

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

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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.

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Anne CHEVALIER

31 August 2019

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Abbreviations

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

MMP	Matrix metalloproteinase	RB	Reticulate body
MOMP	Major outer membrane protein	RIP	Receptor-interacting protein
mT5	Mouse tryptase 5	RLR	retinoic acid-inducible gene 1 like receptor
NCR	Natural cytotoxicity triggering receptor	RNA	Ribonucleic acid
Ndst	<i>N</i> -deacetylase/ <i>N</i> -sulfotransferase	RORγt	RAR-related orphan receptor
NET	Neutrophil extracellular trap	ROS	Reactive oxygen species
NK cell	Natural killer cell	rPrss31	Recombinant protease serine member S31
NLRP	Nucleotide-binding oligomerization domain-like receptor, pyrin domain-containing	rRNA	Ribosomal ribonucleic acid
NO	Nitric oxide	SEM	Standard error of the mean
NOD	Nucleotide-binding oligomerization domain-like	Ser	Serine
Omp	Outer membrane protein	siRNA	Small interfering ribonucleic acid
PAMP	Pathogen-associated molecular pattern	SPF	Specific pathogen free
PAR	Protease-activated receptor	SPG	Sucrose-phosphate-glutamate
PB	Persistent body	STI	Sexually transmitted infection
PBS	Phosphate buffered saline	TCR	T cell receptor
PBS-T	Phosphate buffered saline with 0.05% Tween-20	TGFβ	Transforming growth factor beta
pDC	Plasmacytoid dendritic cell	Th	T helper
PDCA	Plasmacytoid dendritic cell antigen	Thr	Threonine
PFA	Paraformaldehyde	TLR	Toll-like receptor
Pgp	Plasmid glycoprotein	TNF	Tumor necrosis factor
Phe	Phenylalanine	TPSAB	α/β -tryptase
PR	Progesterone receptor	TPSB	β -tryptase
Prss	Protease serine member S	TPSD	δ -tryptase
PRR	Pattern recognition receptor	TPSG	γ -tryptase
qPCR	Quantitative polymerase chain reaction	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 mMCP4, 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.