Sphingosine Kinase-1 Regulates

Neutrophil Recruitment During Allergic Inflammation

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Table of Contents

Thesis abstract

Current health costs for allergy are more than US$300 billion annually worldwide. Although anti-histamines and steroids are the mainstay treatment for allergic inflammation, their effectiveness is varied. Rapid recruitment of neutrophils to sites of inflammation is associated with allergic diseases, such as dermatitis and anaphylaxis, yet they have been largely ignored as a treatment target. Thus, a better understanding of neutrophils in allergic inflammation is required to develop new therapeutic options.

Leukocyte infiltration to a site of inflammation is an important process for the development of allergic diseases and the roles of eosinophils and mast cells have been well described in allergic inflammation. By contrast, neutrophil infiltration has not been fully examined even though they are one of the first responders to be recruited to the inflammatory site(s) and become a dominant producer of histamine over time to enhance the recruitment of other inflammatory cells. Herein, this thesis focuses on the better understanding of neutrophil infiltration during allergic inflammation.

The classical paradigm of leukocyte recruitment suggests that this localization of cells, including neutrophils, from the circulation to a site of inflammation occurs via specific interactions of adhesion molecules. During allergic inflammation, the adhesion molecule P-selectin, which is pre-formed and pre-stored in Weibel Palade

bodies of vascular endothelial cells (ECs), is rapidly exocytosed to the EC surface to recruit neutrophils. The mechanisms underpinning rapid P-selectin exocytosis by ECs are not fully understood.

This study investigated the hypothesis that sphingosine kinase (SK) is a regulator for P-selectin-induced neutrophil recruitment during allergic inflammation. SK is a highly conserved lipid kinase, which is ubiquitously expressed at varying levels in different cell types, and catalyses the phosphorylation of sphingosine to form the bioactive molecule sphingosine-1-phosphate (S1P). Fingolimod (Gilenya) is an FDA approved oral drug currently used to treat multiple sclerosis via its effects on SK and S1P axis. Herein, I reveal that Fingolimod can inhibit histamine-induced neutrophil recruitment *in vitro* and *in vivo*. In addition, I show that topical application of Fingolimod can attenuate the production of inflammatory chemoattractants and ear swelling in two mouse models of allergic inflammation. Finally, I demonstrate that Fingolimod blocks calcium influx in histamine-treated ECs. Taken together, I have begun to unravel previously unknown processes underpinning neutrophil recruitment during allergic inflammation.

Overall, this study provided evidence that SK can be a therapeutic target to prevent and treat allergic inflammation via regulation of excessive neutrophil recruitment. Our findings also reveal a new indication for Fingolimod wherein it can

be applied topically to treat allergic-associated diseases. By doing so we may well provide a new treatment option for acute allergic inflammation and possibly fatal anaphylaxis.

Key words: neutrophil, endothelial cells, allergic inflammation, sphingosine kinase, Fingolimod, P-selectin, histamine

Declaration

This body of work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Miss Wai Yan Sun. To the best of my knowledge and belief, this work contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution to Miss Wai Yan Sun without the prior approval of the University of Adelaide.

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This thesis began with a loose collection of ideas when I was working as a research assistant in A/Prof Claudine Bonder's laboratory, and then developed into a more solid plan as my PhD project. During my PhD candidature, I undertook a concurrent degree of B.Pharmacy and aimed to incorporate the pharmacy knowledge into my research. I admit that the past few years were very busy but extremely enjoyable and fruitful, and it could never be possible without the kind support of my principal supervisor, A/Prof Claudine Bonder.

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8. Penko D, Rojas Canales D, Mohanasundaram D, Peiris HS, **Sun WY**, Drogemuller CJ, Keating DJ, Coates PT, Bonder CS, Jessup CF, Endothelial progenitor cells enhance islet engraftment, influence beta cell function and modulate islet connexin 36 expression, *Cell Transplant*., accepted 2013 Sep 10.

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Manuscripts currently under review/in preparation

2. Sun WY, Pitson S, Grimbaldeston M and Bonder CS. Epicutaneous application of Fingolimod attenuates allergic inflammation. Manuscript in preparation and to be submitted to *Journal of Clinical Investigation* August 2014.

1. Parham KA, Dobbins JR, Tooley KL, **Sun WY**, Moretti PA, Fells JI, Tigyi, G, Pitson SM and Bonder CS. Sphingosine 1-phosphate is a ligand for PPARγ which regulates vascular function. *FASEB J*. July 2014. Submitted.

Awards

International:

2014 International Vascular Biology Meeting Travel Award- AUD 2000
Australian Vascular Biology Society, Australia

2011 The EMBL Advanced Training Centre Corporate Partnership Programme (CPP) Registration Fee Fellowships- EUR 295
European Molecular Biology Laboratory, Heidelberg, Germany

National:

2013 The Best Student Publication- AUD 750
The Centre for Cancer Biology, SA Pathology, SA, Australia

2012 The Best of The Best Student Poster Presentation- AUD 1000
The 6th Australian Health and Medical Research Congress, Adelaide, Australia

2012 ASI Postgraduate International travel award- AUD 2000
Australasian Society for Immunology, Australia

2012 The Best Poster Presentation- AUD 500
The Postgraduate Research Meeting, Faculty of Health Sciences, The University of Adelaide, Adelaide, Australia

2011 The Best PhD Poster Presentation- AUD 500
The 41st Australasian Society for Immunology Annual Meeting, Australasian Society for Immunology, Adelaide, Australia

2011 The Best Student Presentation- AUD 250
The 5th Barossa Meeting: Cell Signalling and Molecular Medicine, Adelaide, Australia

2011 The D R Stranks Travelling Fellowships- AUD 3,000
The University of Adelaide, Adelaide, Australia

2011	PhD Student Presentation Second Prize- AUD 100 The 7th Australasian Society for Immunology SA/NT Branch Annual Student Meeting, Australasian Society for Immunology, South Australia, Australia
2011	PhD Student Presentation Second Prize- AUD 1,000 The Centre for Stem Cell Research Annual Scientific Meeting Robinson Stem Cell Institute, The University of Adelaide, Adelaide, Australia
2011	ARI Pty Ltd Best Commercialization Potential Presentation- AUD 500 The Postgraduate Research Conference 2011, Faculty of Health Sciences, The University of Adelaide, Adelaide, Australia
2010	ASI Travel Award- AUD 770 The 40th Australasian Society for Immunology Annual Meeting, Australasian Society for Immunology, Western Australia, Australia
2010	The Best Poster Presentation- AUD 2,000 The Centre for Stem Cell Research Annual Scientific Meeting Robinson Stem Cell Institute, The University of Adelaide, Adelaide, Australia
2010	The Best Poster Presentation- AUD 500 The Postgraduate Research Expo 2010, Faculty of Health Sciences, The University of Adelaide, Adelaide, Australia
2010-2013	PhD Scholarship- AUD 90,000 CRC Biomarker Translation, Victoria, Australia

Conference presentations

International:

16. Sun WY, Pitson SM, Grimbaldeston M and Bonder CS. Epicutaneous application of Fingolimod attenuates allergic inflammation. *The 18th International Vascular Biology Meeting*, *hosted by International Vascular Biology Society,* Kyoto, Japan, 14-17th April 2014
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-Oral presentation

National:

13. Sun WY, Pitson SM, Grimbaldeston M and Bonder CS. Administration of Fingolimod attenuates neutrophil recruitment during allergic inflammation. *The 21st Australian Vascular Biology Society Scientific Meeting (Joint Meeting with ANZ Microcirculation Society), hosted by Australian Vascular Biology Society and Microcirculation Society,* Barossa Valley, SA, Australia, 5-8th September, 2013
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-Poster presentation

11. Sun WY, Abeynaike L, Pitson SM, Hickey M and Bonder CS. Rapid histamine-induced neutrophil recruitment is sphingosine kinase-1 dependent. *The 20th Australian Vascular Biology Society Scientific Meeting, hosted by Australian Vascular Biology Society,* Hyatt Sanctuary Cove, Qld, Australia, 13-16th September 2012
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10. Sun WY and Bonder CS. Activation of endothelial cell in the acute phase of allergic inflammation is sphingosine kinase dependent. *The 41st Australasian Society for Immunology Annual Meeting, hosted by Australasian Society for Immunology,* Adelaide, Australia, 11th -15thNovember 2011
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9. Sun WY, Pitson S and Bonder CS. Regulation of adhesion molecules by sphingosine kinase in inflammatory disease. *The 5th Barossa Meeting: Cell Signalling and Molecular Medicine, hosted by Centre for Cancer Biology*, Novotel Barossa Valley, Australia, 23rd -26thNovember 2011
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8. Sun WY and Bonder CS. Sphingosine kinase is a key regulator for allergic inflammation. *The CRC Biomarker Translation Student Annual Meeting, hosted by CRC Biomarker Translation*, La Trobe University, Australia, 11thNovember 2011
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5. Sun WY, Pitson S and Bonder CS. A novel mechanism regulates adhesion molecule expressions for neutrophil trafficking in the development of allergic inflammation. *The Postgraduate Research Conference, hosted by Faculty of Health Sciences, The University of Adelaide*, Adelaide, 25th August 2011
-*Poster presentation*

4. Sun WY, Pitson S and Bonder CS. Role for sphingosine kinase mediated-adhesion molecule expression during the early phase of allergic inflammation. *The 40th Australasian Society for Immunology Annual Meeting, hosted by Australasian Society for Immunology*, Perth, Australia, 5th-9th December 2010
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3. Sun WY, Pitson S and Bonder CS. Sphingosine kinase regulates adhesion molecule expression in allergic inflammation. *The 2nd CRC Biomarker Translation Annual Meeting, hosted by CRC Biomarker Translation*, Palm Cove, Australia, 28thNovember- 2nd December 2010
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2. Sun WY and Bonder CS. Regulation of adhesion molecule expression by sphingosine kinase in allergy. *The Centre for Stem Cell Research Annual Scientific Meeting 2010, Robinson Stem Cell Institute, The University of Adelaide*, Adelaide, 7th November 2010
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1. Sun WY and Bonder CS. Controlling adhesion molecule expression in allergic disease. *The Postgraduate Research Expo 2010, hosted by Faculty of Health Sciences, The University of Adelaide*, Adelaide, 1st September 2010
-*Poster presentation*

Abbreviations

$[Ca^{2+}]i$	intracellular calcium
ABC transporter	ATP-binding cassette transporter
ACD	citrate-dextrose solution
ACK	Ammonium-Chloride-Potassium
ADAM	A disintegrin and metallopeptidase
Ang	angiopoietin
APC	antigen presenting cell
ATF	activating transcription factor
BCR	B cell receptor
BMMC	bone marrow-derived mast cell
BSA	bovine serum albumin
CAM	cellular adhesion molecule
cAMP	cyclic adenosine monophosphate
CCL	chemokine ligand
CIA	collagen-induced arthritis
CIB	calcium and integrin binding protein
COPD	chronic obstructive pulmonary disease
CSR	class switch recombination

CXCL	C-X-C motif ligand
DHS	dihydro-sphingosine
DMS	d-*erythro*-*N*,*N*-dimethylsphingosine
DNP	2,4-dinitrophenol
EC	endothelial cell
ECM	extracellular matrix
EGF	epidermal growth factor
EPH	ethanol/propylene glycol/water
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FCS	fetal calf serum
FDA	Food and Drug Administration
Fn	fibronectin
FOV	field of view
G-CSF	granulocyte colony-stimulating factor
GDP	guanosine diphosphate
GF	growth factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GTP	guanosine-5'-triphosphate

HAEC	human aortic endothelial cell
HDAC	histone deacetylases
HDL	high density lipopolysaccharide
HSA	human serum albumin
HUVEC	human umbilical vein endothelial cell
ICAM	intercellular adhesion molecule
IFN	interferon
IL	interleukin
IRF1	interferon-regulatory factor 1
LAD	leukocyte adhesion deficiency
LPS	lipopolysaccharides
LT	leukotriene
MadCAM	mucosal vascular addressin cell adhesion molecule
MetOH	methanol
MCP	monocyte chemoattractant protein
MHC	major histocompatibility complex
MS	multiple sclerosis
Naf	Nef associated factor
NSF	*N*-ethylmaleimide-sensitive factor

OVA	ovalbumin
PAR	proteinase-activated receptor
PCA	passive cutaneous anaphylaxis
PDMC	peritoneal-derived mast cells
PHB	prohibitin
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PP2A	protein phosphatase 2A
PPAR	peroxisome proliferator-activated receptors
PPS	pentosan polysulfate sodium
PSGL	P-selectin glycoprotein ligand
PTX	pentraxin
RT	room temperature
S1P	sphingosine-1-phosphate
siRNA	small interfering RNA
SK	sphingosine kinase
SK2L	sphingosine kinase 2 long
SNAP	soluble NSF attachment protein

TGA	Therapeutic Goods Administration
Th	T helper lymphocyte
TNF	tumour necrosis factor
tPA	tissue plasminogen activator
TRAF	TNF receptor-associated factor
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cell
vWF	von Willebrand factor
WPB	Weibel-Palade bodies
WT	wildtype