The role of c-jun N-terminal kinase (JNK) in human T cell function

Michelle Melino

(B. Biotech. with Hons)

Thesis submitted for the degree of Doctor of Philosophy

Department of Microbiology & Immunology

School of Molecular & Biomedical Science

The University of Adelaide

February 2009

TABLE OF CONTENTS

SUMMARY

T cells are involved in cellular pathways which enable the immune system to protect us against infection and cancer. However, the same mechanisms also allow T cells to generate chronic inflammatory conditions, including autoimmunity and allergy. Thus a concerted effort has been made to try to understand how the immune system functions in order to inhibit responses which may have harmful effects on tissues and organs. There is a continued search for new immunosuppressants which can only be accomplished through a better understanding of the pathways that regulate T cell function. This includes the intracellular signalling pathways which modulate T cell proliferation and cytokine production.

While the Mitogen-Activated Protein Kinases (MAPK), extracellular signal-regulated protein kinases (ERK) and p38 have received attention, the role of the stress-activated protein kinases or c-jun N-terminal kinases (JNK) remains controversial. To overcome some of the limitations in studying the role of JNK, a new approach was taken in this thesis. The investigations used recently described peptides (TAT-$JIP_{153-163}$ and TAT-$JIP_{153-172}$) derived from the scaffold protein, JIP-1, which have previously been demonstrated to act as JNK pathway inhibitors. The research characterised the specificity of these inhibitors to enable the appropriate interpretation of data.

Using these inhibitors, we were able to show that JNK regulated human T cell proliferation and cytokine production in T cell responses induced independently of TCR ligation (PHA-PMA) or via the TCR (anti-CD3-anti-CD28 antibodies, Mixed Lymphocyte Reaction (MLR), Tetanus Toxoid and Der p 2). The data demonstrated that JNK primarily regulated the Th1 cytokine patterns (IFNγ, IL2 and LT) with minimal effect on Th2 cytokine production (IL4, IL10) in response to all stimulatory models. However, while the JNK signalling pathway

promoted T cell proliferation and cytokine production in response to PHA-PMA, the pathway depressed these responses following stimulation with anti-CD3-anti-CD28 antibodies and Tetanus Toxoid. Thus activation of JNK with microbial pathogens such as *Pseudomonas aeruginosa* (PA), which non-specifically activate T cells, may promote lymphocyte proliferation and the release of Th1 cytokines, such as IFNγ. In contrast, JNK activation resulting from engagement of the T cell receptor (TCR) (i.e. Tetanus Toxoid), down-regulates Th1 cytokine production. Therefore, it is likely that the JNK signalling pathway may dampen the development of chronic inflammatory conditions resulting from infection with intracellular parasites and autoimmune diseases. In contrast to Tetanus Toxoid, responses to the recombinant house dust mite allergen, *Dermatophagoides pteronyssinus* (Der p 2) were promoted by JNK, leading to an increase in Th1 cytokine production. Thus the results suggest that the use of JNK inhibitors could exacerbate both inflammatory conditions (autoimmunity and allergy) and this may also apply to p38 but not the ERK signalling pathway.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

……. ……...

Michelle Melino Date

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisors, Professor Tony Ferrante and Professor Shaun McColl for all your patience, guidance and support. Thank you for helping me to achieve a goal I never thought would be possible and to Associate Professor Charles Hii, thank you for answering all my questions and teaching me the challenging world of cell signalling.

A special thank you to Professor W.R. Thomas at the University of Western Australia for kindly donating the recombinant allergen and to Kathie Carman for assisting me with all the cytokine work. I am truly thankful for all your time and effort and I could not have completed this study without you.

To all of my friends in the Immunopathology department, the diagnostic staff: Tricia, Kathie, Lily, Tuyen, Monica, Jess and Renee, who welcomed me with open arms from the very first day. Thank you for all your assistance, kindness and support.

Thank you to all of my friends who have shared the research lab with me over the years. To everyone who was there at the very beginning, Laura, Amy, Mel, Christos and James, I would have been lost without your support. To everyone who was with me until the very end, Alex and Yong, I will miss our little corner of the lab and to Dr. Mukaro (Villey), Bernadette (BM) and Mei (Mei, Mei), thank you for always being there, for making me laugh even when I felt like crying and for sharing your morning tea time with me. It was always my favourite part of the day. I am going to miss you all very much.

Finally, to my parents, Didi and all of my family and friends, thank you for all your patience, love and support. This would not have been possible without you.

PUBLICATIONS AND PRESENTATIONS

Publications

Melino, M., C. S. Hii, S. R. McColl and A. Ferrante (2008). "The effect of the JNK inhibitor, JIP peptide, on human T lymphocyte proliferation and cytokine production." J Immunol 181(10): 7300-6.

Costabile, M., C. S. Hii, M. Melino, C. Easton and A. Ferrante (2005). "The immunomodulatory effects of novel beta-oxa, beta-thia, and gamma-thia polyunsaturated fatty acids on human T lymphocyte proliferation, cytokine production, and activation of protein kinase C and MAPKs." J Immunol 174(1): 233-43.

Presentations

“The role of c-jun N-terminal kinase (JNK) in human T cell proliferation and cytokine production.”
San Raffaele Scientific Institute (2008)
Milan, Italy

“Regulation of human T lymphocyte proliferation and cytokine production by c-jun N-terminal kinase (JNK).”
Australasian Society for Immunology (ASI) 37th Annual Conference (2007)
Sydney, Australia

“Regulation of cytokine production by Mitogen-Activated Protein kinases in human T lymphocytes.”

University of Adelaide (2006)

Adelaide, Australia

ABBREVIATIONS

AICD	activation-induced cell death
AP-1	activator of transcription 1
APC	antigen presenting cells
APS	ammonium persulfate
ASK1	apoptosis signal-regulated kinase 1
ATF2	activating transcription factor 2
ATP	adenosine tri-phosphate
BD	Becton Dickinson
BSA	bovine serum albumin
CaMK	calcium/calmodulin-dependent kinase
CARMA-1	caspase recruitment domain containing membrane-associated guanylate kinase protein-1
CBA	cytometric bead array
CDK2	cyclin dependent kinase 2
CDR	complementarity determining regions
CHK2	checkpoint kinase 2
CIA	collagen-induced arthritis
CK1	casein kinase 1
Con A	concanavalin A
COX	cyclooxygenase
DAG	diacylglycerol
DMARD	disease modifying antirheumatic drug
DMSO	dimethyl sulfoxide
DTT	dithiothreitol

DYRK	dual-specificity tyrosine phosphorylated and regulated kinase
EDTA	ethylenediaminetetraacetic acid
ERK	extracellular signal-regulated kinase
FBS	foetal bovine serum
FITC	fluorescein isothiocynate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GM-CSF	granulocyte monocyte-colony stimulating factor
HDM	house dust mite
HIPK2	homeodomain interacting protein kinase 2
HIV	human immunodeficiency virus
HPK1	hematopoietic progenitor kinase 1
HPLC	high-performance liquid chromatography
HRP	horse radish peroxidase
IFN	interferon
Ig	immunoglobulin
IKK	IκB kinase
IL	interleukin
IP3	inositol 1,4,5-trisphosphate
ITAM	immunoreceptor tyrosine-based activation motif
iTreg	induced regulatory T cells
IκB	inhibitor of NFκB
JAK	Janus kinase
JBD	JNK binding domain
JIP-1	JNK interacting protein 1
JNK	c-jun N-terminal kinase

LAT	linker of activated T cells
LT	lymphotoxin
MAPK	mitogen-activated protein kinase
MELK	maternal embryonic leucine zipper kinase
MHC	major histocompatibility complex
MLK3	mixed lineage kinase 3
MLR	mixed lymphocyte reaction
NFAT	nuclear factor of activated T cells
NFκB	nuclear factor of κ-light-chain-enhancer of activated B cells
NK	natural killer cells
NP40	Nonidet-40
NSAID	non steroidal anti-rheumatic drug
p70S6K	p70 ribosomal protein S6 kinase
PA	*pseudomonas aeruginosa*
PBMC	peripheral blood mononuclear cells
PDK	3’ phosphoinositide-dependent kinase
PE	phycoerythrin
PG	prostaglandin
PHA	phytohaemagglutinin
PI3K	phosphatidylinositol 3 kinase
PIP2	phosphatidylinositol-4,5-bisphosphate
PKC	protein kinase C
PLCγ1	phospholipase Cγ1
PMA	12-myristate-13-acetate
PMSF	phenylmethylsulfonyl fluoride

PTK	protein tyrosine kinase
RA	rheumatoid arthritis
Rag1	recombination activating gene 1
RPMI	Roswell Park Memorial Institute
RPMI/ΔAB	RPMI 1640 containing 5 % heat-inactivated blood group AB serum
RPMI/ΔFBS	RPMI 1640 containing 5 % heat-inactivated foetal bovine serum
Rsk1	ribosomal S6 protein kinase 1
SDS	sodium dodecyl sulphate
SGK	serum and glucocorticoid-regulated kinase
siRNA	small interfering RNA
SLE	systemic lupus erythematosus
SLP-76	SH2 domain-containing leukocyte protein of 76 kDa
SOCS	suppressor of cytokine signalling
SOS	son of sevenless
STAT	signal transducer and activator of transcription
TAK1	transforming growth factor β-activated kinase 1
TAT	transactivator of transcription
TCR	T cell receptor
Th	helper T cell
TNF	tumour necrosis factor
Treg	regulatory T cell
ZAP-70	ζ-associated protein-70

INDEX OF FIGURES

INDEX OF TABLES