The Role of Mast Cell Proteases in Respiratory Disease

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Abbreviations

C57	C57BL/6
SPN	*S. pneumoniae* D39
PA14	*P. aeruginosa* PA14
WSN	Influenza A/WSN/33
IAV	Influenza A virus
PAMP	Pathogen associated molecular patterns
PRR	Pattern recognition receptors
DAMP	Damage associated molecular patterns
HMGB1	High mobility group box 1
MASP	Mannose binding lectin associated protease
APC	Antigen presenting cells
ChoP	Adhesin phosphorylcholine
PAF	Platelet activating factor
CbpA	Choline binding protein A
ECM	Extra cellular matrix
PavA	Pneumococcal adhesion and virulence A
MHC	Major histocompatibility complex
MAC	Membrane attack complex
CPS	Capsular polysaccharide
PCV	CPS-protein conjugate vaccines
IPD	Invasive pneumococcal disease
PnPS	Pneumococcal capsular polysaccharide
PGK	Phosphoglycerate kinase

pIgR	Poly immunoglobulin receptors
CBP	Choline binding
CBD	Choline binding domain
NLR	Nod-like receptor
LTA	Lipoteichoic acid
CLIP	Class II invariant chain peptide
ROI	Reactive oxygen intermediates
NET	Neutrophil extracellular traps
TCR	T cell receptor
PLY	Pneumolysin
PGN	Peptidoglycan
BALf	Bronchoalveolar lavage fluid
GAG	Glucosaminoglycan
NDST2	Glucosaminyl N-deacetylase/N-sulphotransferase-2
LPS	Lipopolysaccharide
DC	Dendritic cells
GEF	Guanine nucleotide exchange factors
PGN	Peptidoglycan
mMCP	Mast cell protease
CLP	Cecal ligation and puncture
SCF	Secreted stem cell factor
COX2	Cyclooxygenase 2
hsp40	Heat shock protein 40
NS1	Non-structural protein 1
CPS	Capsular polysaccharide
PCV	CPS-protein conjugate vaccines
PBS	Sterile phosphate buffered saline
SEM	Standard experimental mean
ANOVA	Analysis of variance
NP	Nuclear protein
PKR	RNA- dependent protein kinase
COPD	Chronic obstructive pulmonary disease
CF	Cystic fibrosis
nCFB	Non-CF bronchiectasis

LB	Lauria-Bertani agar
i.n	Intranasally
p.i	Post infection
TNFα	Tumour necrosis factor alpha
IFNγ	Interferon gamma
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL2	Chemokine (C-X-C motif) ligand 2
IL-6	Interleukin 6
IL-1β	Interleukin 1 beta
CFU	Colony forming units
WHO	World Health Organisation
MDCK	Madin-Darby Canine Kidney
PFU	Plaque forming unit

Synopsis

S. pneumoniae, *P. aeruginosa* and Influenza A virus are 3 common respiratory pathogens responsible for extensive disease and mortality. *S. pneumoniae* is responsible for 25% of preventable child deaths, *P. aeruginosa* is the leading cause of Gram negative nosocomial infections whilst seasonal epidemics of Influenza infections result in half a million deaths per year and pandemic outbreaks of the virus such as the 1918 Spanish flu pandemic infected up to 1/3 of the global population resulting in an estimated 50 million deaths.

Mast cells, synonymous with allergy, are long lived tissue resident sentinels of the immune system. They are strategically located at host interface environments, including the lungs where they comprise 2% of the cross sectional area of alveolar walls. Their expression of a wide range of PRRs means they can detect pathogens and release numerous proinflammatory mediators to recruit additional cells or aid in direct killing of invading pathogens[1].

Mature mast cells develop large electron dense granules[2] filled with mast cell restricted proteases, such as chymases, tryptases and carboxypeptidase A3, together with growth factors, lysosomal enzymes, proteoglycans of serglycin, preformed cytokines and biogenic amines such as histamine and serotonin[1]. Mast cells comprise of almost 50% by weight of protease-serglycin complexes[3]. Following mast cell activation and subsequent degranulation these mediators are released from the cell.

The role of these mast cell proteases, mast cell related proteases and mast cell associated factors in infection and disease is unclear.

Using transgenic mice modified to insert or remove a range of differing mast cell proteases, mast cell related proteases or mast cell associated factors this study investigated

the role of mMCP5, mMCP6, mMCP7, NDST2, Prss31, Prss22 and RasGRP4 during *S. pneumoniae*, *P. aeruginosa* and Influenza A virus infections.

The first study looked at the role of these proteases and associated factors in a *S. pneumoniae* model. By interpreting a combination of results from different transgenic mice I show that mMCP7 expression in this disease results in impaired bacterial clearance. Additionally, I demonstrate that mMCP5$^{-/-}$ mice have impaired macrophage recruitment in this infection showing that one role for mMCP5 in this infection is macrophage recruitment.

The second study looked at the role of these proteases and associated factors in a *P. aeruginosa* model. I demonstrate that mMCP6 promotes protective inflammatory responses during this infection with mMCP6$^{-/-}$ presenting with elevated instances of bacteremia. Additionally, I show mMCP7 expression is detrimental during this infection with elevated mortality rates observed together with an impairment in trans epithelial migration. I also repeated the observation that mMCP5$^{-/-}$ mice have a defective macrophage recruitment, and postulate that mMCP5s role in bacterial infections is to promote macrophage recruitment. I also demonstrate Prss22 as playing a negative role during this infection contributing towards bacteraemia, this observation suggests that the use of Prss22 specific inhibitors could be of therapeutic use in combatting bacteraemia during this infection.

The final study investigated the role of these proteases and associated factors in an Influenza A model. I show the mMCP6 limits excessive inflammation during the resolution of this infection by moderating IP-10 concentrations. Additionally, I show Prss31$^{-/-}$ mice are protected from excessive inflammatory responses, I propose that Prss31 promotes IL-10 production resulting in elevated inflammation. I also link Prss22 expression with protection from excessive inflammation during the infection, pointing to a possible therapeutic use of this tryptase. Finally, I highlight an anti-inflammatory role for RasGRP4 during the resolution of Influenza infection protecting mice from excessive inflammation.

These findings have helped me identify potential roles for these proteases and associated factors in a range of disease and point to their roles being infection specific. I have identified key proteases whose functions point to promising avenues for disease treatment.