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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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	Diphenyleneiodonium
FAD	Flavin adenine dinucleotide
GMP	Granulocyte-macrophage progenitors
GVHD	Graft-versus-host disease
HSC	Hematopietic stem cell
IBD	Inflammatory Bowel Disease
IL	Interleukin
HMGB1	High mobility group box 1
HRP	Horseradish peroxidase
ICAM-1	Intracellular adhesion molecule-1
IFNγ	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
MIPα	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