

The Role of Complement in Immunity to *Nippostrongylus brasiliensis*

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

as a portfolio of publications

by

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TABLE OF CONTENTS

ABSTRACT.....	i
DECLARATION.....	iii
ACKNOWLEDGEMENT OF ANY HELP.....	iv
STATEMENT OF AUTHORSHIP-CHAPTER 2.....	v
STATEMENT OF AUTHORSHIP-CHAPTER 3.....	vii
STATEMENT OF AUTHORSHIP-CHAPTER 4.....	x
ACKNOWLEDGEMENTS.....	xvi
PUBLICATIONS.....	xviii
COMMONLY-USED ABBREVIATIONS.....	xix

CHAPTER 1: INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 HEALTH AND ECONOMIC CONSEQUENCES OF PARASITIC HELMINTH INFECTIONS	1
1.2 IMMUNE RESPONSES TO HELMINTH INFECTION	2
1.2.1 Immune recognition and antigen processing	3
1.2.2 Cytokine responses during helminth infection	4
1.2.3 Immunological basis of gastrointestinal helminth expulsion	6
1.3 LEUKOCYTE-MEDIATED KILLING OF HELMINTHS	9
1.3.1 Neutrophils.....	9
1.3.2 Macrophages.....	10
1.3.3 Eosinophils.....	10
1.3.3.1 IL-5 and eosinophils	11
1.3.3.2 Eosinophil recruitment.....	12
1.3.3.3 Eosinophil activation, secretion and degranulation	13
1.3.3.4 Role of eosinophils in disease.....	14
1.3.3.5 Role of eosinophils is killing helminths	15
1.3.3.6 Other roles for eosinophils during helminth infections	17
1.4 THE COMPLEMENT SYSTEM	19
1.4.1 Function of the complement system	19
1.4.2 Pathways to complement activation	20
1.4.3 Complement-dependent immunity to pathogens	21
1.5 ROLE OF COMPLEMENT IN IMMUNITY TO HELMINTHS.....	23
1.5.1 Complement activation by helminths	25
1.5.2 Complement-dependent leukocyte-mediated killing of helminths.....	26
1.5.2.1 Recruitment of effector leukocytes.....	26
1.5.2.2 Adherence and activation of effector leukocytes.....	27
1.5.3 Evasion of complement activation and leukocyte adherence	27
1.6 <i>NIPPOSTRONGYLUS BRASILIENSIS</i> AS A MODEL FOR STUDYING IMMUNITY TO HELMINTHS.....	29
1.6.1 Parasite life cycle	29
1.6.2 Immune responses to <i>N. brasiliensis</i>	30
1.6.2.1 Cellular inflammatory responses	30
1.6.2.2 Cytokine responses	32

1.6.2.3 Role for eosinophils	32
1.7 INTRODUCTION TO THIS STUDY	34
<u>CHAPTER TWO:</u> Quantitation of complement and leukocyte binding to a parasitic helminth species.....	36
LINKAGE TO CHAPTER TWO AND ARTICLE	37
<u>CHAPTER THREE:</u> Loss of complement activation and leukocyte adherence as <i>Nippostrongylus brasiliensis</i> develops within the murine host	39
LINKAGE TO CHAPTER THREE AND ARTICLE	40
<u>CHAPTER FOUR:</u> The role of complement in innate, adaptive and eosinophil-dependent immunity to the nematode <i>Nippostrongylus brasiliensis</i>	43
LINKAGE TO CHAPTER FOUR AND ARTICLE	44
<u>CHAPTER FIVE: DISCUSSION AND CONCLUSION</u>	
5.1 GENERAL DISCUSSION	46
5.1.1 Summary of main findings	46
5.1.2 Complement and eosinophil-dependent immunity to helminths	47
5.1.2.1 Eosinophil-dependent resistance to helminths.....	47
5.1.2.2 Role of complement <i>in vivo</i> and <i>in vitro</i>	49
5.1.2.3 Complement-dependent eosinophil recruitment to parasite-infected skin	51
5.1.2.4 Complement-independent eosinophil recruitment.....	51
5.1.2.5 Eosinophil versus neutrophil recruitment	53
5.1.2.6 Complement-independent leukocyte adherence to helminths	54
5.1.2.7 Eosinophil degranulation	55
5.1.2.8 Larval aggregation	57
5.1.3 Evasion of complement activation by helminths	58
5.1.4 Pulmonary cellular responses following helminth infection	59
5.1.4.1 Restricted early cellular inflammation in the lungs	59
5.1.4.2 Delayed cellular inflammation in the lungs.....	60
5.1.5 Secondary immune response to helminth infection.....	61
5.1.6 Future directions for studies using complement-deficient/IL-5 Tg mice	62
5.1.7 Issues for design of anthelmintic vaccines	62
5.2 CONCLUSION.....	63
REFERENCES.....	65

ABSTRACT

Approximately two billion people are infected with helminths worldwide. In order to develop a vaccine against these pathogens, more needs to be known about the immune response to helminths. Eosinophils are important for resistance to some helminth species and their recruitment to infected tissues, attachment to parasites and degranulation may all be critical processes for immunity. Complement may contribute to these processes via generation of chemotactic factors (C3a and C5a) or opsonisation of the parasite with C3b/iC3b. The importance of complement during helminth infection is unclear, though complement does promote leukocyte-mediated killing of several helminth species *in vitro*. The aim of the present study was to investigate the role of complement in immunity of mice to *Nippostrongylus brasiliensis*, with a focus on whether complement facilitates eosinophil-dependent resistance to this parasite. A new fluorescence-based method for quantifying *in vitro* complement deposition and leukocyte adherence on *N. brasiliensis* was developed. C3 from human serum was deposited on infective-stage L3 via the classical or lectin complement pathways. In contrast, the alternative complement pathway mediated binding of mouse C3 and eosinophil-rich mouse peritoneal leukocytes to L3. Interestingly, the ability of complement and leukocytes to bind to the parasite changed as it matured. Larvae recovered from the skin 30 min post-injection (p.i.) were coated with C3, however those harvested 150 min p.i. exhibited reduced C3 binding capacity. Binding of C3 and eosinophils to larvae recovered from the lungs 24-48 h p.i. (L4) was also diminished compared to that seen on L3. Adult intestinal worms bound C3 and leukocytes only when treated *ex vivo* with serum and cells. Mice lacking in classical (C1q-deficient), alternative (factor B-deficient) or all complement pathways (C3-deficient) were then employed to determine if complement was important for resistance of mice to *N. brasiliensis*. IL-5 Tg mice deficient in individual complement genes were generated to assess whether complement contributed to eosinophil-dependent resistance to the parasite. Factor B-deficient mice exhibited impaired C3 deposition on larvae, eosinophil recruitment, eosinophil degranulation and larval aggregation

in the skin 30 min p.i. Eosinophil recruitment was similarly abolished by treatment of mice with the C5aR inhibitor PMX53. However at 150 min p.i., larval aggregation, eosinophil and neutrophil recruitment, leukocyte adherence and eosinophil degranulation were largely complement-independent. Ablation of factor B or C3 caused minor but significant increases in lung-larval burden during primary, but not in secondary, infections. Critically, a lack of C3 or factor B in IL-5 Tg mice failed to greatly impair the strong innate anti-parasite resistance typical of these animals, suggesting that eosinophils can provide immunity to *N. brasiliensis* infection in the absence of complement. This was unexpected, given the evidence from this and previous studies which suggested that *in vitro*, complement is important for promoting eosinophil-dependent killing of *N. brasiliensis* and other helminth species. The mechanism(s) by which eosinophils kill *N. brasiliensis* remain unknown, but may involve the coordination of the complement system with complement-independent factors that act in the early stages of infection. Critically, the influence of complement is limited, because soon after entry into the host, the parasite develops the ability to resist complement activation.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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I acknowledge the help of:

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PUBLICATIONS

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Other publication arising from Ph.D. studies:

1. Knott ML, Matthaei KI, **Giacomin PR**, Wang H, Foster PS, Dent LA (2007). Impaired resistance in early secondary *Nippostrongylus brasiliensis* infections in mice with defective eosinophilopoiesis. *International Journal for Parasitology* **37** (12): 1367-1378

Previous publications

1. Keating DJ, Rychkov GY, **Giacomin P**, Roberts ML (2005). Oxygen-sensing pathway for SK channels in the ovine adrenal medulla. *Clinical and Experimental Pharmacology and Physiology*, **32** (10): 882-887
2. McKay D, Brooker R, **Giacomin P**, Ridding M, Miles T (2002). Time course of induction of increased human motor cortex excitability by nerve stimulation. *Neuroreport*, **13** (10): 1271-1273

Manuscripts in preparation

1. **Giacomin PR**, Gauld AD, Cava M, Iddewalla D, Gordon DL and Dent LA. Excretory/secretory proteins from *Toxocara canis* infective larvae reduce eosinophil-dependent innate resistance to *Nippostrongylus brasiliensis* infection

COMMONLY-USED ABBREVIATIONS

Abbreviation	full definition
AAM	alternatively-activated macrophage
ADCC	antibody-dependent cellular cytotoxicity
AMCase	acidic mammalian chitinase
BAL	bronchoalveolar lavage
CCR3	chemokine receptor 3
CR	complement receptor
CVF	cobra venom factor
DAF	decay accelerating factor
ES	excretory/secretory
EPO	eosinophil peroxidase
Ig	immunoglobulin
IL	interleukin
i.p.	intra-peritoneal
L3	third-stage larvae
L4	fourth-stage larvae
MAC	membrane attack complex
MASP	MBL-associated serine protease
MBL	mannan binding lectin
MBP	major basic protein
MPO	myeloperoxidase
NK	natural-killer
p.i.	post-infection
PRR	pattern recognition receptor
s.c	sub-cutaneous
STAT6	signal transducer and activator of transcription 6
Th	T-helper
Tg	transgenic
VLA	very-late antigen
WT	wildtype