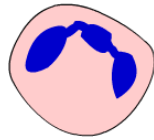


**A NOVEL PROINFLAMMATORY ROLE
FOR ANNEXIN A1 IN NEUTROPHIL
TRANSENDOTHELIAL MIGRATION**



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**This thesis is submitted for the degree of
Doctor of Philosophy, University of Adelaide**

January 2009

ABSTRACT

Neutrophil extravasation into tissues is an essential process required for the inflammatory response. Upon receiving an inflammatory cue, neutrophils begin accumulating on the luminal surface of the endothelium. Neutrophil recruitment is initiated by selectin-mediated tethering and rolling of neutrophils along the endothelial monolayer, followed by integrin-mediated firm adhesion. Adherent neutrophils then traverse the endothelium in a process known as transendothelial migration. The events mediating the rolling and adhesion steps are well characterised, but research into the molecular mechanisms regulating transendothelial migration is an area of intense focus. A previous study conducted in our laboratory found that the activation of endothelial extracellular signal-regulated kinase (ERK) 1/2 was required for neutrophil transmigration. Furthermore, it was found that endothelial ERK was activated in response to a soluble protein produced by fMLP- or IL-8-stimulated neutrophils.

In the present study, the soluble ERK-activating neutrophil protein was identified as annexin A1, which was selected as a possible candidate following mass spectrometry analysis of proteins secreted from activated neutrophils. Annexin A1 antibodies (Abs) were found to block endothelial ERK activation induced by conditioned medium harvested from stimulated neutrophils. Annexin A1 Abs were additionally able to inhibit neutrophil transmigration across human umbilical vein endothelial cell (HUVEC) monolayers in an *in vitro* transmigration assay. Following the purification of recombinant annexin A1, it was demonstrated that it could activate endothelial ERK in a similar manner to neutrophil conditioned medium. Upon further investigation, ERK activation was found to be induced by a truncated form of annexin A1 present in the protein preparation rather than the full length protein. Calpain I, a calcium dependent protease that is activated upon neutrophil stimulation and is known to cleave annexin A1 within the N-terminal domain, was shown to process full length inactive recombinant annexin A1 into an unidentified product that could activate endothelial ERK. A calpain I inhibitor was also found to prevent stimulated neutrophils from secreting an ERK-activating protein, thus further suggesting a role for calpain I in this process. As full length annexin A1 has been reported to signal through the formyl peptide receptor (FPR) family, a pan-FPR antagonist was incubated with endothelial cells and was found to inhibit ERK activation induced by neutrophil conditioned medium, indicating that pro-inflammatory annexin A1 is also a FPR ligand.

Endothelial projections termed “transmigratory cups” form around neutrophils during extravasation, of which ICAM-1 is a major component. Using an assay that examined transmigratory cups during neutrophil transmigration, it was found that annexin A1 Abs could inhibit neutrophil adhesion and transmigration through HUVEC monolayers by interfering with transmigratory cup formation around neutrophils, as shown by monitoring ICAM-1 during the process. Quantification of transmigrating neutrophils highlighted that the majority of neutrophils were emigrating via a transcellular pathway, which is in opposition to many *in vitro* studies where paracellular transmigration predominates.

The results generated from this study identified a novel pro-inflammatory role for annexin A1 in neutrophil transendothelial migration. Preliminary experiments suggested that the pro-inflammatory annexin A1 responsible for endothelial ERK activation was a truncated form. Calpain I appears to be a likely candidate responsible for the generation of this uncharacterised, truncated annexin A1 product, however further experiments are required to confirm this hypothesis. Pro-inflammatory annexin A1 represents a new target for the treatment of inflammatory disorders.

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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Samantha Williams.

ACKNOWLEDGEMENTS

I wouldn't be composing this acknowledgements section if it wasn't for my supervisor Yeesim Khew-Goodall. You are a remarkable scientist and thank you for being such a fantastic supervisor. Thank you for dealing with my ups and downs and thank you for basically letting me do whatever the hell I wanted to do. Although I didn't realise it at the time, you were showing me that you had confidence in me and in my ideas. There are only a small number of supervisors who are relaxed enough to allow their PhD students determine the direction of their own projects and luckily for me, you are one of them. Although I sometimes went about my experiments in a convoluted way, I got there in the end and the process of making mistakes has been an invaluable learning experience for me. That instant of realisation where one recognises a whole lot of time has been wasted because something wasn't considered or thought out properly is a gut-wrenching feeling, but experiencing those moments has made me an infinitely better scientist. You never made me feel stupid and always listened to my ideas and I cannot put into words how much I valued that. Until writing this thesis (combined with the benefit of 1.5 years off!) I didn't fully appreciate how good the results actually are. I'm finally proud of what I achieved during my PhD and I cannot thank you enough Yeesim for your guidance, patience and for being an all-round awesome lady.

I would also like to thank my co-supervisor, now boss, Stuart Pitson. Your teachings into the realms of protein chemistry were pivotal to many of the results gained during my PhD. Despite having your own lab to run, you were always willing to help, sometimes with great haste if there was an FPLC malfunction! Thank you for employing me before my thesis was completed- working in a different area has been a very welcome change of scenery and has made this thesis a much more positive, readable document. Thank you also for providing the subtle reminders about finishing my thesis, however your not-so-subtle declaration that my continued employment hinged on its completion before the end of the year really did the trick. I also appreciate your patience in tolerating my zombie-like state at work for the past few months, although I suspect you probably enjoyed the unfamiliar silence.

Thank you to my wonderful friend Leila. You are one of the most dedicated, diligent people I know and having you in the lab was a constant reminder of what I should aim to be. Thank you for suffering through my incessant chatter, loud music, whinging, stealing your equipment and bugging you to come to the pub when you wanted to write your lab book up, amongst the many other things I'm not aware of because you were too nice to say anything.

Thank you also to the staff and students in the Division of Human Immunology- I am convinced it is the best department ever to work in. I thoroughly enjoy the tea room conversations, which are always entertaining, lively and guaranteed to put at least one person off their food. Thank you to Jennifer Gamble and Mathew Vadas from the now extinct Vascular Biology Laboratory for their collaboration, which allowed me to obtain HUVEC every week to perform my experiments. Following on from that, I would like to thank Jenny Drew and Anna Sapa for passing on your expertise on the fine art of culturing of HUVEC, for doing the icky extraction of HUVEC from umbilical cords and for sneaking me extra lines when you could. You are both champions. A big thank you also to Hayley Ramshaw for offering to proof-read this thesis (no amount of chocolate could numb that pain!) and Midge for her little contributions.

I must also acknowledge Douwe Egberts for the assistance that their product, Moccona Espresso Blend Coffee, has given me in writing this thesis. Without a half-decent tasting brand of instant coffee, I would have had no other alternative than to drink the real stuff and consequently would have died from caffeine poisoning.

Finally to my husband Craig: Told you I'd finish it! :P 1337 +3 chair buff to thesis writing FTW! Now let teh pwnage begin!!~1!

“When you’re going through hell, keep going.”

-Winston Churchill

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ABBREVIATIONS

Ab	Antibody
Ac2-26	Acetylated N-terminal annexin A1 residues 2-26
ANOVA	Analysis of the variance
AnxA1	Annexin A1
BALF	Bronchoalveolar lavage fluid
Boc2	Boc-Phe-Leu-Phe-Leu-Phe
BSA	Bovine serum albumin
CHO	Chinese Hamster Ovary
CM	Conditioned medium
CMV	Cytomegalovirus
DAPI	4'-6-Diamidino-2-phenylindole
DMSO	Dimethylsulphoxide
EM	Electron microscope
ERK	Extracellular signal-regulated kinase
ERM	Ezrin radixin moesin
ESAM	Endothelial cell-selective adhesion molecule
FBS	Foetal bovine serum
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
fMLP	Formyl-Met-Leu-Phe
FPLC	Fast protein liquid chromatography
FPR	Formyl peptide receptor
FPRL	Formyl peptide receptor like
GFP	Green fluorescent protein
HEK293	Human embryonic kidney 293 cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPA	Hypothalamic Pituitary Adrenal
HRP	Horse Radish Peroxidase

HSA	Human serum albumin
HUVEC	Human Umbilical Vein Endothelial Cells
ICAM	Intercellular Adhesion Molecule
IL	Interleukin
JAM	Junctional adhesion molecule
KLH	Keyhole limpet hemocyanin
KO	Knock out
LAD	Leukocyte Adhesion Deficiency
LB	Luria Bertani
LFA-1	Lymphocyte function-associated antigen-1
LPS	Lipopolysaccharide
LTB4	Leukotriene B4
LXA4	Lipoxin A4
mAb	Monoclonal antibody
Mac-1	Macrophage antigen-1
MAPK	Mitogen activated protein kinase
MCS	Multiple cloning site
MEK	Mitogen-activated protein kinase kinase
MLCK	Myosin Light Chain Kinase
MMP	Matrix Metalloprotease
MNEI	Monocyte/Neutrophil Elastase Inhibitor
MOPS	3-(N-morpholino) propanesulfonic acid
MPO	Myeloperoxidase
MQ	Milli Q
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NET	Neutrophil Extracellular Traps
O.D.	Optical density
pAb	Polyclonal antibody
PAF	Platelet activating factor
PBS	Phosphate buffered saline

PBS-T	PBS/0.1% Tween 20
PCR	Polymerase chain reaction
PDZ	Post-synaptic density-95/discs large/zonula occludens-1
PECAM-1	Platelet Endothelial Cell Adhesion Molecule-1
PSGL-1	P-selectin glycoprotein ligand-1
PKC	Protein kinase C
PMA	Phorbol myristate acetate
PMS	Phenazine methosulfate
PVDF	Polyvinylidene fluoride
ROS	Reactive Oxygen Species
SAA	Serum Amyloid A
SEM	Standard error of the mean
SMP	Skim Milk Powder
TCA	Trichloroacetic acid
TGF- β	Transforming growth factor beta
TNF- α	Tumour necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor