

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

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TABLE OF CONTENTS

Summary.....	i
Declaration.....	iii
Acknowledgements.....	iv
Publications and presentations.....	v
Abbreviations.....	vii
Index of Figures.....	xi
Index of Tables.....	xvi
Chapter One.....	1
Introduction.....	1
1.1 General Introduction.....	2
1.2 T cell development.....	3
1.3 CD4 ⁺ T cell classification.....	4
1.4 Th1 and Th2 differentiation.....	7
1.5 Th1 and Th2 cytokine patterns.....	7
1.6 Cytokines which impact on helper T cells.....	11
1.7 T cells in allergy.....	11
1.8 T cells in autoimmunity.....	13
1.9 Mechanism of T cell activation.....	16
1.10 The MAPK pathways in T cell proliferation and cytokine production.....	20
1.11 Role of ERK in T cell proliferation and cytokine production.....	21
1.12 Role of p38 in T cell proliferation and cytokine production.....	25
1.13 Role of JNK in T cell proliferation and cytokine production.....	30
1.14 The TAT-JIP peptide.....	37

1.15	Concluding remarks	43
1.16	Aims, hypotheses and significance	43
	Chapter Two	45
	Materials and Methods.....	45
2.1	Materials	46
2.2	Buffers	48
2.3	Purification of human PBMC	51
2.4	Purification of human T cells.....	52
2.5	Purification of murine splenic T cells.....	54
2.6	Determination of cell purity.....	54
2.7	PHA-PMA and anti-CD3-anti-CD28 induced activation	57
2.8	Tetanus Toxoid induced lymphocyte responses	57
2.9	Mixed Lymphocyte Reaction.....	58
2.10	Allergen induced activation	58
2.11	Cytokine determination.....	59
2.12	Measurement of phosphorylated JNK and phosphorylated jun by western blotting	61
2.12.1	Sample preparation	61
2.12.2	Lowry's Protein assay	61
2.12.3	Western Blot	62
2.13	siRNA	62
2.14	Kinase profiler assays	63
2.15	Statistical Analysis.....	64
	Chapter Three	65
	Role of JNK in T cell responses induced by PHA-PMA.....	65
3.1	Introduction.....	66
3.2	PHA-PMA induced JNK activation in human T cells	68

3.3	Effect of TAT-JIP ₁₅₃₋₁₆₃ on the JNK pathway in human T cells	72
3.4	Effect of the TAT-JIP ₁₅₃₋₁₆₃ peptide on human T cell function	74
3.5	Effect of the TAT-JIP ₁₅₃₋₁₆₃ peptide on murine T cell function	78
3.6	Effect of the pharmacological JNK inhibitor, SP600125 on human T cell function	80
3.7	Summary	83
	Chapter Four	84
	Role of JNK in T cell responses induced via the TCR	84
4.1	Introduction.....	85
4.2	Effect of the TAT-JIP ₁₅₃₋₁₆₃ peptide on the JNK pathway in TCR-induced T cells	87
4.3	Effect on human T cell function in response to anti-CD3-anti-CD28 antibodies	90
4.4	Effect on T cell responses in the mixed lymphocyte reaction	96
4.5	Effect on antigen-induced T cell responses	99
4.6	Effect on allergen-induced T cell responses	102
4.7	Summary	105
	Chapter Five.....	108
	Relationship between JNK, ERK and p38 in T cell function	108
5.1	Introduction.....	109
5.2	Role of ERK and p38 in PHA-PMA-induced T cell responses	110
5.3	The effect of ERK, p38 and JNK inhibition on PHA-PMA-induced T cell responses	116
5.4	Role of ERK and p38 in anti-CD3-anti-CD28-induced T cell responses.....	120
5.5	The effect of ERK, p38 and JNK inhibition on anti-CD3-anti-CD28-induced T cell responses	125
5.6	Summary	129
	Chapter Six	132
	Specificity of the TAT-JIP ₁₅₃₋₁₆₃ peptide	132

6.1	Introduction.....	133
6.2	Effect of JIP-1-derived peptides on CDK2, CK1, p70S6K, Rsk1, SGK and DYRK activity	134
6.3	Effect of the TAT-JIP ₁₅₃₋₁₇₂ peptide on PHA-PMA and anti-CD3-anti-CD28-induced T cell responses.	147
6.4	Investigating the role of JNK using RNA interference.....	157
6.5	Summary.....	161
	Chapter Seven.....	163
	Discussion.....	163
7.1	Introductory remark	164
7.2	Targeting the JNK signalling pathway with the TAT-JIP peptides.....	165
7.3	Role of JNK in T cell proliferation.....	168
7.4	Role of JNK in T cell cytokine production.....	170
7.5	Interaction between members of the MAPK family in T cell function	173
7.6	The relationship between Th1, Th2, Th17 and Tregs.....	179
7.7	Infection and immunity, allergy and autoimmunity	180
7.8	Concluding remarks.....	181
	References.....	184

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-JIP₁₅₃₋₁₆₃ and TAT-JIP₁₅₃₋₁₇₂) 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 (IFN γ , 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 IFN γ . 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.

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Michelle Melino

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Date

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PUBLICATIONS AND PRESENTATIONS

Publications

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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 isothiocyanate
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	I κ B kinase
IL	interleukin
IP3	inositol 1,4,5-trisphosphate
ITAM	immunoreceptor tyrosine-based activation motif
iTreg	induced regulatory T cells
I κ B	inhibitor of NF κ B
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
NF κ B	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	<i>pseudomonas aeruginosa</i>
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
PLC γ 1	phospholipase C γ 1
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/ Δ AB	RPMI 1640 containing 5 % heat-inactivated blood group AB serum
RPMI/ Δ FBS	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

Fig. 1.1. Summary of CD4 ⁺ helper T cell subsets.	6
Fig. 1.2. Mechanism of T cell activation.	19
Fig. 1.3. The ERK1/ERK2 cascade.	23
Fig. 1.4. The p38 cascade.	28
Fig. 1.5. The JNK cascade.	34
Fig. 1.6. The chemical structure of SP600125.	35
Fig. 1.7. JIP-1 is a scaffold protein for the JNK signalling pathway.	40
Fig. 2.1. Flow chart of experimental procedure.	53
Fig. 2.2. Dot plot of T cell analysis by flow cytometry.	56
Fig. 2.3. Examples of standard curves for human cytokine production.	60
Fig. 3.1. JNK is phosphorylated in human T cells in response to PHA-PMA stimulation.	70
Fig. 3.2. Jun is phosphorylated in human T cells in response to PHA-PMA stimulation.	71
Fig. 3.3. Inhibition of jun phosphorylation by TAT-JIP ₁₅₃₋₁₆₃ in intact human T cells in response to PHA-PMA stimulation.	73
Fig. 3.4. Inhibition of human T cell proliferation by the TAT-JIP ₁₅₃₋₁₆₃ peptide.	75
Fig. 3.5. The control peptide did not inhibit T cell proliferation in response to PHA-PMA stimulation.	76
Fig. 3.6. Inhibition of human T cell cytokine production by the TAT-JIP ₁₅₃₋₁₆₃ peptide.	77
Fig. 3.7. Inhibition of T cell proliferation by TAT-JIP ₁₅₃₋₁₆₃ in mouse splenic T cells.	79
Fig. 3.8. SP600125 does not inhibit human T cell proliferation in response to PHA-PMA stimulation.	81
Fig. 3.9. SP600125 did not inhibit jun phosphorylation in human T cells.	82

Fig. 4.1. Jun is phosphorylated in human T cells in response to anti-CD3-anti-CD28 antibodies.....88

Fig. 4.2. Inhibition of JunB phosphorylation by the TAT-JIP₁₅₃₋₁₆₃ peptide in human T cells in response to anti-CD3-anti-CD28 antibodies.....89

Fig. 4.3. Enhancement of T cell proliferation by the TAT-JIP₁₅₃₋₁₆₃ peptide in response to anti-CD3-anti-CD28 antibody stimulation.....92

Fig. 4.4. Enhancement of cytokine production by the TAT-JIP₁₅₃₋₁₆₃ peptide in response to anti-CD3-anti-CD28 antibody stimulation.93

Fig. 4.5. Inhibition of T cell proliferation by SP600125 in response to anti-CD3-anti-CD28 antibodies.....94

Fig. 4.6. The effect of SP600125 on cytokine production in response to anti-CD3-anti-CD28 stimulation.95

Fig. 4.7. Enhancement of cell proliferation by the TAT-JIP₁₅₃₋₁₆₃ peptide in the MLR.....97

Fig. 4.8. Enhancement of IFN γ production by the TAT-JIP₁₅₃₋₁₆₃ peptide in the mixed lymphocyte reaction.....98

Fig. 4.9. Enhancement of lymphocyte proliferation by the TAT-JIP₁₅₃₋₁₆₃ peptide in response to Tetanus Toxoid.100

Fig. 4.10. Enhancement of cytokine production by the TAT-JIP₁₅₃₋₁₆₃ in response to antigen stimulation.101

Fig. 4.11. Inhibition of lymphoproliferation by the TAT-JIP₁₅₃₋₁₆₃ peptide in response to Der p 2.103

Fig. 4.12. Inhibition of cytokine production by TAT-JIP₁₅₃₋₁₆₃ peptide in response to Der p 2.104

Fig. 5.1. Enhancement of T cell proliferation by PD98059 in response to PHA-PMA stimulation.112

Fig. 5.2. Effect of the ERK pathway inhibitor, PD98059 on T cell cytokine production in response to PHA-PMA stimulation.	113
Fig. 5.3. Inhibition of T cell proliferation by the p38 pathway inhibitor, SB203580 in response to PHA-PMA stimulation.	114
Fig. 5.4. Inhibition of T cell cytokine production by SB203580 in response to PHA-PMA stimulation.	115
Fig. 5.5. Inhibition of T cell proliferation by a combination of ERK, p38 and JNK inhibitors in response to PHA-PMA stimulation.	117
Fig. 5.6. Inhibition of T cell cytokine production by p38 and JNK inhibitors in response to PHA-PMA stimulation.	118
Fig. 5.7. Inhibition of T cell cytokine production by a combination of ERK, p38 and JNK inhibitors in response to PHA-PMA stimulation.	119
Fig. 5.8. Inhibition of T cell proliferation by PD98059 in response to anti-CD3-anti-CD28 antibodies.	121
Fig. 5.9. Inhibition of T cell cytokine production by PD98059 in response to anti-CD3-anti-CD28 antibodies.	122
Fig. 5.10. Enhancement of T cell proliferation by SB203580 in response to anti-CD3-anti-CD28 antibodies.	123
Fig. 5.11. Enhancement of IL2 production by SB203580 in response to anti-CD3-anti-CD28 antibodies.	124
Fig. 5.12. The effect of combining ERK, p38 and JNK inhibitors on T cell proliferation in response to anti-CD3-anti-CD28 antibodies.	126
Fig. 5.13. The effect of combining p38 and JNK inhibitors on T cell proliferation in response to anti-CD3-anti-CD28 antibodies.	127
Fig. 5.14. Inhibition of T cell cytokine production by a combination of ERK, p38 and JNK inhibitors in response to CD3-CD28 stimulation.	128

Fig. 6.1. TAT-JIP ₁₅₃₋₁₆₃ inhibits CDK2/cyclin A activity.....	135
Fig. 6.2. TAT-JIP ₁₅₃₋₁₆₃ inhibits p70S6K activity.....	136
Fig. 6.3. TAT-JIP ₁₅₃₋₁₆₃ inhibits SGK activity.....	137
Fig. 6.4. TAT-JIP ₁₅₃₋₁₆₃ does not inhibit CK1 activity.....	138
Fig. 6.5. TAT-JIP ₁₅₃₋₁₆₃ does not inhibit DYRK activity.....	139
Fig. 6.6. TAT-JIP ₁₅₃₋₁₆₃ does not inhibit Rsk1 activity.....	140
Fig. 6.7. TAT-JIP ₁₅₃₋₁₇₂ does not inhibit CDK2/cyclin A activity.....	141
Fig. 6.8. TAT-JIP ₁₅₃₋₁₇₂ does not inhibit p70S6K activity.....	142
Fig. 6.9. TAT-JIP ₁₅₃₋₁₇₂ does not inhibit SGK activity.....	143
Fig. 6.10. TAT-JIP ₁₅₃₋₁₇₂ does not inhibit CK1 activity.....	144
Fig. 6.11. TAT-JIP ₁₅₃₋₁₇₂ does not inhibit DYRK activity.....	145
Fig. 6.12. TAT-JIP ₁₅₃₋₁₇₂ inhibits Rsk1 activity.....	146
Fig. 6.13. Inhibition of human T cell proliferation by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to PHA-PMA.	149
Fig. 6.14. Inhibition of human T cell cytokine production by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to PHA-PMA.	150
6.15. Enhancement of human T cell proliferation by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to anti-CD3-anti-CD28 antibodies.....	151
Fig. 6.16. Enhancement of cytokine production by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to CD3-CD28 stimulation.....	152
6.17. Enhancement of human T cell proliferation by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to Tetanus Toxoid.	153
Fig. 6.18. Enhancement of cytokine production by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to Tetanus Toxoid stimulation.	154
Fig. 6.19. Inhibition of lymphoproliferation by the TAT-JIP ₁₅₃₋₁₇₂ peptide in response to Der p 2.	155

Fig. 6.20. Inhibition of cytokine production in TAT-JIP ₁₅₃₋₁₇₂ treated PBMC in response to Der p 2.	156
Fig. 6.21. The effect of siRNA on JNK1 and GAPDH expression.	159
Fig. 7.1. Summary of the role of the MAPK in human T cell function in response to PHA-PMA (A) and anti-CD3-anti-CD28 antibodies (B).....	176
Fig. 7.2. Summary of the role of the MAPK in human T cell function in response to Tetanus Toxoid.....	177
Fig. 7.3. Summary of the role of the MAPK in human T cell function in response to Der p 2 allergen.....	178

INDEX OF TABLES

Table 1.1: Effect of ERK inhibition on T cell function.	24
Table 1.2. Effect of p38 inhibition on T cell function.	29
Table 1.3. Effect of JNK inhibition on T cell function.	36
Table 1.4. The amino acid sequences for the TAT peptide and the long and short JIP-1-derived peptides.	41
Table 1.5. Recent studies involving the use of JIP-derived peptides.	42
Table 4.1. Summary of the effect of the TAT-JIP ₁₅₃₋₁₆₃ peptide on T cell function in TCR-induced models.	107
Table 5.1. Comparison of the effect of MAPK inhibition on T cell proliferation in response to PHA-PMA and CD3-CD28 stimulation.	130
Table 5.2. Comparison of the effect of MAPK inhibition on T cell cytokine production in response to PHA-PMA and CD3-CD28 stimulation.	131
Table 6.1 Comparison of the effect of the JIP-1 derived peptides on human T cell function in response to PHA-PMA, anti-CD3-anti-CD28 antibodies, Tetanus Toxoid and Der p 2.....	162
Table 7.1. Comparison between the effect of SP600125, TAT-JIP ₁₅₃₋₁₆₃ and TAT-JIP ₁₅₃₋₁₇₂ on CDK2/cyclin A, CK1, p70S6K, Rsk1, SGK and DYRK activity.	167