The roles of mast cells and mast cell proteases during *Chlamydia* reproductive tract infection

Anne CHEVALIER

B.Sc. (*Bordeaux*); M.Sc. (*Montpellier*)

Discipline of Immunology and Microbiology
School of Biomedical Science and Pharmacy
Faculty of Health and Medicine
The University of Newcastle
Newcastle, NSW, Australia

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy (Immunology and Microbiology)

August 2019

Statement of originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains no material which has been accepted, or is being examined, 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.

I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Anne CHEVALIER

31 August 2019

Acknowledgements

I would like to express my gratitude to all of those who have made the completion of my PhD studies possible by their time, guidance and support.

Firstly, I would like to thank my primary supervisor, Professor Philip Hansbro, and my co-supervisors, Associate Professor Jay Horvat and Professor Darryl Knight, for offering me the opportunity to do this PhD. I would also like to thank them for their support, guidance and expertise throughout my PhD studies. I would also like to convey my special thanks to Professor Jay Horvat for his contribution, his help with the animal work, for the multitude advices he gave me, for his persistent encouragements and for spending time reviewing my thesis.

I would like to thank the University of Newcastle and the Hunter Medical Research Institute for providing me the opportunity to do my PhD and by giving me access to the resources necessary to conduct this research.

I would also like to thank the member of my confirmation panel, Professor Jonathan Hirst and Associate Professor Mark Baker, for their help and insightful advices.

I would also like to thank collaborators, Professor Richard Stevens and Associate Professor Michele Grimbaldeston, for their advice and for providing some of the mice and reagents used in my PhD studies.

I would also like to extend my acknowledgments to past and present members of the Horvat and Hansbro teams, as well as other colleagues from the Hunter Medical Research Institute, thank you for the daily help in the lab and for your support during hard times. I would specifically like to thank Kamal and Andi, for providing advices, expertise and help in the early stage of my PhD. I would like to thank the past and present member of the "*Chlamydia* infection study group", Huw, Daniel and Jemma, for their help with tissue collection as well as their advices and expertise. I would also like to thank Richard and Henry for their help and advices with experimental work.

I would like to thank my friends, who supported me during my PhD studies and helped me going through the hard time I have been facing. I would like to specifically thank Camille for

always being here for me, during both good and bad time. Thank you for taking the decision to come to Newcastle and for all the good memories we had in the last 3 years. I would also like to give special thanks to David, for his advices and for always finding ways to make me feel better when I was down. Finally, special thanks to Dee for all the laughs we had together and for giving me motivation throughout my PhD.

Last but not least, I would like to thanks my family for their support, encouragements and patience during my PhD. More specially, I would like to thank my mother, my father and my sister for coming to visit me at the opposite side of the world and for always being here for me.

Conference presentations arising from my PhD studies

First author oral presentations:

A. Chevalier, J.R. Mayall, H.W. McCarthy, R.L. Stevens, J.C. Horvat, P.M. Hansbro. *Mast cell deficiency is protective against Chlamydia-induced pathology of the female reproductive tract* - 6th Annual Australian Society for Medical Research (ASMR) Medical Research Week, Newcastle Satellite Scientific Meeting (June 2019).

A. Chevalier, J.R. Mayall, H.W. McCarthy, D.P. Hampsey, R.Y. Kim, W.M. Huston, R.L. Stevens, J.C. Horvat, P.M. Hansbro. *The role of mast cells and their proteases during Chlamydia infection*. International Union for Sexually Transmitted Infections (IUSTI) Asia-Pacific Congress, Auckland (November 2018).

A. Chevalier. *The role of mast cells and their proteases during Chlamydia reproductive tract infection*. Three-minute thesis, Faculty of Health and Medicine, University of Newcastle (July 2018).

A. Chevalier, D.P. Hampsey, J.R. Mayall, R.Y. Kim, D.A. Knight, W.M. Huston, R.L. Stevens, J.C. Horvat, P.M. Hansbro. *The role of mast cells and their proteases during Chlamydia female reproductive tract infection*. Australian *Chlamydia* Conference, Sydney (November 2017).

Presentations that I have contributed to during my candidature:

H.W. McCarthy, J.R. Mayall, J.C. Horvat, **A. Chevalier**, S.L. Masters, M.A. Cooper, A.B. Robertson, K. Schroder, R.Y. Kim, P.M. Hansbro. *The NLRP3 inflammasome induces natural killer cell responses in the uterus during Chlamydia FRT infections*. 6th Annual Australian Society for Medical Research (ASMR) Medical Research Week, Newcastle Satellite Scientific Meeting (June 2019).

J. Mayall, N. Mangan, **A. Chevalier**, D. Hampsey, A. Brown, M. Starkey, R. Kim, S. Maltby, P. Foster, P. Hertzog, J. Horvat, P. Hansbro. *The role of direct and indirect IFN-ε-mediated*

responses during Chlamydia infections. Australian *Chlamydia* Conference, Sydney (November 2017).

J. Mayall, N. Mangan, A. Brown, **A. Chevalier**, M. Starkey, R. Kim, P. Hertzog, P. Hansbro, J. Horvat. *IFN-ε regulated innate immune responses in the female reproductive tract during Chlamydia infection (P948).* American Association of Immunologists (AAI) Annual Meeting, Washington (May 2017).

J. Mayall, N. Mangan, **A. Chevalier**, B. Rae, M. Starkey, R. Kim, P. Hertzog, J. Horvat, P. Hansbro. *IFN-ε-regulated gene expression patterns in protection against female reproductive tract infection*. 16th International Congress of Immunology (ICI), Melbourne (August 2016).

J. Mayall, N. Mangan, **A. Chevalier**, M. Starkey, R. Kim, A. Brown, S. Maltby, P. Foster, P. Hertzog, J. Horvat, P. Hansbro. *IFN-ε-mediated local and systemic NK cell responses during Chlamydia infection*. ASI Annual Scientific Meeting, Canberra (December 2015).

Table of Contents

List of figures

List of tables

Abbreviations

ABR	Australian BioResources
Ala	Alanine
ANOVA	Analyses of variance
Arg	Arginine
Asp	Aspartic acid
ATP	Adenosine triphosphate
BMMC	Bone marrow mast cell
CAH	Central Animal House
cDNA	Complementary DNA
CMA1	Chymase 1
Cmu	*Chlamydia muridarum*
CPA3	Carboxypeptidase A3
CPAF	Chlamydial protease-like activating factor
CTMC	Connective tissue mast cell
CXCL	Chemokine (C-X-C motif) ligand
CXCR	Chemokine (C-X-C motif) rececptor
DAMP	Damage-associated molecular pattern
dbi	Days before infection
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
dpi	Day post infection
DPPI	Dipeptidyl peptidase I
DTT	DL-dithiothreitol
EB	Elementary body
EDTA	Ethylenediaminetetraacetic acid
ER	Oestrogen receptor
FACS	Flow cytometry and cell sorting
FcεR	High affinity immunoglobulin E receptor
FCS	Fetal Calf Serum
FOXP3	Forkhead box P3
FRT	Female reproductive tract
GAG	Glycosaminoglycan
GATA3	GATA Binding Protein 3
Gly	Glycine
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HEPES	2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulphonic acid
His	Histidine
HIV	Human immunodeficiency virus
HMRI	Hunter Medical Research I nstitute
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
HPV	Human papillomavirus
IFN	Interferon
ifu	Inclusion forming unit
Ig	Immunoglobulin
IL	Interleukin
LGV	Lymphogranuloma venereum
LPS	Lipopolysaccharide
Lys	Lysine
MC	Mast cell
MC_C	Mast cell chymase$^+$ tryptase$^-$
MC_T	Mast cell chymase$^-$ tryptase$^+$
MC_{TC}	Mast cell chymase$^+$ tryptase$^+$
Mcl	Myeloid cell leukemia
mDC	Myeloid dendritic cell
Met	Methionine
MHC	Major histocompatibility complex
MITF	*mi* transcription factor
MMC	Mucosal mast cell
mMCP	Mouse mast cell protease

MMP	Matrix metalloproteinase
MOMP	Major outer membrane protein
mT5	Mouse tryptase 5
NCR	Natural cytotoxicity triggering receptor
Ndst	*N*-deacetylase/*N*-sulfotransferase
NET	Neutrophil extracellular trap
NK cell	Natural killer cell
NLRP	Nucleotide-binding oligomerization domain-like receptor, pyrin domain-containing
NO	Nitric oxide
NOD	Nucleotide-binding oligomerization domain-like
Omp	Outer membrane protein
PAMP	Pathogen-associated molecular pattern
PAR	Protease-activated receptor
PB	Persistent body
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline with 0.05% Tween-20
pDC	Plasmacytoid dendritic cell
PDCA	Plasmacytoid dendritic cell antigen
PFA	Paraformaldehyde
Pgp	Plasmid glycoprotein
Phe	Phenylalanine
PR	Progesterone receptor
Prss	Protease serine member S
PRR	Pattern recognition receptor
qPCR	Quantitative polymerase chain reaction
RB	Reticulate body
RIP	Receptor-interacting protein
RLR	etinoic acid-inducible gene 1 like receptor
RNA	Ribonucleic acid
RORγt	RAR-related orphan receptor
ROS	Reactive oxygen species
rPrss31	Recombinant protease serine member S31
rRNA	Ribosomal ribonucleic acid
SEM	Standard error of the mean
Ser	Serine
siRNA	Small interfering ribonucleic acid
SPF	Specific pathogen free
SPG	Sucrose-phosphate-glutamate
STI	Sexually transmitted infection
TCR	T cell receptor
TGFβ	Transforming growth factor beta
Th	T helper
Thr	Threonine
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TPSAB	α/β-tryptase
TPSB	β-tryptase
TPSD	δ-tryptase
TPSG	γ-tryptase
Treg	Regulatory T cell
Trp	Tryptophan
Tyr	Tyrosine
UniSA	University of South Australia
Val	Valine
WT	Wild type

Synopsis

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI), with approximately 130 million cases of infection occurring annually worldwide. Although *Chlamydia* infections are relatively simple to diagnose and treat, the majority of infected women do not develop any symptoms, hence they often go undiagnosed and untreated. Over time, untreated infections may ascend from the vagina into the upper female reproductive tract (FRT) and cause severe complications including pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility. The host immune responses to *Chlamydia* infections are very complex and a greater understanding of the host responses, including immune cells and factors, that contribute to clearance of infection *versus* those that underpin infection-associated pathology is required.

Mast cells (MCs) are large, tissue-resident, immune cells of haematopoietic origin that are present in the FRT. They are characterised by their numerous intracellular secretory granules that hold a wide variety of preformed inflammatory mediators, including histamine, serglycin proteoglycans and MC proteases. Upon MC activation, these preformed mediators are released into the extracellular matrix through a mechanism called degranulation. Although MCs are well recognised for their detrimental role in allergy, they are also key mediators of immune responses to an extensive number of pathogens. However, the role that MCs play during STIs remains largely unknown.

To address this, my PhD studies aimed to investigate the role(s) of MCs and MC proteases during *Chlamydia* FRT infections, using a suite of genetically modified mice that are deficient in MCs or in specific MC proteases and a murine model of *Chlamydia* FRT infection.

I show that the number of uterine MCs and their expression of the MC proteases, mouse MC protease (mMCP)4, mMCP5, mMCP6 and carboxypeptidase (Cpa)3 are regulated by female sex hormones and/or stage of oestrous cycle in the absence of *Chlamydia* FRT infection. Whilst the number, phenotype and degranulation of MCs is not changed 3 days post infection (dpi) with *Chlamydia*, the number of MCs and their expression of mMC4, mMCP5, mMCP6 and CPA3 are

slightly reduced at 14dpi. In contrast, I show that the expression of protease serine member S31 (Prss31), a unique MC protease that possesses a membrane anchor that binds it to the plasma membrane of MCs upon degranulation, is independent of female sex hormones and *Chlamydia* FRT infection. Together, these data reveal that female sex hormones and *Chlamydia* FRT infection can affect the number and phenotype of MCs in the FRT.

Moreover, my studies reveal a novel role for MCs in mediating *Chlamydia* FRT infection. I show that MC-deficient mice are protected against *Chlamydia*-induced pathology and have slightly reduced eosinophils, neutrophils, monocytes and macrophages in their uterus, suggesting a role for MCs in contributing to the recruitment of innate immune cells, associated with development of *Chlamydia*-induced pathology, in the upper FRT.

By using intravaginal treatments with the MC stabiliser cromolyn, I show evidence that MC degranulation is detrimental, especially during the early stages of *Chlamydia* FRT infection. Mice that received cromolyn throughout the early stages of *Chlamydia* infection have reduced infection at 3dpi. However, this protective effect is not maintained at later stages of infection. Importantly, mice that receive cromolyn treatment during the early stages of infection are protected against infection-induced pathology during the later stages. These observations show that the inhibition of MC degranulation does not recapitulate the effects observed in MC-deficient mice, suggesting that some of the factors released through degranulation might have differential effects to other factors released by MCs through other pathways.

I next sought to identify the role(s) of some of the key factors, specifically factors that are stored in the secretory granules of MCs that are released during MC degranulation, in the pathogenesis of *Chlamydia* FRT infection. In my first series of experiments, *N*-Deacetylase/*N*-Sulfotransferase 2 (Ndst2)-deficient mice were subjected to *Chlamydia* FRT infection. These mice lack the important enzyme for *N*-deacetylation and *N*-sulfation of heparan sulfate in MCs, which causes abnormal storage of the MC mediators that are normally bound to heparin in the secretory granules of MCs, including histamine and the MC proteases, mMCP4, mMCP5, mMCP6 and Cpa3. Interestingly, I show that Ndst2-deficient mice are more susceptible to

infection, while being protected against *Chlamydia*–induced pathology. This increase in susceptibility to infection and protection against pathology is associated with a decrease in the number of innate and adaptive cells present in the uterus, suggesting that the factors that are affected by Ndst2 deficiency, play important role in the induction of the recruitment of immune cell associated with clearance of infection (E.g. $CD4^+$ T cells) and with development of *Chlamydia*–associated pathology (E.g. neutrophils).

I next sought to assess the individual roles played by specific MC proteases in the pathogenesis of *Chlamydia* FRT infection. To do this, mMCP5-, mMCP6-, Prss31-deficient and mMCP6-deficient/mMCP7-sufficient mice were infected with *Chlamydia* FRT. I show that whilst mMCP6-deficient mice have similar course of infection as wild type (WT) control mice, mMCP5-deficient mice are protected against infection at 3dpi. Interestingly, the presence of mMCP7 (which is naturally deficient in the WT control mice used) in mMCP6-deficient mice slightly protected against the early stages of infection as well as infection-induced pathology during the later stages of infection compared to WT controls. I also show that Prss31-deficient mice are more susceptible to infection early, but have no change in infection-induced pathology at later stages of *Chlamydia* infection. My studies also show that absence of Prss31 results in a decrease in immune cell recruitment to the uterus during infection. Importantly, daily intravaginal treatment with recombinant Pss31 protects against infection and infection-induced pathology.

Together, my studies show important role(s) for MCs and MC degranulation in the pathogenesis of *Chlamydia* FRT infection. My studies also show that different MC proteases may play different roles in infection and infection-induced disease. Importantly, whilst the mechanisms involved remain to be elucidated, my studies highlight that MC-mediated responses may be therapeutically manipulated in order to treat/prevent *Chlamydia* FRT infection and/or infection-induced FRT pathology.