985 cells	53
V. Confocal microscopy	53
A. Live cell confocal videomicroscopy.....	53
B. Immunofluorescence microscopy	54
VI. NKT cell activity.....	55
A. In vitro NKT activation assay.....	55
B. Purification and adoptive transfer of NKT cells.....	55
VII. Efferocytosis	56
A. Sodium periodate-induced peritoneal inflammation	56
B. Isolation of neutrophils and induction of apoptosis.....	56
C. In vitro efferocytosis assays.....	57
D. Diaminobenzidine histochemistry (DAB) for myeloperoxide (MPO).....	58
E. Intracellular staining for cytokines in macrophages	58
F. Intracellular staining for cytokines in CD4+ T cells.....	59
VIII. Quantitative RT-PCR	60
IX. ELISA	60
X. Statistical analyses.....	61

CHAPTER THREE: RESULTS	62
Part I: Efferocytosis induces an IL-4-dependent macrophage-iNKT cell circuit to suppress sterile inflammation	62
1. Human peripheral blood neutrophils undergo spontaneous apoptosis ex vivo and are readily ingested by mouse peritoneal exudate macrophages	62
2. Efferocytosis of apoptotic neutrophils induce macrophages produce IL-4 in vitro	66
3. Efferocytosis of apoptotic neutrophils induce macrophages to produce IL-4 in vivo.....	72
4. Invariant NKT cells are activated to produce IL-4 to resolve sterile inflammation in the mouse peritoneum.....	80
5. Efferocytosing macrophages increased CD1d expression and activate iNKT cells	86
6. Mice lacking IL-4 displayed impaired resolution of sterile inflammation.....	92
7. IL-4 signaling to macrophages contributed to alternative activation of peritoneal macrophages and was important for the resolution of inflammation	97
Part II: Efferocytosis activates the phagocyte NADPH oxidase in macrophages	104
1. The NADPH oxidase in macrophages is activated during efferocytosis	104
2. Complement receptor 3 and TLR4/MyD88 are both required to activate the phagocyte NADPH oxidase in efferocytosing macrophages	109

3. p40 ^{phox} is required for optimal activation of the phagocyte NADPH oxidase in efferocytosing macrophages	116
4. ROS derived from the phagocyte NADPH oxidase are required for efficient degradation of ingested apoptotic cells and suppression of proinflammatory response in macrophages.....	118
Part III: The efferocytosis-induced IL-4-dependent macrophage-iNKT cell circuit is defective in gp91 ^{phox} -deficient X-CGD mice	
1. X-CGD mice exhibited enhanced peritoneal inflammation and delayed resolution.....	125
2. Activation of peritoneal iNKT cells was impaired in X-CGD mice	132
Part IV. A Rac-gp91 ^{phox} interaction is important for electron transfer on the phagocyte NADPH oxidase and assembly of the oxidase on phagosome membrane	
1. Disrupted interaction with Rac and gp91 ^{phox} led to defective NADPH oxidase activity in PLB-985 neutrophils	138
2. An intact Rac-binding domain in gp91 ^{phox} was not required for assembly of the phagocyte NADPH oxidase on plasma membrane in PMA-stimulated neutrophils	141
3. Rac-gp91 ^{phox} interaction was dispensable for recruitment of p67 ^{phox} to SOZ-phagosomes but was required for efficient Rac accumulation to SOZ or IgG bead –phagosomes	143
4. Rac-gp91 ^{phox} interaction was sufficient to target Rac1 to phagosome membrane in COS7 cells lacking p67 ^{phox}	150

5. Rac-gp91 ^{phox} interaction partially modulated electron transfer from NADPH to FAD on the phagocyte NADPH oxidase.....	155
6. RacQ61L could not overcome the loss of Rac-gp91 ^{phox} interaction to activate the NADPH oxidase.....	158
CHAPTER FOUR: DISCUSSION	161
Part I: Efferocytosis induces an IL-4-dependent macrophage-iNKT cell circuit to suppress sterile inflammation.....	161
1. Efferocytosing macrophages are an early cellular source of IL-4 to alternatively activate macrophages during the acute response to tissue injury	163
2. IL-4 in clearance of apoptotic cells and immunologic tolerance.....	165
3. Expression of IL-4 in macrophages.....	166
4. IL-4 producing-efferocytosing macrophages are antigen-presenting cells capable of both activating and promoting a type-2 cytokine response from iNKT cells.....	168
5. CD1d-dependent activation of iNKT cells by efferocytosing macrophages.....	169
6. The efferocytosing macrophage–iNKT cell circuit as a novel mechanism to resolve sterile inflammation.....	170
Part II: Efferocytosis activates the phagocyte NADPH oxidase in macrophages	172
1. CR3 in efferocytosis and activation of the phagocyte NADPH oxidase.....	174

2. The phagocyte NADPH oxidase in efferocytosis and processing of ingested apoptotic cells	176
Part III: The efferocytosis-induced IL-4-dependent macrophage-iNKT cell circuit is defective in gp91 ^{phox} -deficient mice with CGD	178
1. Exaggerated acute infiltration of neutrophils into the inflamed peritoneum of X-CGD mice	180
2. Increased IL-4 production by X-CGD efferocytosing macrophages and giant cell formation in X-CGD macrophages	181
3. The phagocyte NADPH oxidase in processing of apoptotic cells and the macrophage-iNKT cell circuit	182
Part IV: A Rac-gp91 ^{phox} interaction is important for electron transfer and assembly of the phagocyte NADPH oxidase on phagosome membrane	185
1. Differential mechanisms to recruit and retain Rac2 on the plasma membrane and phagosome membrane	185
2. Rac-gp91 ^{phox} interaction serves as an additional checkpoint before initiation of electron flow on the NADPH oxidase	188
CHAPTER FIVE: FUTURE DIRECTIONS	190
Part I: Efferocytosis induces an IL-4-dependent macrophage-iNKT cell circuit to suppress sterile inflammation	190
1. To further delineate the contribution of IL-4 from macrophages to the resolution of sterile inflammation using macrophage-specific IL-4-deficient mice	190

2. To identify glycolipids involved in the activation of iNKT cells by efferocytosing macrophages.....	191
Part II: Efferocytosis activates the phagocyte NADPH oxidase in macrophages.....	192
1. To further characterize CR3-mediated efferocytosis and activation of the phagocyte NADPH oxidase.....	192
2. To define the function of ROS generated by the phagocyte NADPH oxidase in efferosome maturation and processing of apoptotic cells.....	193
Part III: The efferocytosis-induced IL-4-dependent macrophage-iNKT cell circuit is defective in CGD mice	195
1. To characterize NKT cell function in CGD mice and CGD patients.....	195
2. To study CD1d trafficking in X-CGD macrophages for activation of NKT cells	195
Part IV: A Rac-gp91 ^{phox} interaction is important for electron transfer and assembly of the phagocyte NADPH oxidase on phagosome membrane	197
1. To define the dependence of p67 ^{phox} for Rac2 localization to the plasma membrane and phagosome membrane using p67 ^{phox} – deficient neutrophils	197
2. To determine the importance of the interaction between Rac and gp91 ^{phox} in the activity of the phagocyte NADPH oxidase in macrophages.....	198
REFERENCES	199
CURRICULUM VITAE	

LIST OF FIGURES

Figure 1. Tissue injury – induced sterile inflammation.....	33
Figure 2. Assembly and activation of phagocyte NADPH oxidase	37
Figure 3. Chrystal structure of Rac GTPase (Rac1 or Rac 2)	40
Figure 4. A two-step model for Rac regulation of the activation of the phagocyte NADPH oxidase and electron transfer	41
Figure 5. Induction of apoptotic human neutrophils	64
Figure 6. Ingestion of human apoptotic neutrophils by mouse macrophages	65
Figure 7. Efferocytosing macrophages produce IL-4 in vitro	68
Figure 8. IL-4 signaling in efferocytosing macrophages	69
Figure 9. IL-4 expression in efferocytosing macrophages	70
Figure 10. Efferocytosis of mouse apoptotic neutrophils induces macrophages to produce IL-4 in vitro	71
Figure 11. Sodium periodate – induced inflammation in mouse peritoneum	75
Figure 12. Efferocytosing macrophages produce IL-4 in vivo	76
Figure 13. Characterization of intracellular staining for IL-4	77
Figure 14. Detection of IL-4 in peritoneal lavage and IL-4 signaling in macrophages	78
Figure 15. Sodium periodate induced GFP+ PEMs in 4get mice on day 3.....	79
Figure 16. NKT cells are present in mouse peritoneum and are activated following peritonitis.....	83
Figure 17. iNKT cells in inflamed peritoneum produced IL-4 and IFN γ during the resolution of inflammation	84

Figure 18. $J\alpha 18^{-/-}$ mice were rescued by adoptive transfer of WT NKT cells	85
Figure 19. Differential IL-4 production by thymic, liver, and peritoneal NKT cells upon activation ex vivo	88
Figure 20. Peritoneal exudate macrophages increased CD1d expression and were able to activate iNKT cells independently of IL-4	89
Figure 21. IL-4 is not required for peritoneal exudate macrophages to activate iNKT cells	90
Figure 22. Apoptotic cells induced macrophages to upregulate CD1d and activate iNKT cells	91
Figure 23. Mice lacking IL-4 displayed more exaggerated inflammation following periodate challenge	94
Figure 24. Adoptive transfer of WT splenic NKT cells was unable to rescue <i>Il4^{-/-}</i> mice.....	95
Figure 25. IL-4 from macrophages augments type-2 cytokine response in NKT cells.....	96
Figure 26. IL-4 signaling to macrophages contributed to alternative activation of peritoneal macrophages.....	100
Figure 27. Sodium periodate-induced sterile inflammation in WT BALB/C mice	101
Figure 28. IL-4 to signaling to myeloid cells was critical to resolve sterile inflammation	102
Figure 29. IRF4 from myeloid cells was important to resolve sterile inflammation	103
Figure 30. Normal ingestion of human apoptotic neutrophils by X-CGD macrophages.....	106

Figure 31. Efferocytosis of apoptotic neutrophils activated the NADPH oxidase to generate ROS in macrophages	107
Figure 32. Activation of the phagocyte NADPH oxidase in efferocytosing macrophages requires complement 3 receptor (CR3) and TLR4/MyD88 signaling	113
Figure 33. Aged apoptotic human neutrophils expressed iC3b on cell surface	115
Figure 34. p40 ^{phox} was required for optimal activation of the phagocyte NADPH oxidase in efferocytosing macrophages	117
Figure 35. The phagosome NADPH oxidase was required for efficient degradation of ingested apoptotic cells	121
Figure 36. Delayed recruitment of lysosomal marker Lamp1 to efferosomes in X-CGD PEMs	124
Figure 37. Apoptotic neutrophils induced increased production of proinflammatory cytokines in X-CGD macrophages.....	125
Figure 38. Elevated inflammation in X-CGD mice in response to challenge with sodium periodate	128
Figure 39. Increased efferocytosing macrophages and IL-4-producing macrophages in X-CGD mice as a result of elevated inflammation	129
Figure 40. Increased IL-4 produced in X-CGD macrophages induced X-CGD macrophages to form giant cells.....	130
Figure 41. Impaired cytokine response of day 4 X-CGD NKT cells	134

Figure 42. Day 4 peritoneal exudate macrophages in X-CGD mice displayed reduced CD1d expression and defective activation of iNKT hybridoma cells.....	135
Figure 43. Adoptive transfer of WT splenic NKT cells was unable to rescue X-CGD mice.....	136
Figure 44. Partial intrinsic defect in activation of X-CGD splenic NKT cells	137
Figure 45. Expression of gp91 ^{phox} mutants in PLB-985 X-CGD cells.....	139
Figure 46. Disrupted interaction between Rac and gp91 ^{phox} resulted in defective NADPH oxidase activity in PLB-985 neutrophils	140
Figure 47. Assembly of NADPH oxidase on the plasma membrane did not require Rac-gp91 ^{phox} interaction	142
Figure 48. Rac-gp91 ^{phox} interaction was dispensable for recruitment of p67 ^{phox} to phagosomes during phagocytosis of SOZ.....	146
Figure 49. Rac2-gp91 ^{phox} interaction was required for efficient recruitment of Rac2 to phagosomes	148
Figure 50. Immunostaining for both Rac1 and Rac2 on IgG bead – phagosomes.....	149
Figure 51. Expression of transgenic WT or gp91 ^{phox} Y425A in COS7 cell lines	152
Figure 52. Rac-gp91 ^{phox} interaction was sufficient to target Rac1 to phagosome membrane in COS7 cells lacking p67 ^{phox}	153
Figure 53. Electron transfer to FAD was defective in permeabilized PLB-985 gp91 Y425A cells.....	157
Figure 54. Rac1Q61L was unable to overcome the requirement of Rac-gp91 ^{phox} binding for oxidase activation in COS cells.....	160

Figure 55. Efferocytosis-induced macrophage-iNKT cell circuit in sterile inflammation	162
Figure 56. Activation of the phagocyte NADPH oxidase in efferocytosing macrophages	173
Figure 57. Impaired efferocytosing macrophage-iNKT cell circuit in mice with X-CGD	179

ABBREVIATIONS

α -GalCer	α -galactosylceramide
CGD	Chronic Granulomatous Disease
CTL	Cytotoxic T lymphocytes
DAMPs	Danger-associated molecular patterns
DC	Dendritic cell
DIC	Differential interference contrast.
DPI	Diphenyleiodonium
FAD	Flavin adenine dinucleotide
GMP	Granulocyte-macrophage progenitors
GVHD	Graft-versus-host disease
HSC	Hematopoietic stem cell
IBD	Inflammatory Bowel Disease
IL	Interleukin
HMGB1	High mobility group box 1
HRP	Horseradish peroxidase
ICAM-1	Intracellular adhesion molecule-1
IFN γ	Interferon gamma
INT	Iodonitrotetrazolium
IRF4	Interferon regulatory factor 4
MCP1	Monocyte chemoattractant protein 1
MHC I or II	Major histocompatibility complex class I or II molecule
MIP α	Macrophage inflammatory protein α

MPO	Myeloperoxidase
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitro-tetrazolium blue
NCF2	Neutrophil cytosolic factor2
NKT	Natural Killer T
NETs	Neutrophil extracellular traps
PAMPs	Pathogen associated molecular pattern
PE	Phosphatidylethanolamine
PEM	Peritoneal exudate macrophage
PI3P	Phosphatidylinositol 3-phosphate
PRRs	Pathogen recognition receptors
PX	Phox-homolog
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T2D	Type 2 Diabetes
TCR	T cell receptor
Tfh cells	Follicular helper T cells
SLE	System lupus erythematosus