Selective activation of tumour necrosis factor receptor-mediated intracellular signalling pathways

VIOLET RUDO SAMANTHA MUKARO

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Department of Immunopathology

Children, Youth and Women's Health Service

Faculty of Health Sciences

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Summary

Tumour necrosis factor (TNF) is a pleiotropic cytokine that has been shown to play a major role in defence against infections and malignancy, and regulation of the innate and adaptive immune responses. Despite its beneficial role, the cytokine has been implicated in the pathophysiology of a range of diseases including sepsis, cerebral malaria and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. While blocking the activity of excessive TNF has become a therapeutic approach to managing patients with these diseases, there are concerns since this also decreases resistance against infection and cancer. Attempts to target intracellular signalling pathways used by TNF, such as the p38 mitogen activated protein kinase (MAPK) have also met with limitations and studies have been discontinued due to toxicity. Since most proteins exert their biological activity through the interaction between very small regions of their folded surfaces to their cognate receptors, smaller peptides which mimic the shape of the proteins at these points of contact with the receptors can be used to mimic and/or block the actions of these proteins. We have previously demonstrated that the TNF mimetic peptides TNF_{70-80} and $TNF_{132-150}$ exhibited distinct biological activities, which in combination represented the spectrum of biological activities displayed by TNF. Research in this thesis sought to use these properties to develop new targets for development of anti-inflammatory agents. The mimetic TNF_{70-80} was shown to bind and act as a ligand for the TNF receptor I (TNFRI) and selectively activated the p38 MAPK pathway, and not the c-Jun NH_2-terminal kinase (JNK) and extracellular-signal-regulated kinase 1 and 2 (ERK1/ERK2) pathways. In contrast $TNF_{132-150}$ selectively activated the JNK and ERK1/ERK2 pathways. This is consistent with the biological properties of

these peptides. The basis for the activation of a restricted signalling pathway by TNF_{70-80} was related to a reduced capability to recruit adapter proteins. The peptide mimetic ligated TNFR was able to functionally couple TNF receptor associated factor 2 (TRAF2) to the p38 and NF-κB pathway but was unable to effect the coupling of germinal centre kinase (GCK) and apoptosis signal-regulating kinase (ASK1) to TRAF2, probably explaining the lack of activation of JNK and ERK1/ERK2 pathways. Using the ability of TNF_{70-80} to activate p38, we identified the region to which TNF_{70-80} binds to the TNFRI. Synthetic peptides representing the 206-211 amino acid residues of the TNFRI were made and examined for anti-TNF effects *in vitro* and *in vivo*. These TNFR mimetic peptides were found to selectively block TNF induced p38 activation and associated functions of neutrophil superoxide production, CD11b upregulation and cytokine production. Similar results were found with the monocytic cell line, Mono Mac 6. These TNFRI-derived peptides were found to inhibit leukocyte infiltration into inflammatory sites in acute and chronic inflammation models. Our findings open new opportunities for the development of therapeutics which selectively target the TNFR-p38 signalling pathway in chronic inflammatory diseases.

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.

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Violet R.S. Mukaro Date

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Publications, presentations and awards

Publications

1. Mukaro, V. R., M. Costabile, K. J. Murphy, C. S. Hii, P. R. Howe, and A. Ferrante. 2008. Leukocyte numbers and function in subjects eating n-3 enriched foods: selective depression of natural killer cell levels. Arthritis Res Ther 10 (3): R57.

2. Marantos, C., V. Mukaro, J. Ferrante, C. Hii, and A. Ferrante. 2008. Inhibition of the Lipopolysaccharide-Induced Stimulation of the Members of the MAPK family in Human Monocytes/Macrophages by 4-Hydroxynonenal, a Product of Oxidized Omega-6 Fatty Acids. Am J Pathol. 173(4):1057-66.

3. Mukaro, V., X. Gao, C. Haddad, G. Mayne, H. Sundqvist, R. Flower, Z. H Huang, C. S. T. Hii, and A. Ferrante. 2008. Selective signaling via p38 MAP kinase by the TNF peptide mimetic TNF_{70-80} involving the TNF receptor. In preparation.

4. Yeh, M, V. Mukaro, C. Marantos, C. Hii and A. Ferrante. 2008. Regulation of neutrophil chemotaxis and phagocytosis by c-jun NH2 terminal kinases. Submitted.

5. Thathaisong, U., V. Mukaro, C. Marantos, C. S. T. Hii, A. Ferrante and N. N. Gorgani. 2008. Arachidonic Acid Inhibits Complement Receptor Immunoglobulins (CRIg) mRNA Expressed During Human Monocytes Maturation to Macrophages and a Role of Protein Kinase C. In preparation.

Abstracts presented at conferences

1. Mukaro, V., Z. Huang, X. Gao, C. Haddad, G. Mayne, H. Sundqvist, R. Flower, C. S. T. Hii, and A. Ferrante. Selective activation of tumour necrosis factor receptor-mediated intracellular signaling pathways. Australian Society for Medical Research Week, SA Scientific Meeting, Adelaide, Australia June 4 2008.

2. Mukaro, V., Z. Huang, X. Gao, C. Haddad, G. Mayne, H. Sundqvist, R. Flower, C. S. T. Hii, and A. Ferrante. Selective activation of TNFR-induced intracellular signalling. Cambridge Healthtech Institute's 15th International Molecular Medicine Tri-Conference. San Francisco, CA, USA March 25-28, 2008.

3. Mukaro, V., M. Costabile, K. J. Murphy, C. S.T. Hii, P. R.C. Howe and A. Ferrante. Effect of long term consumption of omega-3 enriched foods on circulatory leukocyte numbers and function: Selective depression of natural killer

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Awards

Australasian Society for Immunology (ASI) student travel bursary- 2007

Faculty of Health Sciences Postgraduate Travelling Fellowship- 2008

Abbreviations

ACTH	adenocortiotropic hormone
ADAM	a disintegrin and matrix metalloprotease domains
APC	antigen presenting cells
AREs	adenosine-uridine rich elements
ASK1	apoptosis signal-regulating kinase
A-SMase	acid sphingomyelinase
ATF	activation transcription factors
BAL	bronchoalveolar lavage
CAMS	cell adhesion molecules
CAPK	ceramide-activated protein kinase
cIAP	cellular inhibitor of apoptosis protein
CINC	cytokine-induced neutrophil chemoattractant
CM	cerebral malaria
COPD	chronic obstructive pulmonary disease
COX	cyclo-oxygenases
CR	complement receptor
CRD	cysteine rich domains
DC	dendritic cells
DC-SIGN	DC specific-ICAM-3-grabbing non-integrin
DD	death domain
DED	death effector domain
DISC	death-inducing signal complex
DMEM	Dulbecco's Modified Eagle's Medium

DTH	delayed-type hypersensitivity
EPO	erythropoietin
ERK	extracellular-signal-regulated kinases
FAD	flavin adenine nucleotide
FADD	Fas-associated death domain
FAN	factor associated with N-SMase activation
FCS	foetal calf serum
FLICE	FADD-like ICE
fMLP	formyl-methionine-leucine-phenylalanine
FSH	follicle stimulating hormone
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GCK	germinal centre kinase
GH	growth hormone
GM-CSF	granulocyte-macrophage colony stimulating factor
GROα	growth-related gene product
HBSS	Hanks' Balanced salt solution
HF	heart failure
HSP	heat shock proteins
HUVECs	human umbilical vein endothelial cells
ICAM	intracellular-adhesion-molecule
IFNγ	interferon gamma
IgG	immunoglobulins
IκB	inhibitor kappa B
IKK	IκB kinase

IL	interleukin
IP	interferon-γ-inducible protein
ITAMS	immunoreceptor tyrosine-based activation motifs
JNK	c-Jun NH_2-terminal kinase
KC	keratinocyte chemoattractant
LIF	leukaemia inhibitory factor
LOX	lipoxygenases
LPS	lipopolysaccharide
LT	lymphotoxin
LTBI	latent tuberculosis infection
MAPK	mitogen activated protein kinase
MCP	monocyte chemotactic protein
M-CSF	macrophage CSF
MEF2C	myocyte enhancing factor 2C
MEKK1	MAPK kinase kinase
MHC	major histocompatability complex
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinase
MNL	mononuclear cells
MPO	myeloperoxidase
MPS	mononuclear phagocyte system
mTNF	membrane bound TNF
mTOR	mammalian target of rapamycin
NADPH	nicotinamide adenine nucleotide phosphate

NEMO	NF-κB essential modulator
NF-κB	nuclear factor kappa B
NIK	NF-κB inducing kinase
NK	natural killer cells
NOS	nitric oxide synthase
NSD	neutral sphingomyelinase domain
N-SMase	neutral sphingomyelinase
PAMPs	pathogen-associated molecular patterns
PDK1	phosphoinositide dependent kinase
PECAM-1	platelet endothelial adhesion molecule-1
PI	3'-phosphoinositide
PI3K	phosphatidylinositol-3-kinase
PLAD	pre-ligand assembly domain
PRR	pattern recognition receptors
RA	rheumatoid arthritis
RIP	receptor interacting protein
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
S1P	sphingosine-1-phosphate
SLE	systemic lupus erythematosus
SMase	sphingomyelinase
SODD	silencer of death domain
SphK	sphingosine kinases
SRBC	sheep red blood cell

STAT	signal transduction and activators of transcription
T regs	regulatory T cells
TACE	TNFα converting enzyme
TANK	TRAF-associated NF-κB activator
TGFα	transforming growth factor alpha
TLR	toll-like receptors
TMB	3',3',5',5'-tetramethylbenzidine
TNF	tumour necrosis factor
TNF-RM	TNF rich medium
TNFRI	TNF receptor I
TNFRII	TNF receptor II
TPO	thrombopoietin
TRADD	TNF receptor-associated death domain
TRAF2	TNF receptor associated factor 2
TSH	thyroid stimulating hormone
VEGF	vascular endothelial growth factor

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