

**The role of the P2X<sub>7</sub> receptor in the  
intestinal inflammatory response to the  
parasite, *Toxoplasma gondii***

by

**Alana Maree Zakrzewski**

BMedSc (Hons) (UTS)

A thesis submitted for the degree of Doctor of Philosophy

Institute for the Biotechnology of Infectious Diseases,  
Faculty of Science, University of Technology, Sydney,  
Australia

2011

## 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.

Production Note:  
Signature removed prior to publication.

Alana Zakrzewski

## Acknowledgements

---

My life completely changed when in 2007, as an undergraduate student, I enrolled in the subject "Parasitology" headed by Professor Nick Smith. Since meeting Nick I have grown from a shy undergraduate student, too afraid to do a 10 minute oral presentation, to a confident, capable scientist all thanks to Nick.

Nick, you have had such an amazing impact on my life and I'm truly grateful for everything you have done for me, you are my mentor and my friend and I don't think I will ever be able to thank you enough.

After meeting Nick and being accepted as his Honours student in 2008 I was fortunate enough to meet and work with Dr Kate Miller. Kate is a wonderful scientist and has taught me so much over the past 4 years. She has given me so much help and support, not only in the lab but in all aspects of my life. I am enormously thankful for everything she has done for me and don't think I could have completed my PhD without her.

I would like to thank everyone at UTS/IBID that has helped me throughout my PhD. In particular, Dr Michael Johnson for always being able to help me whenever I've needed him and Dr Marilyn Katrib for always being so lovely and willing to help wherever she can.

I thank Professor Bob Bao from the University of Sydney for all of his help with the histology aspects of my thesis. It was a real pleasure working with Bob and a very fruitful collaboration that I am very grateful for.

I honestly could not have completed this PhD without the help and friendship of Dr Amanda Hudson, Philippa Sharman, Rowan Ikin and Dr Michael Lees. You have all helped me in so many ways over the years I can't even begin to list them. I am whole heartedly thankful to all of you.

Finally I would like to thank my family and, my soon-to-be in-laws, the Wylie family for their constant support, love and encouragement throughout my PhD (in particular my grandparents!). And last but certainly not least, thank you to Pete for getting me through my PhD. No matter what I've been through you've always been there for me with constant love and support and I'm so blessed to have you in my life.

This thesis is dedicated to my father who would have been so proud of everything I have achieved and has been the biggest inspiration in helping me achieve it.

## 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<sub>7</sub> 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<sub>7</sub> 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<sub>7</sub> receptor deficient mice exhibit severe histopathology and dysregulated nitric oxide production in *Toxoplasma gondii* induced ileitis. International Congress of Parasitology, 16<sup>th</sup>-20<sup>th</sup> 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<sub>7</sub> receptor. Gordon Conference- Biology of Host-Parasite Interactions, 27<sup>th</sup> June-2<sup>nd</sup> 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<sub>7</sub> 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<sub>7</sub> receptor in toxoplasmosis? Australian Society for Parasitology and ARC/NHMRC Research Network for Parasitology Annual Conference, 12<sup>th</sup>-15<sup>th</sup> 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<sub>7</sub> receptor in immunopathology associated

with infection with *Toxoplasma gondii*. 10<sup>th</sup> International Congress on Toxoplasmosis, 19<sup>th</sup>-23<sup>rd</sup> 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<sub>7</sub> receptor-mediated killing of *Toxoplasma gondii* in human and murine cells. 10<sup>th</sup> International Congress on Toxoplasmosis, 19<sup>th</sup>-23<sup>rd</sup> 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<sub>7</sub> 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<sub>7</sub> receptor in the innate immune response to the protozoan parasite *Toxoplasma gondii* RNSH/UTS/USyd/Kolling Institute XXIVth Annual Scientific Research Meeting 13-14<sup>th</sup> 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<sub>7</sub> 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<sub>7</sub> 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.

## Table of Contents

---

CERTIFICATE.....	i
ACKNOWLEDGEMENTS.....	ii
PUBLICATIONS.....	v
TABLE OF CONTENTS.....	viii
LIST OF FIGURES AND TABLES.....	xii
ABBREVIATIONS.....	xiv
ABSTRACT.....	xvi

### Chapter 1- INTRODUCTION

<b>1.1 <i>Toxoplasma gondii</i></b> .....	<b>2</b>
1.1.1 <i>The Life cycle of Toxoplasma gondii</i> .....	2
1.1.2 <i>Toxoplasma gondii infections in humans</i> .....	4
1.1.3 <i>The immune response to Toxoplasma gondii</i> .....	4
<b>1.2 <i>ATP as a danger signal</i></b> .....	<b>9</b>
<b>1.3 <i>P2 Receptors</i></b> .....	<b>9</b>
<b>1.4 <i>The P2X receptor family</i></b> .....	<b>10</b>
<b>1.5 <i>The P2X<sub>7</sub> receptor</i></b> .....	<b>11</b>
1.5.1 <i>P2X<sub>7</sub> receptor activation</i> .....	12
1.5.2 <i>Intracellular pathways triggered by P2X<sub>7</sub> receptor activation</i> .....	13
1.5.3 <i>P2X<sub>7</sub> receptor polymorphisms</i> .....	15
1.5.4 <i>The P2X<sub>7</sub> receptor and infectious disease</i> .....	18
1.5.5 <i>The P2X<sub>7</sub> receptor and intracellular bacteria</i> .....	18
1.5.6 <i>The P2X<sub>7</sub> receptor and extracellular bacteria</i> .....	23
1.5.7 <i>The P2X<sub>7</sub> receptor and intracellular parasites</i> .....	24
1.5.8 <i>The P2X<sub>7</sub> receptor and Toxoplasma gondii</i> .....	26
<b>1.6 <i>Hypothesis and Aims</i></b> .....	<b>29</b>

### Chapter2 - Materials and Methods

<b>2.1 Materials</b> .....	<b>31</b>
2.1.1 – <i>Consumables</i> .....	31
2.1.2 – <i>Media and buffers</i> .....	33
2.1.3 – <i>Antibodies and ELISA kits</i> .....	34



2.1.4 – Cell lines, parasites and mice.....	34
2.1.5 – Instruments and equipment.....	34
2.1.6 – Software.....	35
<b>2.2 Methods.....</b>	<b>35</b>
2.2.1 – Ethics.....	35
2.2.2 – Parasites.....	35
2.2.3 – Mice.....	35
2.2.4 – In vitro tissue culture.....	36
2.2.4.1 – Vero culture.....	36
2.2.4.2 – <i>Toxoplasma gondii</i> culture.....	36
2.2.4.3 – Murine bone marrow macrophage culture.....	37
2.2.5 – In vitro parasite viability assay.....	38
2.2.6 – Cyst purification and preparation.....	39
2.2.7 – Mice and infections.....	40
2.2.8 – Sample collection and preparation for processing.....	41
2.2.8.1 Serum.....	41
2.2.8.2 Ileum collection and assessment of pathology.....	41
2.2.8.3 Preparation of intestinal homogenates for parasite burden.....	44
2.2.8.4 Preparation of spleens for parasite burden.....	44
2.2.9 – Assessment of in vivo parasite burden.....	45
2.2.9.1 Immunoperoxidase staining.....	45
2.2.9.2 Microtitre limiting dilution assay.....	46
2.2.9.3 Plaque forming assay.....	46
2.2.10 – Inflammatory cytokine analysis.....	47
2.2.10.1 Ileum homogenates for cytokine analysis.....	47
2.2.10.2 BD Cytometric Bead Array.....	47
2.2.10.3 Enzyme-linked immunosorbent assay.....	49
2.2.10.4 Inflammation multi-target ELISA.....	50
2.2.11 – Measurement of reactive nitrogen intermediates.....	51

## **Chapter 3- The effect of the P2X<sub>7</sub> receptor on toxoplasmic ileitis**

<b>3.1 Introduction.....</b>	<b>53</b>
<b>3.2 Methods and Results.....</b>	<b>54</b>
3.2.1 Susceptibility of mice to infection.....	54
3.2.2 Pathology.....	56
3.2.2.1 Gross pathology.....	56

3.2.2.2 Histopathology.....	58
<b>3.3 Discussion.....</b>	<b>60</b>
<b>Chapter 4- P2X<sub>7</sub> receptor-mediated control of <i>T. gondii</i> <i>in vitro</i> and <i>in vivo</i></b>	
<b>4.1 Introduction.....</b>	<b>65</b>
<b>4.2 Methods and Results.....</b>	<b>66</b>
4.2.1 <i>In vitro</i> assessment of parasite viability.....	66
4.2.2 <i>In vivo</i> assessment of parasite burden.....	66
<b>4.3 Discussion.....</b>	<b>70</b>
<b>Chapter 5- The effect of the P2X<sub>7</sub> receptor on the modulation of the intestinal inflammatory response to oral infection with <i>Toxoplasma gondii</i></b>	
<b>5.1 Introduction.....</b>	<b>75</b>
<b>5.2 Methods and Results.....</b>	<b>76</b>
5.2.1 IFN- $\gamma$ .....	76
5.2.2 MCP-1.....	78
5.2.3 TNF.....	78
5.2.4 IL-6.....	81
5.2.5 IL-12.....	81
5.2.6 IL-1 $\beta$ .....	84
5.2.7 IL-18.....	84
5.2.8 TGF- $\beta$ .....	87
5.2.9 IL-10.....	87
5.2.10 Reactive Nitrogen Intermediates.....	87
<b>5.3 Discussion.....</b>	<b>91</b>
<b>Chapter 6- Effect of the P2X<sub>7</sub> receptor on intracellular inflammatory pathways following oral infection with <i>Toxoplasma gondii</i></b>	
<b>6.1 Introduction.....</b>	<b>95</b>
<b>6.2 Methods and Results.....</b>	<b>96</b>
6.2.1 NF $\kappa$ B p65.....	96
6.2.2 Phospho-NF $\kappa$ B p65.....	97
6.2.3 Phospho-SAPK/JNK.....	98
6.2.4 Phospho-p38.....	99
6.2.5 Phospho-IK $\beta$ .....	100

**6.3 Discussion.....101**

**Chapter 7- General Discussion**

**7.1 Key Findings and Future Experiments.....105**

**7.2 Significance.....109**

**References.....113**

## List of Figures and Tables

---

### Chapter 1- Introduction

Figure 1.1- The life cycle of <i>Toxoplasma gondii</i> .....	3
Figure 1.2- Sequence of events following oral infection with <i>Toxoplasma gondii</i> .....	6
Figure 1.3- Structural diagram of the P2X receptors.....	11
Figure 1.4- Intracellular signalling pathways in immune cells stimulated by P2X <sub>7</sub> receptor activation.....	14
Table 1.1- Important change-in-function polymorphisms in the P2X <sub>7</sub> receptor that have been identified in humans.....	16
Table 1.2- P2X <sub>7</sub> receptor-mediated effects of extracellular ATP on intracellular pathogens macrophages.....	20

### Chapter 2- Materials and Methods

Figure 2.1- Six well template for <i>T. gondii</i> viability assay.....	39
Table 2.1: Pathology scoring system for gross ileal pathology.....	42
Table 2.2- Tissue processing schedule for ileal sections from infected and control mice.....	43
Table 2.3: Ileal histopathology scoring system.....	43
Table 2.4- Dilution factors used in microtitre limiting dilution assay used to assess parasite burden in mice orally infected with Me49 <i>T. gondii</i> .....	46

### Chapter 3- The effect of the P2X<sub>7</sub> receptor on toxoplasmic ileitis

Figure 3.1- Cumulative weight changes in male BALB/c, C57BL/6J and P2X <sub>7</sub> R <sup>-/-</sup> mice following oral infection with 10 <i>T. gondii</i> Me49 cysts .....	55
Figure 3.2- Time to euthanasia of male BALB/c, C57BL/6J and P2X <sub>7</sub> R <sup>-/-</sup> mice following oral infection with 10 <i>T. gondii</i> Me49 cysts .....	57
Figure 3.3- Gross ileal pathology in male BALB/c, C57BL/6J and P2X <sub>7</sub> R <sup>-/-</sup> mice following oral infection with 10 <i>T. gondii</i> Me49 cysts.....	58
Figure 3.4- Representative photomicrograph portraying a Haematoxylin and Eosin stained ileal section from a healthy uninfected mouse.....	59
Figure 3.5- Microscopic analysis of Haematoxylin and Eosin stained ileal sections.....	61
Figure 3.6- Ileal histopathology scores in BALB/c, C57BL/6J and P2X <sub>7</sub> R <sup>-/-</sup> mice following oral infection with 10 <i>T. gondii</i> Me49 cysts.....	62

## **Chapter 4- P2X<sub>7</sub> receptor-mediated control of *T. gondii* in vitro and in vivo**

Figure 4.1- <i>T. gondii</i> Me49 viability following P2X <sub>7</sub> receptor activation of murine BMM.....	67
Figure 4.2- Immunoperoxidase stained ileal sections from BALB/c (A), C57BL/6J (B) and P2X <sub>7</sub> R <sup>-/-</sup> (C) mice using anti- <i>Toxoplasma gondii</i> antibodies.....	68
Figure 4.3- Ileal parasite burden in mice orally infected with <i>T. gondii</i> Me49 as assessed using a microtitre limiting dilution assay.....	69
Figure 4.4- Ileal parasite burden in mice orally infected with <i>T. gondii</i> Me49 as assessed using a plaque formation assay.....	70
Figure 4.5- Splenic parasite burden in mice orally infected with <i>T. gondii</i> Me49 as assessed using a microtitre limiting dilution assay.....	71

## **Chapter 5- The effect of the P2X<sub>7</sub> receptor on the modulation of the intestinal inflammatory response to oral infection with *Toxoplasma gondii***

Figure 5.1- Ileal IFN- $\gamma$ concentrations in <i>T. gondii</i> Me49 infected mice.....	77
Figure 5.2- Ileal MCP-1 concentrations in <i>T. gondii</i> Me49 infected mice.....	79
Figure 5.3- Ileal TNF concentrations in <i>T. gondii</i> Me49 infected mice.....	80
Figure 5.4- Ileal IL-6 concentrations in <i>T. gondii</i> Me49 infected mice.....	82
Figure 5.5- Ileal IL-12 concentrations in <i>T. gondii</i> Me49 infected mice.....	83
Figure 5.6- Ileal IL-1 $\beta$ concentrations in <i>T. gondii</i> Me49 infected mice.....	85
Figure 5.7- Serum IL-18 concentrations in <i>T. gondii</i> Me49 infected mice.....	86
Figure 5.8- Ileal TGF- $\beta$ concentrations in <i>T. gondii</i> Me49 infected mice.....	88
Figure 5.9- Ileal IL-10 concentrations in <i>T. gondii</i> Me49 infected mice.....	89
Figure 5.10- Ileal RNI concentrations in <i>T. gondii</i> Me49 infected mice.....	90

## **Chapter 6- Effect of the P2X<sub>7</sub> receptor on intracellular inflammatory pathways following oral infection with *Toxoplasma gondii***

Figure 6.1- Ileal NF $\kappa$ B p65 levels in <i>T. gondii</i> Me49 infected mice.....	97
Figure 6.2- Ileal Phospho-NF $\kappa$ B p65 levels in <i>T. gondii</i> Me49 infected mice.....	98
Figure 6.3- Ileal Phospho-SAPK/JNK levels in <i>T. gondii</i> Me49 infected mice.....	99
Figure 6.4- Ileal Phospho-p38 levels in <i>T. gondii</i> Me49 infected mice.....	100
Figure 6.5- Ileal Phospho-IK $\beta$ a levels in <i>T. gondii</i> Me49 infected mice.....	101

## 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- $\kappa$ B	Nuclear factor- $\kappa$ B
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- $\beta$	Transforming growth factor- $\beta$
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<sub>7</sub> 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<sub>7</sub> receptor knockout mice (which are C57BL/6J mice whose gene for the P2X<sub>7</sub> receptor has been deleted). Following infection, experiments were done to assess the effect of the absence of the P2X<sub>7</sub> 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<sub>7</sub> receptor rendered mice acutely susceptible to oral infection with *T. gondii* ME49. P2X<sub>7</sub> 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<sub>7</sub> receptor could induce killing of *T. gondii* ME49. However, intestinal parasite burdens were no different in mice with or without the P2X<sub>7</sub> receptor, indicating that the inflammatory pathology in the intestines of P2X<sub>7</sub> receptor knockout mice was not due to uncontrolled replication of *T. gondii*.

The pathology and weight loss experienced by P2X<sub>7</sub> receptor knockout mice infected with *T. gondii* was associated with significantly elevated ileal levels of the pro-inflammatory cytokines, IFN- $\gamma$ , MCP-1, TNF, IL-6, IL-12, IL-1 $\beta$  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- $\beta$  between the three strains of mice, ruling out any dysregulation of anti-inflammatory pathway activation in the development of toxoplasmic ileitis in P2X<sub>7</sub> receptor-deficient mice.

The single most outstanding difference between the P2X<sub>7</sub> receptor knockout mice and mice with functional P2X<sub>7</sub> 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, NF $\kappa$ B. A series of *in vivo* experiments demonstrated that, without the P2X<sub>7</sub> receptor, mice lack the ability to regulate key elements of NF- $\kappa$ B 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<sub>7</sub> 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.