A novel role for the TRAIL signalling pathway in the pathogenesis of Eosinophilic Oesophagitis

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B Biomed Sci (Hons)

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Statement of Originality

I hereby certify that to the best of my knowledge that this thesis is my own written work and contains no material previously published or written by another person except where due references and acknowledgements are made. It contains no material that has been previously submitted by me for the award of any other degree or diploma in any university or other tertiary institution

Leon Sokulsky

Thesis by publication

I hereby certify that this thesis is in the form of three separate papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.

Acknowledgements

It goes without question that completing a doctorate of philosophy is a taxing and challenging experience. However, one of the best things about a PhD candidacy is that you rarely venture forth alone. The work I have presented here in this thesis would not have been possible if it weren't for the support of the following people.

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List of Publications

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Leon A. Sokulsky, Jason LN. Girkin, Malcolm R. Starkey, Philip M. Hansbro, Paul S. Foster, Adam M. Collison and Joerg Mattes. *A unique role for IL-13 in Eosinophilic oesophagitis by inducing eosinophilia through MID-1 and STAT6*. **Journal of Allergy and Clinical Immunology** (*Submitted under review*) (2016).

*both authors contributed equally

List of Abbreviations:

AAD- Allergic Airways Disease

AHR- Airways Hyperreactivity

Asp F- *Aspergillus Fumigatus*

BAL- Bronchoalveolar Lavage

CCL11- C-C motif chemokine ligand 11 (Eotaxin-1)

CCL24- C-C motif chemokine ligand 24 (Eotaxin-2)

CCL26- C-C motif chemokine ligand 26 (Eotaxin-3)

DISC- Death Inducing Signalling Complex

EoE- Eosinophilic oesophagitis

FADD- Fas Associated Death Domain

GORD- Gastro-Oesophageal Reflux Disease

IgE- Immunoglobulin E

IKK- I Kappa B

IL- Interleukin

ILC2- Innate Lymphoid Cell Type 2

IL-13Rα- Interleukin IL Receptor α

MAPK- Mitogen-Activated Protein Kinase

MID-1- Midline-1

NFκB- Nuclear Factor Kappa B

OVA- Ovalbumin

PP2A- Protein Phosphatase 2A

PP2Ac- Protein Phosphatase 2A catalytic subunit

PPI- Proton Pump Inhibitor

PPI-REE- Proton Pump Inhibitor Responsive Eosinophilic Oesophagitis

RIP- Receptor Activating Protein

STAT- Signal Transducer and Activator of Transcription

TGF-β- Transforming Growth Factor-β

Th2- T helper 2

TRAIL- TNF-Related Apoptosis Inducing Ligand

TRAIL-/-- TRAIL Deficient

TRAIL-R- TRAIL Receptor

TSLP- Thymic Stromal Lymphopoietin

VCAM-1- Vascular Cellular Adhesion Molecule-1

VEGF-A- Vascular Endothelial Growth Factor-A

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Thesis Abstract

Eosinophilic Oesophagitis (EoE) is an allergen mediated disorder of the oesophagus, associated with eosinophilic infiltration of the oesophageal epithelial layer and remodelling of oesophageal scaffolding. There has been a significant rise in EoE prevalence over the past ten years, however, therapeutic strategies to counter hallmark EoE features have remained relatively unchanged from steroid therapy and dietary restrictions. The apoptotic factor TRAIL has previously been implicated in allergic asthma as a driver for immune cell infiltration, remodelling and airway hyperreactivity. The upregulation of TRAIL through allergen exposure results in the induction of MID-1 and subsequent downregulation of PP2A: a negative regulator of NF-κB and MAP kinase inflammatory pathways. Given the similarities between EoE and asthmatic inflammation, this thesis will explore the role of the TRAIL signalling pathway through the analysis of EoE oesophageal human biopsies and the employment of EoE *in vivo* models.

In chapter 1, TRAIL and MID-1 expression was found to be elevated in EoE patient biopsies and *in vivo* modelling of *Asp F*-driven EoE demonstrated an activation of the TRAIL signalling pathway. TRAIL and MID-1 deficiency resulted in an ablation of EoE hallmark features *in vivo*, including reduced eosinophil infiltration, fibrosis, eotaxins, Th2 cytokines and TSLP, with TSLP recapitulation found to restore disease properties despite TRAIL deficiency. Chapter 2 further analyses the TRAIL signalling pathway in human EoE, demonstrating a correlation in TRAIL and MID-1 protein and mRNA as well as showing a reduction of PP2A activity in EoE patients. Additionally, chapter 2 demonstrated that TRAIL deficiency ablated ovalbumin driven EoE and that restoring PP2A activity via salmeterol therapy was comparable to corticosteroid treatment *in vivo*. Finally, in chapter 3, the impact of the Th2 cytokine on the TRAIL signalling pathway was assessed *in vivo*, where MID-1

silencing was found to ablate eotaxin-1 expression and completely abolish eosinophilia into the oesophagus. Given the upregulation of MID-1 in TRAIL deficient, but not STAT6 dependent mice, it is likely that MID-1 can operate independently of TRAIL via STAT6 in IL-13 driven inflammation.

Overall, this thesis has taken multiple approaches in addressing TRAIL's role in the perpetuation of EoE hallmark features, through the analysis of human biopsies to the employment of multiple EoE mouse models. The studies conducted in this thesis have broadened our understanding of this emerging disorder and highlighted potential therapeutic strategies to combat this disease.