Host-parasite interactions in primary and secondary infections with *Nippostrongylus brasiliensis* and *Heligmosomoides bakeri*

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by

Michelle Louise Knott

THE UNIVERSITY OF ADELAIDE AUSTRALIA

SUB CRUCE LUMEN

Discipline of Microbiology and Immunology

School of Molecular and Biomedical Science

The University of Adelaide

South Australia

Australia

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ABSTRACT

Parasitic helminth infections are a significant problem worldwide. Some helminth species are becoming resistant to current therapies and new forms of treatment and/or vaccines are required. *Nippostrongylus brasiliensis* is a tissue-invasive parasitic helminth that infects rodents, and the lifecycle of this parasite is similar to that of the human hookworms. The aims of this study were to investigate primary and secondary immune responses to *N. brasiliensis*, focusing on the pre-lung phase of infection. The roles of cytokines, chemokines, signalling pathways and leukocytes such as eosinophils were investigated in the skin, lungs and small intestine. The roles of eosinophils were also investigated in primary infections with the intestinal nematode *Heligmosomoides bakeri*.

Interleukin (IL)-5 is important for eosinophil development and maturation and for protection during some helminth infections. Over-expression of IL-5 provides potent protection in primary infections with *N. brasiliensis* in the pre-lung phase of infections. Although mice deficient in IL-5 or eosinophils might therefore be predicted to be more susceptible to *N. brasiliensis*, IL-5-deficient (IL-$5^{-/-}$) and eosinophil-deficient (ΔdblGATA) mice showed similar infection patterns as wildtype (WT) mice during primary *N. brasiliensis* infections. Intestinal worm and/or egg numbers however were elevated in mice with defective eosinophilopoiesis compared with WT animals. In secondary infections, despite skin inflammatory responses (4 hours p.i.) being similar in WT, IL-$5^{-/-}$ and ΔdblGATA animals, at day 2 p.i., lung larval burdens in the two latter hosts were significantly higher than in the resistant WT controls. However, parasites were expelled from intestines of all mice by day 7 of secondary infections. These data suggest that in the pre-gut phase of secondary infection, IL-5 and eosinophils play an important role in resistance to *N. brasiliensis*. Despite this, eosinophils do not appear to be essential for protection mediated within the gut during secondary infections, even though IL-5 and eosinophils do appear to confer some protection in the gut during primary infections.

Complement is required for the recruitment of eosinophils into the skin in the first 150 minutes of primary infection with *N. brasiliensis* (Giacomin *et al.*, 2008a), however other chemotactic factors appear to be involved after this time. Although eotaxin is

chemotactic for eosinophils in some tissues, the importance of eotaxin and signalling pathways involved in expression of this chemokine has not been previously characterized in *N. brasiliensis* infections. Signal transducer and activator of transcription (STAT)6 is a key transcription factor in the IL-4/IL-13 signalling pathway and these cytokines can induce expression of eotaxin in some tissues. Expulsion of *N. brasiliensis* adult worms during primary infections is profoundly impaired in STAT6-deficient ($STAT6^{-/-}$) mice. IL-5 Tg mice are highly resistant to primary infections with *N. brasiliensis* and in the current study, it was shown that ablation of eotaxin-1 or STAT6 in IL-5 Tg mice did not impair the strong innate resistance typically seen in the pre-lung phase of *N. brasiliensis* infections. While recruitment of eosinophils to the skin (4 hours p.i.) was reduced in these mice compared with IL-5 transgenic (Tg) mice, protective capacity was preserved in both primary and secondary infections. Further, eotaxin-$1^{-/-}$ single mutant mice were strongly resistant to secondary *N. brasiliensis* infections, with few larvae migrating to the lungs on day 2 p.i. In contrast, $STAT6^{-/-}$, IL-$13^{-/-}$, IL-4R$\alpha^{-/-}$ and IL-$13^{-/-}$/IL-4R$\alpha^{-/-}$-double deficient mice had significantly higher secondary lung larval burdens than WT mice and parasite egg production was prolonged in all of these strains. These data suggest a role for this signalling pathway in protection during the early stages of secondary infections with this parasite. In both primary and secondary infections, eosinophils were recruited to the skin in all gene knock out strains in comparable numbers to those seen in WT mice, and this suggests that alternative eosinophil recruitment pathways may compensate for the absence of these factors. Adding to the extensive work on the intestinal phase of *N. brasiliensis*, this work clearly indicates for the first time that early pre-lung events are crucial in determining the outcome of infection, and should be the focus of future studies with *N. brasiliensis*. In contrast, when STAT6- and eotaxin-1-deficient mice were infected with another intestinal nematode, *H. bakeri*, the mutant strains were as susceptible as WT mice, with parasite eggs present in similar numbers on all days examined. Resistance mechanisms that operate in the intestine during *N. brasiliensis* infections do not therefore appear to extend to a parasite that can infect naïve hosts for many months.

The FVB/N mouse strain was introduced into this study whilst exploring the potential roles of eosinophils in the intestinal phase of *N. brasiliensis* infections. The impact of intestine-specific expression of transgenes encoding IL-5 and eotaxin-1 were examined and although both lines of Tg mice were highly resistant to *N. brasiliensis*, naïve WT

FVB/N mice also showed very potent innate resistance. Very few worms were observed in the small intestine of WT FVB/N animals on day 7 p.i., whereas skin larval and leukocyte numbers (4 hours p.i.) and lung larval burdens (day 2 p.i.) were similar in WT FVB/N and WT CBA/Ca mice. Interestingly, lung larvae recovered from WT FVB/N animals were significantly smaller in size (days 1-2 p.i.) and less motile than lung larvae recovered from WT CBA/Ca mice. Further, there were significantly fewer eggs (day 6 p.i.) and worms (day 7 p.i.) in the former. However, WT FVB/N mice were no more resistant to infections with *H. bakeri* than WT CBA/Ca mice, with parasite eggs detected in comparable numbers until day 116 p.i. Resistance mechanisms operating against *N. brasiliensis* in WT FVB/N mice would not therefore appear to extend to *H. bakeri* infections.

Eosinophils can provide potent protection in some helminth infections and this study builds on our previous work and that of other groups. We have now shown that early pre-lung events may be critical in determining host resistance against *N. brasiliensis*. In secondary *N. brasiliensis* infections, cytokine signalling pathways that protect against adult worms in the late intestinal phase of infection also play a role in resistance during the early pre-lung phase, when the parasite is still at the larval stage. Eosinophils are also of importance for protection against this parasite, and future studies should focus on the early events to further characterize resistance mechanisms. This information may prove useful for the development of successful vaccines against hookworms and other nematodes.

DECLARATION

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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 eosinophilopoeisis. *International Journal for Parasitology* **37** (12): 1367-78

2. **Knott ML**, Matthaei KI, Foster PS, Dent LA (2009). The roles of eotaxin and the STAT6 signalling pathway in eosinophil recruitment and host resistance to the nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides bakeri*. *Molecular Immunology*. **46** (13): 2717-22

3. **Knott ML**, Hogan SP, Wang H, Matthaei KI, Dent LA (2009). FVB/N mice are highly resistant to primary infection with *Nippostrongylus brasiliensis*. *Parasitology* **136** (1): 93-106

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STATEMENTS OF AUTHORSHIP-CHAPTER 2

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PUBLICATIONS

Within thesis:

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Published abstracts:

1. **Knott ML**, Matthaei KI and Dent LA (2005). FVB/N mice are highly resistant to *Nippostrongylus brasiliensis*. *Tissue Antigens* **66** (5): 343-604

COMMONLY-USED ABBREVIATIONS

Abbreviation	full definition
AAM	alternatively activated macrophage
CAM	classically activated macrophage
CCR3	chemokine receptor 3
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
EPO	eosinophil peroxidase
GM-CSF	granulocyte-macrophage colony-stimulating factor
ICAM	intercellular adhesion molecule
Ig	immunoglobulin
IL	interleukin
IL-4Rα	interleukin-4 receptor α
Jak	janus kinase
KO	knock out
L3	third-stage larvae
L4	fourth-stage larvae
LT	leukotriene
mAb	monoclonal antibody
MBP	major basic protein
MCP	monocyte-chemotactic protein
MIP	macrophage inflammatory protein
mMCP-1	mouse mast cell protease-1
p.i.	post-infection
PAF	platelet activating factor
RANTES	regulated on activation, normal T cell expressed and secreted
s.c.	subcutaneously
STAT6	signal transducing and activator of transcription 6
Tg	transgenic
Th2	T-helper 2
VCAM	vascular cell adhesion molecule
WT	wildtype