

# **The Role of Mast Cell Proteases in Respiratory Disease**

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Andrew Deane

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## Abbreviations

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

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

<b>LB</b>	Lauria-Bertani agar
<b>i.n</b>	Intranasally
<b>p.i</b>	Post infection
<b>TNF<math>\alpha</math></b>	Tumour necrosis factor alpha
<b>IFN<math>\gamma</math></b>	Interferon gamma
<b>CXCL1</b>	Chemokine (C-X-C motif) ligand 1
<b>CXCL2</b>	Chemokine (C-X-C motif) ligand 2
<b>IL-6</b>	Interleukin 6
<b>IL-1<math>\beta</math></b>	Interleukin 1 beta
<b>CFU</b>	Colony forming units
<b>WHO</b>	World Health Organisation
<b>MDCK</b>	Madin-Darby Canine Kidney
<b>PFU</b>	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<sup>1</sup>.

Mature mast cells develop large electron dense granules<sup>2</sup> 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<sup>1</sup>. Mast cells comprise of almost 50% by weight of protease-serglycin complexes<sup>3</sup>. 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<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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.