The role of the $P2X_7$ receptor in the intestinal inflammatory response to the parasite, *Toxoplasma gondii*

by

Alana Maree Zakrzewski

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Institute for the Biotechnology of Infectious Diseases,

Faculty of Science, University of Technology, Sydney,

Australia

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Certificate

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Alana Zakrzewski

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Publications

Journal Articles

Some of the research reported in this thesis has been published:

- Lees M, Fuller SJ, McLeod R, Miller CM, Zakrzewski AM, Boulter NR, Mui E, Witola W, Coyne JJ, Hargrave A, Jamieson S, Blackwell J, Wiley J and Smith NC (2010) $P2X_7$ Receptor-Mediated Killing of an Intracellular Parasite, *Toxoplasma gondii*, by Human and Murine Macrophages. *Journal of Immunology*. 184, 7040-7046
- Miller CM, Boulter NR, Fuller SJ, Zakrzewski AM, Lees MP, Saunders BM, Wiley JS and Smith NC. (2011) The role of the $P2X_7$ receptor in infectious diseases. Accepted for publication *PLoS Pathogens*.

Conference Presentations and Abstracts

- Zakrzewski AM, Miller CM, Ikin RI, Fuller SJ, Wiley JS and Smith NC. $P2X_7$ receptor deficient mice exhibit severe histopathology and dysregulated nitric oxide production in *Toxoplasma gondii* induced ileitis. International Congress of Parasitology, 16^{th}-20^{th} August 2010, Melbourne
- Zakrzewski AM, Smith NC, Ikin RI, Fuller SJ, Wiley JS and Miller CM. Control of nitric oxide production in toxoplasmic ileitis is dysregulated in mice lacking the $P2X_7$ receptor. Gordon Conference- Biology of Host-Parasite Interactions, 27^{th} June-2^{nd} July 2010, Salve Regina University, Rhode Island, USA.
- Zakrzewski AM, Ikin RJ, Smith NC, Lees M, Boulter N, Fuller S, Wiley J and Miller CM. The $P2X_7$ receptor mediates killing of, and modulates inflammatory responses to, the protozoan parasite, *Toxoplasma gondii*. RNSH/UTS/USyd/Kolling Institute XXVIth Annual Scientific Research Meeting 24-25th November 2009 Sydney, N.S.W.
- Zakrzewski AM, Ikin RJ, Smith NC, Lees M, Boulter N, Fuller S, Wiley J and Miller CM. A dual role for the $P2X_7$ receptor in toxoplasmosis? Australian Society for Parasitology and ARC/NHMRC Research Network for Parasitology Annual Conference, 12^{th}-15^{th} July, 2009, University of Sydney
- Smith NC, Zakrzewski AM, Ikin RJ, lees M, Boulter N, Fuller S, Wiley J and Miller CM. The role of the $P2X_7$ receptor in immunopathology associated

with infection with *Toxoplasma gondii.* 10th International Congress on Toxoplasmosis, 19th-23rd June, 2009, Kerkrade, The Netherlands

- Zakrzewski AM, Smith NC, Miller CM, Boulter N, Fuller S, Wiley J, Jamieson S, Blackwell J, Mui E, Witola W, Coyne JJ, Hargrave A, McLeod R and Lees M. $P2X_7$ receptor-mediated killing of *Toxoplasma gondii* in human and murine cells. 10th International Congress on Toxoplasmosis, 19th-23rd June, 2009, Kerkrade, The Netherlands

- Zakrzewski AM, Miller CM, Ikin RJ, Boulter N, Fuller S, Wiley J and Smith NC. The course of infection with *Toxoplasma gondii* in mice lacking $P2X_7$ receptor function. 2008 ASP & ARC/NHMRC Research Network for Parasitology Annual Conference 6-9 July, Adelaide, SA

- Zakrzewski AM, Miller CM, Ikin RJ and Smith NC. The role of the $P2X_7$ receptor in the innate immune response to the protozoan parasite *Toxoplasma gondii* RNSH/UTS/USyd/Kolling Institute XXIVth Annual Scientific Research Meeting 13-14th November 2007 Sydney, N.S.W.

Invited Seminars

Some of the research reported in this thesis has been presented in seminars at:

The University of Zurich, Switzerland

Zakrzewski AM, Miller CM, Ikin R, lees M, Boulter N, Fuller S, Wiley J and Smith NC. The role of the $P2X_7$ receptor in immunopathology associated with infection with *Toxoplasma gondii*

The National Institutes of Health, Bethesda, USA.

Zakrzewski AM, Smith NC, Ikin RI, Fuller SJ, Wiley JS and Miller CM. Control of nitric oxide production in toxoplasmic ileitis is dysregulated in mice lacking the $P2X_7$ receptor.

Awards and Prizes

- 2010 ICOPA Committee Open Poster Prize
- 2010 UTS Science Faculty 3 minute thesis winner
- 2010 UTS 3 minute thesis People's Choice Award
- Young Investigator Award at the 2009 RNSH / UTS / USyd / Kolling Institute Scientific Research Meeting. A $4000 international travel grant awarded for the best oral presentation.
- 2009 Australia Society for Parasitology student award for best 15 minute oral presentation. A year's subscription to the Journal 'Trends in Parasitology' awarded for the best student oral presentation at the annual conference.
- 2009 Vice-Chancellor's University Conference Fund award
- 2009 University of Technology Faculty of Science Post Graduate Conference Fund award
- 2009 Institute for the Biotechnology of Infectious Diseases Conference Fund award
- University of Technology Sydney, doctoral scholarship recipient, 2008.
- Institute for the Biotechnology of Infectious Diseases, top-up scholarship recipient, 2008.
- Best Poster Prize at the 2007 RNSH / UTS / USyd / Kolling Institute Scientific Research Meeting. A $750 cheque awarded for the best poster presentation at the conference.

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
ATCC	American type culture collection
ATP	Adenosine triphosphate
BBG	Brilliant blue G
BCG	Bacillus Calmette-Guérin
BMM	Bone marrow-derived macrophage
BzATP	Benzoyl-benzoyl adenosine triphosphate
CBA	Cytometric bead array
CNS	Central nervous system
CREB	c-adenosine monophosphate responsive element binding protein
DAMP	Damage associated molecular patterns
DC	Dendritic cell
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorting
FBS	Foetal bovine serum
FSW	FACS stain/wash solution
GM-CSF	Granulocyte macrophage-colony stimulating factor
HBSS	Hank's balanced salt solution
HIV	Human immunodeficiency virus
HSP	Heat shock protein
iDCs	Immature dendritic cells
IFN	Interferon
IKK	Inhibitor kappa kinases
IL	Interleukin
JNK	c-Jun N-terminal kinases
MAPK	Mitogen-activated protein kinase

Mins	Minutes
MSP	Macrophage stimulating protein
MyD88	Myeloid differentiation factor 88
NBS	Newborn bovine serum
NED	N-(1-naphthyl) ethylene diamine
NF-κB	Nuclear factor-κB
NK	Natural killer
NO	Nitric oxide
oATP	Oxidised adenosine triphosphate
PAMP	Pathogen associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PLD	Phospholipase D
PRR	Pattern recognition receptor
PS	Penicillin/streptomycin
RNI	Reactive nitrogen intermediates
ROS	Reactive oxygen species
ROI	Reactive oxygen intermediates
RPMI	Roswell Park Memorial Institute
SAPK	Stress-activated protein kinase
TCA	Trichloroacetic acid
TGF-β	Transforming growth factor-β
TLR	Toll-like receptor
TNF	Tumour necrosis factor

Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It is found throughout the world, infecting a variety of warm-blooded animals; overall it infects one in three people worldwide, though local prevalence rates vary widely.

Experimental oral infection of susceptible strains of mice with cysts of *T. gondii* is known to provoke an acute inflammatory response in the intestine, known as toxoplasmic ileitis. The hypothesis tested in this thesis was that the purinergic $P2X_7$ receptor plays a critical role in intestinal inflammation. This was tested by infecting mice with 10 cysts of the ME49 strain of *T. gondii* – three strains of mouse were used: BALB/c mice, which are known to be resistant to toxoplasmic ileitis; C57BL/6J mice, which are classified as susceptible to toxoplasmic ileitis; and $P2X_7$ receptor knockout mice (which are C57BL/6J mice whose gene for the $P2X_7$ receptor has been deleted). Following infection, experiments were done to assess the effect of the absence of the $P2X_7$ receptor on: (a) weight loss and intestinal pathology; (b) parasite control; (c) changes in pro- and anti-inflammatory mediators; and (d) activation of key transcription factors and intracellular signalling pathways.

In vivo studies showed that absence of the $P2X_7$ receptor rendered mice acutely susceptible to oral infection with *T. gondii* ME49. $P2X_7$ receptor knockout mice lost weight at a much faster rate and had significantly higher levels of intestinal pathology (including swelling, angiogenesis, intestinal villous breakdown and infiltration of inflammatory cells) than either C57BL/6J or BALB/c mice.

In vitro studies confirmed that activation of the $P2X_7$ receptor could induce killing of *T. gondii* ME49. However, intestinal parasite burdens were no different in mice with or without the $P2X_7$ receptor, indicating that the inflammatory pathology in the intestines of $P2X_7$ receptor knockout mice was not due to uncontrolled replication of *T. gondii*.

The pathology and weight loss experienced by $P2X_7$ receptor knockout mice infected with *T. gondii* was associated with significantly elevated ileal levels of the pro-inflammatory cytokines, IFN-γ, MCP-1, TNF, IL-6, IL-12, IL-1β and IL-18, compared with C57BL/6J or BALB/c mice. There were no significant differences observed in the

production of the anti-inflammatory cytokines IL-10 and TGF-β between the three strains of mice, ruling out any dysregulation of anti-inflammatory pathway activation in the development of toxoplasmic ileitis in $P2X_7$ receptor-deficient mice.

The single most outstanding difference between the $P2X_7$ receptor knockout mice and mice with functional $P2X_7$ receptors was an overproduction of nitric oxide. Transcription of *iNOS,* which codes for the enzyme that generates nitric oxide, is activated by the transcription factor, NFκB. A series of *in vivo* experiments demonstrated that, without the $P2X_7$ receptor, mice lack the ability to regulate key elements of NF-κB activation, which may have contributed to the over-production of nitric oxide and inflammatory cytokines seen in these mice.

The experiments presented in this thesis improve understanding of the role played by the $P2X_7$ receptor in the intestinal immune response and pathology induced by infection and reveal a previously unrecognised role for this receptor in the regulation of intestinal inflammation.