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The role of IL-4Rα signaling during *Toxoplasma gondii* infection

by

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A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

2010

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: Date:

ACKNOWLEDGEMENTS

First I would like to thank Professor Frank Brombacher and Professor James Alexander for offering me the opportunity to do a PhD here. To my primary supervisor Professor James Alexander, thank you for the constant support and guidance throughout the past few years. It has been a great learning curve. I would also like to thank my second supervisor Dr Craig Roberts for his support throughout my time here, I have acquired a lot of knowledge and skills under your supervision. Dr Fiona Henriques, thank you for your support and contribution to my work. I would also like to thank Dr Fiona Roberts for her contribution with the histology. I am very thankful for the technical assistance from the staff at the BPU, Gordon, Carol, Allan, George, thank you so much. Mrs Adrienne McGachy, thank you for always being your wonderful self and keeping my morale up. I cannot thank you enough for always being available to help with those T-cell assays! I would also like to thank my lab mates, past and present, who I have learnt a lot from and have been a pleasure to work with: Leigh, Emma, Fiona, Caroline, Sara, Karen, Selina, Chesney and everyone else in the lab. I would also like to thank Roderick Williams for giving advice and some much needed words of wisdom. Lastly, this would not have been possible without the support of my family. My mom and dad, SiPhuti and Daddy, your unconditional love and unwavering support are what kept me going. *Ga ke gola ke batla go tshwana le lona*. *Ke leboga Modimo ka go ntlhopela lona*. Thank you to the rest of my family and friends, my sister, uncles and aunts for being there for me. Masego thanks for always being there to listen. I'm blessed to have you all in my life. SiPhuti le Daddy, this is for you, for being my absolute rock stars. *Ke a leboga.*

ABSTRACT

A type-1 cytokine response is critical for protective immunity against *Toxoplasma gondii* infection. Nevertheless, a persistent overproduction of type-1 cytokines can be detrimental to the host and cause fatal immunopathology. The extent to which type-2 cytokines can modulate the disease exacerbating versus protective responses remains largely unresolved. Previous studies have shown that IL-4 and IL-4Rα deficient BALB/c mice are highly susceptible to *T. gondii* infection. IL-4 can modulate type-1 inflammatory responses by counter-regulating the effects of IFN-γ on $CD4^+$ T cells and/or macrophages. In order to analyze the protective mechanism(s) and functional target(s) of IL-4 during *T. gondii* infection the outcomes of *T. gondii* infection in macrophage/neutrophil specific IL-4Rα$^{-/-}$ BALB/c ($LysM^{cre}$IL-4Rα$^{-/lox}$) mice, $CD4^+$ T cell specific IL-4Rα$^{-/-}$ (Lck^{cre}IL-4Rα$^{-/lox}$) mice, $CD4^+CD8^+$T cell specific IL-4Rα$^{-/-}$ ($NLck^{cre}$IL-4Rα$^{-/lox}$) mice and their wild type (IL-4Rα$^{-/lox}$) littermates as well as global IL-4Rα$^{-/-}$ mice were compared. Overall female mice were more susceptible to infection compared with male mice as measured by mortality and this was associated with delayed parasite specific type-1 cytokine responses. *T. gondii* infection in $LysM^{cre}$IL-4Rα$^{-/lox}$ mice resulted in an augmented type-1 cytokine response and excessive lung pathology which caused increased mortality which was similar to global IL-4Rα$^{-/-}$ mice and more severe than IL-4Rα intact (WT) mice. On the other hand Lck^{cre}IL-4Rα$^{-/lox}$ were relatively resistant to infection and had a similar phenotype to WT mice, whilst $NLck^{cre}$IL-4Rα$^{-/lox}$ mice exhibited increased susceptibility during the chronic phase of

infection. Splenocytes from NLck^{cre}IL-4R$\alpha^{-/lox}$ mice but not Lck^{cre}IL-4R$\alpha^{-/lox}$ had impaired parasite-specific IFN-γ production. *T. gondii* was demonstrated to induce various elements of alternative macrophage activation independently of IL-4Rα while RT-PCR analysis revealed that markers of alternative macrophage activation YM1, Arginase1 and FIZZ1 may contribute to disease protective and/or exacerbative processes during infection. Collectively, these findings illustrate the multifaceted innate and adaptive IL-4/IL-13-mediated responses employed to influence *T. gondii* infection.

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ABBREVIATIONS

Arg1	Arginase1
BAL	Broncheoalveolar lavage
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation
cDNA	Complementary Deoxyribonucleic Acid
CNS	Central Nervous System
ConA	Concavalin A
DC	Dendritic cell
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FIZZ1	Found in inflammatory zone-1
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
HP	High Power
IDO	Indoleamine 2,3-dioxygenase
IEL	Intraepithelial cells
IFN-	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-4R	Interleukin-4 Receptor
iNOS	inducible Nitric oxide synthase
I.P	Intraperitoneal

kDA	Kilo Daltons
KO	Knock-out
LP	Low Power
LPS	Lipopolysaccharide
MHC	Major Histocompatability Complex
MIF	Macrophage migration Inhibitory Factor
mRNA	Messenger Ribonucleic Acid
MyD88	Myeloid Differentiation Factor 88
MØ	Macrophage
NeMØ	Nematode elicited macrophages
NK	Natural Killer
NO	Nitric oxide
NOS2	Nitric oxide synthase-2
p	p-value
p.i	post infection
PMN	Polymorphonuclear leukocytes
PV	Parasitophorous Vacuole
PV cuffing	Perivascular cuffing
RELM-α	Resistin-like molecule-α
RNA	Ribonucleic acid
RNI	Reactive nitrogen intermediates
rpm	Revolutions per minute

SCID	Severe combined Immune Deficient
STAT	Signal transducer and activator of transcription
Tbp	TATA-box Binding protein
TE	Toxoplasmic encephalitis
TgCyst	*Toxoplasma gondii* Cyst matrix antigen
TGF	Tumour Growth Factor
Th	T helper
TLA	*Toxoplasma* Lysate Antigen
TLR	Toll-Like Receptor
TNF	Tumour Necrosis Factor
SEM	Standard Error of Mean