The role of class IA PI3Kδ in experimental autoimmune encephalomyelitis

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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	Carboxyfluorescin 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	IκB 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 histocombatability 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
PLCγ2	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. PI3Kδ 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: PI3Kδ 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 PI3Kδ 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 PI3Kδ is required for the pathogenesis of experimental autoimmune encephalomyelitis

2008: Oral presentation at the Australasian Immunology Retreat (Adelaide, Australia).
Title: Investigating the role of p110δ 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 PI3Kδ in experimental autoimmune encephalomyelitis

2006: Oral presentaion at the second Adelaide Immunology Retreat (Adelaide, Australia).
Title: Investigating the role of p101/PI3Kγ 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 (PI3Kδ) has a significant impact on both the cell-mediated and innate arms of the immune system. The catalytic protein subunit of PI3Kδ, p110δ, 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 p110δ inactivation *in vitro* is becoming clearer, the precise role that p110δ 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 p110δ ($p110\delta^{D910A/D910A}$ mice) it is shown that functional p110δ is required for full expression of experimental autoimmune encephalomyelitis (EAE), a Th17-dependent model of the human autoimmune disease multiple sclerosis (MS). In p110δ-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 p110δ than was the Th1 response. The decrease in T cell activation was not due to a defect in dendritic cell (DC) function because p110δ-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 $p110\delta^{D910A/D910A}$ mice, the ability of the p110δ 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 p110δ plays an important role in the development of IL-17-dependent inflammation and suggest that small molecule inhibitors for p110δ may be useful therapeutics for the treatment of IL-17-driven pathologies.