Effect of growth factors on T-lymphocyte induced keratinocyte apoptosis

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For my family and Tom

"Happy is he who gets to know the reasons for things."

Virgil (70-19 BCE)
Roman poet.

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Declaration

" I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text"

Ilse S. Daehn

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Publications arising from this project

(see Appendix)

Daehn I, Varelias A, Rayner T. Sodium butyrate induced keratinocyte apoptosis. *Apoptosis*. 2006 Aug;11(8):1379-90.

Ruzehaji G, Daehn I, Varelias A, Rayner T. Exploring cellular interactions relevant to wound healing. *Primary Intention*. 2006 Feb;14(1):22-30.

Abbreviatons

α6	alpha-6 integrin
ACD	allergic contact dermatitis
AE	atopic eczema
BSA	bovine serum albumin
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
DMEM	dulbecco's modified eagle's medium
FACS	fluorescence activated cell sorter
FBS	fetal bovine serum
FasL	Fas ligand
FITC	fluorescein isothiocyanate
HLDA	human cell differentiation antigens
HDI	histone deacetylase inhibitor
hrs	hours
ICAM-1	intracellular adhesion molecule
IGF-I	insulin-like growth factor-I
IGFBP	IGF-binding proteins
IFNγ	interferon γ
Ig	immunoglobulin
IL	interleukin

LFA-1	lymphocyte function-associated antigen-1
LR3-IGF	LONG™R3 IGF-I
mAb	monoclonal antibody
mins	minutes
MFI	mean fluorescence intensity
NF-κβ	nuclear factor κβ
NHEK	normal human epidermal keratinocytes
PARP	poly (ADP-ribose) polymerase
PBS	phosphate buffered saline
PE	phycoerythrin
PI	propidium iodide
PMA	phorbol myristate acetate
PS	phosphotidylserine
RT	room temperature
rpm	revolutions per minute
TGFβ	transforming growth factor β
Th	T helper cell
TIMs	topical macrolide immunomodulators
TNFα	tumour necrosis factor α
TRAIL	tumour necrosis related apoptosis-inducing ligand
WGFE	whey growth factor extract
x g	relative centrifugal force (g-force)

THESIS SUMMARY

Atopic eczema is a T-lymphocyte mediated chronic inflammatory skin disorder. The interaction of CD4+ T-lymphocytes with epidermal keratinocytes results in dysregulated, chronic inflammation and altered barrier function. T-lymphocyte induced keratinocyte apoptosis has been proposed as a mechanism by which epidermal integrity is impaired in eczema. Apoptosis of keratinocytes is thought to result from T-lymphocyte associated Fas ligand (FasL) binding to the death receptor Fas on keratinocytes. The primary aim of this project was to characterize the induction of keratinocyte apoptosis by T-lymphocytes and address the hypothesis that insulin-like growth factor-I (IGF-1), transforming growth factor β_1 (TGFβ_1) and a milk derived growth factor extract containing TGFβ and IGF-I (whey growth factor extract; WGFE) protect keratinocytes from T-lymphocyte mediated apoptosis.

To address the aims of this project, an *in vitro* co-culture model was developed combining T-lymphocytes with keratinocytes. Co-cultures were initially established using human Jurkat T-lymphocytes and human HaCaT keratinocytes with more extensive characterisation undertaken using primary CD4+ T-lymphocytes together with HaCaTs or normal human epidermal keratinocytes (NHEK). Annexin V and propidium iodide staining was established as the primary method for measuring keratinocyte apoptosis with this validated using sodium butyrate a known inducer of apoptosis. Changes in nuclear fragmentation and cell morphology were also examined as a key

feature of apoptosis. The involvement of the Fas pathway was investigated by assessing T-lymphocyte FasL expression, keratinocyte Fas expression and downstream caspase activation. Inflammatory cytokines IFNγ and TNFα were also examined due to their ability to induce Fas expression.

Studies performed with T-lymphocytes demonstrated that keratinocyte apoptosis was induced, with this due primarily to direct T-lymphocytes and keratinocytes interactions, rather than soluble mediators in the co-culture milieu. Activated T-lymphocytes were found to have high levels of FasL and to upregulate keratinocyte Fas expression. The increased keratinocyte Fas was associated with increased IFNγ levels in the co-culture media and activation of the caspase cascade. A Fas blocking antibody prevented T-lymphocyte induced keratinocyte apoptosis demonstrating that this was a Fas dependent event.

As the primary function of keratinocytes is to terminally differentiate, the differentiation status of the cells induced to undergo apoptosis was examined. It was demonstrated that T-lymphocytes decrease the intensity of α6 integrin expression by the keratinocytes. This marker identifies undifferentiated basal cells as high expressors of α6, with cells in the early stages of differentiation pathway found to be low expressors of α6. Co-staining with Annexin V demonstrated that the apoptotic keratinocytes were low expressors of α6 and thus cells committed to the early stages of differentiation. This suggested that the T-lymphocytes initiated the onset of keratinocyte terminal differentiation with this linked

to the cells being more susceptible to death induced by T-lymphocyte by activation of the Fas pathway.

The ability of TGFβ_1, IGF-I and WGFE to inhibit T-lymphocyte induced keratinocyte apoptosis was examined. A combination of recombinant TGFβ (10ng) & IGF-I (100ng) was able to significantly inhibit keratinocyte apoptosis. A similar result was obtained with WGFE, and although these growth factor treatments were able to reduce the elevated IFNγ levels in the co-culture media, they did not reduce T-lymphocyte induced Fas upregulation. The TGFβ_1 and IGF-I combination as well as WGFE did however prevent the T-lymphocyte induced shift from α6 bright to dim expressing keratinocytes. As such, the growth factor combinations appeared to protect the keratinocytes from T-lymphocyte mediated apoptosis by preventing them from committing to terminal differentiation.

The studies in this thesis have characterised the Fas associated mechanisms by which T-lymphocytes induce keratinocyte apoptosis and suggest specific growth factor combinations may have the potential to ameliorate the reduced barrier function associated with inflammatory skin conditions such as atopic eczema.