

The role of class IA PI3K δ in experimental autoimmune encephalomyelitis

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A thesis submitted to the University of Adelaide
in fulfilment of the requirements for the degree of
Doctor of Philosophy

July 2010



Declaration

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July 2010

Acknowledgements

First of all I must thank my supervisor Professor Shaun McColl for affording me the opportunity to undertake such an interesting Ph.D. project. Your scientific advice, expertise and guidance have been invaluable, as has been your trust in allowing me some scientific independence. On a personal note, I am very thankful for the patience, kindness, encouragement and understanding that you have always shown me, particularly when I came to you two years into my Ph.D. and said „Guess what! I‘m having a baby“! I would also like to thank you for the time you have dedicated to editing both my thesis and published material and for the patient way that you have helped make my scientific writing much more betterer!

Next I must thank my wonderful colleagues: you have always made life in the lab interesting! Iain, you truly have been an amazing help, both on the giant experiment days and with your scientific advice; you‘re an inspiring role model and good friend to me, thanks. Adriana, your efforts to keep the lab going are no less than amazing, and you are always great for a laugh too! Julie and Matt, thanks for the endless laughs, entertainment and special lab coat dancing! Manuela and Marina, ever-knowledgeable post-docs, thanks for all of your scientific input as well as all of the great chats. Meizhi, all the best for finishing your Ph.D. with a newborn baby - you are Supermum, you can do it! Mark, Yuka, Wendel and Michelle: thanks for all the great chats and laughs and all the very best for the future. Lastly, the departed Jane and Scott: you have both contributed so much towards me enjoying my Ph.D. years and I feel very happy to have worked with you both and for having made such enduring friendships.

Professionally, I must thank Dr. Kamal Puri and Calistoga Pharmaceuticals (Seattle, USA) for providing the IC87114 compound used in this study and for performing all of the GC-MS on plasma samples. Dr. Iain Comerford assisted me on many of the busy days, performed some of the intracellular cytokine staining required for this study, optimised conditions for the Th1- and Th17-skewing cultures, aided with the optimisation of the DC antigen presentation experiment and helped with *in vivo* inhibitor experiments. Mark Bunting contributed to the DC migration and CFA-

immunisation experiments, commonly maintained BMDC cultures, assisted with optimising the DC antigen presentation assay and also helped with *in vivo* inhibitor experiments. This assistance was invaluable, thank you to you all.

Now for my wonderful friends: Kate B, you are awesome, you have no idea how much I will miss you! And thanks for your great advice when you told me that I „only need ONE Ph.D.“! Erin, you have significantly contributed to my sanity and happiness throughout this Ph.D. thing, thanks. Good luck getting finished and getting back to the ski slopes; I hope it happens very soon! Wendy, thanks for all of the great chats over the years, lab life just isn't the same without you. There are many important people who aren't specifically named here, but thank you everyone who has supported me through both my Ph.D. and becoming a mum. You are all irreplaceable and hopefully you know who you are.

Thanks to „Christine“ Mum, Dad (how did you get off that easily?), „idiot head“ Kate (plus Jye Jye and Kobes) and „spacko“ Amy, I really would not be the person that I am today without you guys. Thank you for always supporting me in what I do, I love you all forever. Archie, Eva, Quinn, Lisa, Hayden, Carson and Hope, thanks for your endless love, support and patience (well, actually, I wouldn't really say that Lisa was „patient“ *per se*), I can't wait to spend more time with all of you! Thanks also to the rest of my wonderful family in Australia and Canada - I am so lucky to be surrounded by such an amazing bunch of level-headed, caring and happy people.

Todd, thank you so much for your support during my Ph.D., it has been second to none. I am the luckiest girl in the world to be married to you; you are my best friend and having you in my life for the last 10 years has been an amazing blessing. I know that you wanted to be acknowledged both first and last on this page - it didn't happen, but trust me, I agree that you deserve it! And last but most certainly never the least, Lily. You are the brightest light in my life; you make me smile, laugh and feel happy every single day. Always remember that, just like Mummy, you can grow up to be whatever you want to be. I love you Todd and Lily - thanks.

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Abbreviations

ADP	Adenosine di-phosphate
AML	Acute myeloid leukaemia
APC	Antigen presenting cell
APL	Acute promyelocytic leukaemia
ARF	ADP ribosylation factors
ARNO	ARF nucleotide binding site opener
ATP	Adenosine tri-phosphate
AV	Annexin V
BBB	Blood brain barrier
BCR	B cell receptor
BD	Becton Dickinson
BMDC	Bone marrow-derived dendritic cell
BrdU	5-Bromo-2'-Deoxyuridine - Sigma
Btk	Bruton's tyrosine kinase
BSA	Bovine serum albumin
CEF	Chicken embryo fibroblast
CFA	Complete Freund's adjuvant
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CIA	Collagen-induced arthritis
CNS	Central nervous system
ConA	Concanavalin A
CTL	Cytotoxic T lymphocyte
DAG	Diacyl glycerol
DC	Dendritic cell
DEPC	Diethyl Pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA-PK	DNA-dependent protein kinase
DNP	Dinitrophenyl
EAE	Experimental Autoimmune Encephalomyelitis
Fab	Fragment, antigen binding
Fc	Fragment, crystalisable

FCS	Foetal calf serum
fMLP	N-formyl-methionyl-leucyl-phenylalanine
GAP	GTPase-activating proteins
GDP	Guanosine di-phosphate
GEF	Guanine nucleotide exchange factors
GM-CSF	Granulocyte macrophage – Colony stimulating factor
GPCR	G protein-coupled receptor
GRP	General receptor for phosphoinositides
GTP	Guanosine tri-phosphate
HBSS	Hank's balanced salt solution
IFA	Incomplete Freund's adjuvant
IFN- γ	Interferon gamma
IHC	Immunohistochemistry
IKK	I κ B kinase
Ins(1,4,5) P_3	Inositol(1,4,5)-trisphosphate
Itk	Inducible T cell kinase
KO/KI	Knock-out/knock-in
LPS	Lipopolysaccharide
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
MOG	Myelin Oligodendrocyte Glycoprotein
MRCRB	Mouse red cell removal buffer
mTOR	Mammalian target of rapamycin
NK cell	Natural killer cell
OVA	Ovalbumin
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PH	Plekstrin homology
PI3K	Phosphoinositide 3-kinase
PI	Propidium iodide
PIPkins	Proline-rich domain-containing inositol 5-phosphatase kinases

PIP ₂	Phosphatidylinositol(4,5)-bisphosphate (PtdIns(4,5)P ₂)
PIP ₃	Phosphatidylinositol(3,4,5)-trisphosphate (PtdIns(3,4,5)P ₃)
PKB	Protein kinase B
PKC	Protein kinase C
PLC	Phospho-lipase C
PLCγ ₂	Phospholipase C gamma 2
PLP	Proteolipid Protein
PMN	Polymorphonucleocyte
PP-MS	Primary progressive multiple sclerosis
PRR	Proline rich region
PtdIns	Phosphatidylinositol
PtdIns(4,5)P ₂	Phosphatidylinositol(4,5)-bisphosphate (PIP ₂)
PtdIns(3,4,5)P ₃	Phosphatidylinositol(3,4,5)-bisphosphate (PIP ₃)
PTEN	Phosphatase and tensin homolog deleted on chromosome ten
RA	Rheumatoid arthritis
RR-MS	Relapsing-remitting multiple sclerosis
RTK	Receptor tyrosine kinase
SA	Streptavidin
SHIP	Src homology 2 domain containing inositol polyphosphate phosphatase
SIP	Standard isotonic Percoll
SP-MS	Secondary progressive multiple sclerosis
TAE	Tris Acetate-EDTA
TCR	T cell receptor
Tc	T cytotoxic cell
Tg	Transgenic
Th	T helper cell
TNF	Tumour necrosis factor
Treg	Regulatory T cells (CD4 ⁺ /CD25 ⁺ /FoxP3 ⁺)

Publications arising from this work

Manuscripts

Sarah Haylock-Jacobs*, Iain Comerford*, Scott Townley, Mark Bunting, & Shaun McColl. PI3K δ is required for Th17 differentiation and the pathogenesis of experimental autoimmune encephalomyelitis. *Manuscript submitted to The Journal of Immunology, January 2010.*

Adrian Liston, Rachel Kohler, Scott Townley, **Sarah Haylock-Jacobs**, Iain Comerford, Adriana Caon, Julie Webster, Jodie Harrison, Jeremy Swann, Iain Clark-Lewis, Heinrich Korner & Shaun McColl. Inhibition of Chemokine Receptor 6 (CCR6) function reduces the severity of experimental autoimmune encephalomyelitis via effects on the priming phase of the immune response, *The Journal of Immunology*, 2009, 182 (5), 1321-30.

Rachel Kohler, Iain Comerford, Scott Townley, **Sarah Haylock-Jacobs**, Iain Clark-Lewis & Shaun McColl. Antagonism of the chemokine receptors CXCR3 and CXCR4 reduces the pathology of experimental autoimmune encephalomyelitis, *Brain Pathology*, 2008, 18(4), 504-16.

Iain Comerford, Robert Nibbs, Wendell Litchfield, Mark Bunting, Yuka Harata-Lee, **Sarah Haylock-Jacobs**, Steve Forrow & Shaun McColl. The atypical chemokine receptor CCX-CKR scavenges CCL21 in vivo and suppresses experimental autoimmune encephalomyelitis by regulating T cell priming in the spleen. *Manuscript submitted to Blood, January 2010.*

Iain Comerford*, **Sarah Haylock-Jacobs***, Wendel Litchfield, Geoff Hill, Heinrich Korner & Shaun McColl. Uncoupled regulation of cell surface CCR6 expression and IL-17 production by type 17 CD4⁺ and CD8⁺ T cells. *Manuscript in preparation.*

Manuela Klingler-Hoffmann, Julie Brazzatti, Erik Procko, Adriana Caon, **Sarah Haylock-Jacobs**, Angel Lopez, Mark Guthridge, Reinhard Wetzker & Shaun McColl. Essential roles of p101 in cell migration. *Manuscript in preparation.*

Conference proceedings

- 2009:** Oral presentation at the Australasian Immunology Retreat (Adelaide, Australia).
Title: PI3K δ is important for Th17 generation and EAE
- 2009:** Poster presentation at the Australasian Society for Medical Research conference (Adelaide, Australia).
Title: Activity of the catalytic subunit of PI3K δ is required for the pathogenesis of experimental autoimmune encephalomyelitis
- 2008:** Poster presentation at the Australasian Society for Immunology Annual Scientific Meeting (Canberra, Australia).
Title: Activity of the catalytic subunit of PI3K δ is required for the pathogenesis of experimental autoimmune encephalomyelitis
- 2008:** Oral presentation at the Australasian Immunology Retreat (Adelaide, Australia).
Title: Investigating the role of p110 δ in EAE
- 2008:** Poster presentation at the Canadian Society for Immunology conference (Montreal, Canada).
Title: The role of chemokine receptor CCR7 in experimental autoimmune encephalomyelitis
- 2007:** Oral presentaion at the third Adelaide Immunology Retreat (Adelaide, Australia).
Title: Investigating the role of PI3K δ in experimental autoimmune encephalomyelitis
- 2006:** Oral presentaion at the second Adelaide Immunology Retreat (Adelaide, Australia).
Title: Investigating the role of p101/PI3K γ in cell migration
- 2005:** Poster presentation at the Australasian Society for Immunology Scientific Meeting (Melbourne, Australia).
Title: The role of chemokine receptor CCR7 in experimental autoimmune encephalomyelitis

Abstract

Through its role in cells of haematopoietic origin, the class IA phosphoinositide 3-kinase delta (PI3K δ) has a significant impact on both the cell-mediated and innate arms of the immune system. The catalytic protein subunit of PI3K δ , p110 δ , has been implicated in leukocyte activation and survival, Th1 and Th2 differentiation as well as the development of autoimmunity in a model of rheumatoid arthritis. While the impact of p110 δ inactivation *in vitro* is becoming clearer, the precise role that p110 δ plays *in vivo* remains poorly understood, particularly in regard to Th17 differentiation and models of autoimmunity. Here, using mice that express a catalytically inactive form of p110 δ (p110 $\delta^{\text{D910A/D910A}}$ mice) it is shown that functional p110 δ is required for full expression of experimental autoimmune encephalomyelitis (EAE), a Th17-dependent model of the human autoimmune disease multiple sclerosis (MS). In p110 δ -inactivated mice, T and B cell activation and function during EAE were markedly reduced, and fewer T and B cells were observed in the central nervous system (CNS) throughout disease. Th17 cell generation was demonstrably more dependent on p110 δ than was the Th1 response. The decrease in T cell activation was not due to a defect in dendritic cell (DC) function because p110 δ -inactivated DCs migrated, became activated and presented antigen normally. However, there was a significant increase in the proportion of T and B lymphocytes undergoing apoptosis at early stages of EAE. Due to the promising findings observed in the p110 $\delta^{\text{D910A/D910A}}$ mice, the ability of the p110 δ inhibitor, IC87114, to reduce EAE pathogenesis was investigated. While IC87114 was shown to be a potent inhibitor of Th1 and Th17 activation and differentiation *in vitro*, administration of this compound failed to reduce EAE disease under the dosing regimen used. Despite this, these findings indicate that p110 δ plays an important role in the development of IL-17-dependent inflammation and suggest that small molecule inhibitors for p110 δ may be useful therapeutics for the treatment of IL-17-driven pathologies.