

# Skin Peptides: Biological Activity and Therapeutic Opportunities

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Received 14 May 2007; revised 13 August 2007; accepted 15 August 2007

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21198

**ABSTRACT:** The skin provides an effective barrier to the loss of body fluids and environmental assault. In addition to the physical barrier provided by the stratum corneum, the skin also contains a chemical barrier consisting of antimicrobial peptides (AMPs), which control microbial growth on the surface. These AMPs also have multiple roles as mediators of inflammation with effects on epithelial and inflammatory cells, influencing cell proliferation, wound healing, cytokine/chemokine production and chemotaxis. This review describes the range of peptides found in the skin, both constitutive and those induced in response to injury. The role these peptides play in normal skin function and in various skin conditions is described. A better understanding of their role in normal and skin disease may offer new strategies in skin disease, dermatology and as cosmeceuticals. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 97:2524–2542, 2008

**Keywords:** antimicrobial peptides; wound healing; psoriasis; dermatitis; dermatology; cosmeceutical

## INTRODUCTION

The integrity of the skin is essential as it provides a barrier to prevent loss of body fluid and exposure to various forms of environmental assault. When the barrier function is severely compromised, as in generalised psoriasis, up to 6 L of water may be lost per day through the skin.<sup>1</sup> This review describes the range of peptides, both constitutive and those induced in response to injury, found in the skin. Considerable research has been undertaken to identify and isolate the expression of these peptides. The role these peptides play in normal skin function and in various skin conditions is described. The skin produces peptides

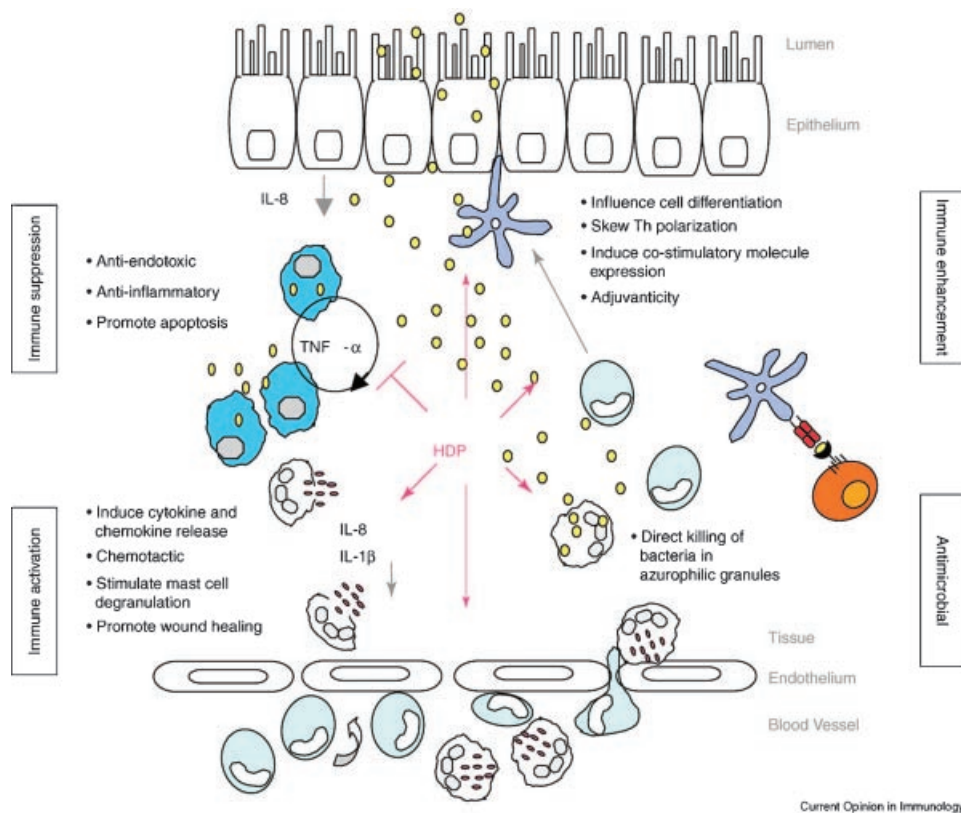
with broad antimicrobial activity that contribute to its innate resistance to invading pathogens (Fig. 1). In addition skin peptides play an integral role in stimulating and regulating wound healing and inflammation. Altering the availability of these peptides at their target sites within the skin may offer new therapeutic approaches in skin disease and dermatology, wound healing and for cosmetic applications.

## PEPTIDES—ACTIVITY IN THE SKIN

The *stratum corneum*, a nonviable, desiccated layer of the skin, acts as a first line of defence against invading microorganisms<sup>2</sup> and contributes significant resistance to molecular transport both from and into the body.<sup>3</sup> However, this physical barrier is susceptible to injuries or may be compromised by some diseases that allow the entry of opportunistic microbial agents into

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*Journal of Pharmaceutical Sciences*, Vol. 97, 2524–2542 (2008)  
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**Figure 1.** Functions of cationic host defence (antimicrobial) peptides within cytoplasmic granules (reproduced from Ref. 119 with permission).

the skin.<sup>4</sup> When these microorganisms gain access to the otherwise sterile internal environment, they activate a complex immune defence system.<sup>5</sup> Human skin is also known to produce some antimicrobial agents that form an innate epithelial chemical shield.<sup>6</sup> These endogenous antimicrobial peptides (AMPs) are molecules produced by the epithelial surface of the host.<sup>5</sup> Two major classes of peptides have been identified in mammalian skin: defensins and cathelicidins. These peptides act as effector molecules and exhibit activity against a broad range of microorganisms such as bacteria, fungi and viruses.<sup>7–9</sup> They are also involved in host repair and adaptive immune responses<sup>5</sup> (Fig. 1). Many AMPs have recently been isolated, including lysozyme, RNase 7, elafin, psoriasin, dermicidin (DCD) 1L, granulysin, antileukoprotease and human cationic antimicrobial protein (hCAP18).<sup>7,10</sup> Table 1 provides a summary of the biological activity of skin peptides.

### ANTIMICROBIAL PEPTIDES (AMPS)

Cathelicidins and defensins are the two major groups of epidermal AMPs that possess inherent

antimicrobial activity.<sup>11</sup> Like most AMPs they are positively charged, with the positively charged amino acids localised to one side of the molecule and opposite to the most hydrophobic groups.<sup>5</sup> The AMPs active in the skin are summarised in Table 1. Granulysin is also an AMP but whilst it has action in the skin it is not synthesised in the skin. Other peptides and proteins that exert antimicrobial activity within the skin are psoriasin, RNase 7, adrenomedullin (AM), antileukoprotease and DCD.

### Cathelicidins

The expression of cathelicidins and mouse cathelicidin-related antimicrobial peptide (CRAMP) in skin keratinocytes varies with infection and/or injury. Only one cathelicidin, human cationic antibacterial protein (hCAP18; MW 18 kDa) has been identified in humans, although about 30 cathelicidin members are present in mammalian species.<sup>10</sup> hCAP expression has been detected in human skin keratinocytes at sites of inflammation in the skin and in specialised keratinocytes of the nail, neonatal skin and in eccrine glands.<sup>12,13</sup>

**Table 1.** Biological Activity of Various Endogenous and Synthetic Peptides

Peptide	Abbreviation	Source	Biological Activity	References
Therapeutic peptides Adrenomedullin	AM	Keratinocytes of the epidermis and hair follicles, cells of the glands and secretory ducts of normal skin and in skin tumours of different histologies	Antibacterial and antifungal activity. May be involved in the wound repair process and functions as a growth regulator. Role in skin homeostasis, carcinogenesis and acne vulgaris	42
Alpha defensins	HNP-(1, 2, 3 and 4)	Neutrophil granules, macrophages, monocytes, T cells, paneth cell granules	Antimicrobial effect, macrophage phagocytosis, complement activation	23
Antileukoprotease	ALP	Human callus and detected in supernatants of cultured human primary keratinocytes	Antimicrobial activity against several microorganisms and maintains homeostasis	43,44
Antiflammin-1 and Antiflammin-2		Synthetic nonapeptides	Potent anti-inflammatory action	75
Cathelicidin	LL-37	Keratinocytes, epithelial cells, neutrophils, monocytes, T cells, mast cells	Antimicrobial effect, cytokine and chemokine production by monocytes and keratinocytes	18
Copper peptides	For example, Gly-His-Lys-Cu	Synthetic	Antioxidant, growth and regulation of hair follicles and increases collagen disposition	58
Dermicidin 1L	DCD-1L	Synthetic: recombinant dermcidin-1L was expressed in <i>Escherichia coli</i> as a fusion protein and purified by affinity chromatography	New class of potential broad-spectrum antimicrobial and antitumour drug	48
Elafin	SKALP	Isolated from psoriatic skin	Specific inhibitor of human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), proteinase3	77,78
Granulysin		Psoriatic plaques	Broad spectrum antimicrobial	36
Growth hormone	GH	Expressed in whole human skin (normal and BCC)	Associated with fibroblast activity, sebum production and induction of IGF-1 production	64
Human beta defensin	H $\beta$ D (1-4)	Suprabasal keratinocytes of the skin and sweat ducts within the dermis	Wound healing and skin diseases such as atopic eczema	6,29
Human neutrophil elastase	HNE	Detected in psoriatic lesions	Physiological functions in host defence against bacterial infections and matrix remodelling following tissue injury	76

Interleukins	IL	Released from an intracellular preformed pool in keratinocytes	Involved in wound healing	60
Peptide T		Synthetic	Antichemotactic activity—therapeutic efficacy in psoriasis	72
Proopiomelanocortin and melanocortin peptides	POMC	Found in the basal layer of the intermolecular epidermis	Stimulate eumelanin synthesis, antipyretic and anti-inflammatory	67,68
Psoriasis		Discovered in psoriatic skin lesions and constitutive expression in healthy skin keratinocytes	Psoriasis preferentially kills <i>E. coli</i> , but has a weak antimicrobial activity against <i>S. aureus</i> , <i>P. aeruginosa</i> and <i>Staph. epidermidis</i>	39
RNase 7		Expressed in healthy skin (isolated from skin derived stratum corneum)	Broad spectrum antimicrobial	40
Skin peptide	SPYY	Isolated from the amphibian skin	Antifungal and antiparasitic activity	81,79
Soluble RGD peptides	RGD	Released during the proteolytic remodelling of the extracellular matrix	Wound healing—internalised into a cell and able to bind and activate caspase-3 inducing apoptosis	54
Transforming growth factor	TGF- $\alpha$ , TGF- $\beta$	Intercellular space of all the layers of the epidermis and the subepidermal area of the dermis	Regulator of both cell growth and differentiation	66
Cosmetic peptides				
Pal-KTTS		Pentapeptide fragment	Stimulates collagen I and III and fibronectin production	104
Acetyl hexapeptide-3		Synthetic peptide	Improvement in periorbital rhytides	107
Palmitoyl pentapeptide-3		It is a fatty acid mixed with amino acids	Superior antiwrinkle effects	www.sederma.fr
Syn-Ake		Synthetic tri-peptide	Antiwrinkle/antiaging effect	www.centerchem.com
Antioxidative collagen derived peptides	For example, Arg-Ser-Arg-Lys	Tetrapeptide	Antioxidant and decreases cellular activity	110
Carnosine		Naturally occurring histidine containing dipeptide	Wound healing and acts as an antiaging peptide	111,113

Cathelicidin peptide (human cathelicidin; LL-37) is produced by the neutrophils, mast cells and keratinocytes in response to inflammatory processes. LL-37 also acts as a chemoattractant for neutrophils, monocytes, T cells and mast cells. Mast cells can produce LL-37 thereby leading to a positive feedback cycle.<sup>14,15</sup> Elevated levels of LL-37 have been reported in psoriatic skin ( $\approx 304 \mu\text{M}$ ) and wounded skin.<sup>2</sup> LL-37 plays an important role in the repair of damaged tissue and wound closure by promoting wound vascularisation and reepithelisation.<sup>16</sup> It also induces IL-8, IL-18 and IL-20 production by human keratinocytes through MAP kinase pathway.<sup>17</sup> IL-18, an interferon (IFN)- $\gamma$  inducer, is a proinflammatory cytokine intracellularly produced from a biologically inactive precursor, pro-IL-18. It is produced by keratinocytes and its expression is highly enhanced in skin diseases, like psoriasis in which human  $\beta$ -defensins (h $\beta$ D) and LL-37 are highly expressed.

The potential role of human cathelicidins in host susceptibility to herpes simplex virus (HSV) infection has been investigated.<sup>18</sup> Eczema herpeticum (ADEH) is a disseminated HSV 1 or 2 infection that occurs in some patients with atopic dermatitis (AD).<sup>19</sup> The difference in cathelicidins expression between patients with uncomplicated AD and patients with ADEH has been examined. Cathelicidin expression was evaluated in skin biopsy specimens from patients with AD without a history of HSV skin infection and from patients with ADEH. Glycoprotein D, measured by RT-PCR, was used as a marker of HSV-2 replication. LL-37 exhibited activity against HSV-2 in an antiviral assay ( $p < 0.01$ ) and concentrations of LL-37 as low as  $25 \mu\text{M}$  significantly reduced the levels of HSV gene expression in previously infected keratinocytes. In addition, higher levels of HSV-2 replication in cathelicidin-deficient (Cnlp2/2) mouse skin compared with skin from their wild-type counterparts were observed. This confirmed the importance of cathelicidin in antiviral skin host defence. Lower levels of cathelicidin expression were found in skin with eczema herpeticum as compared to AD.

## Defensins

Defensins are cationic peptides (MW 3–5 kDa), characterised by six cysteine residues that form characteristic disulphide bridges. They are divided into alpha, beta and theta subfamilies based on the alignment of the disulphide bonds.

AMPs of the defensin family exhibit broad activity against gram-negative bacteria, fungi, mycobacteria and enveloped viruses and have been isolated from neutrophil granules, macrophages and some specialised epithelial cells of the small intestine.<sup>20</sup>

## $\alpha$ -Defensins

Six alpha defensins have been identified, of which four are known as human neutrophil peptides (HNP)-1, 2, 3 and 4, as they are associated with human neutrophils. The other two are called human defensins (HD)-5 and 6. They are expressed in the paneth cells of the intestine and in the epithelial cells of the female genitourinary tract.<sup>21</sup> Alpha defensins exert their action on both microbes and the host. They have potent antiviral activity and HNPs 1–3 have been shown to increase the expression of tumour necrosis factor (TNF)  $\alpha$  and interleukin-1 in human monocytes activated by *Staphylococcus aureus*.<sup>22</sup>

The expression of alpha defensins has been studied in squamous cell carcinomas of the human tongue and compared with autogenous nontumour tissue. HPLC-MS and amino acid sequencing was utilised for separation, structural identification and quantitation of the HNPs. MALDI-MS analysis of HPLC fractions from both tumour and nontumour tissue detected peptide masses for HNP-1, HNP-2 and HNP-3 which were confirmed by amino acid sequencing. When analysis of paired tumour and nontumour tissue samples was performed, the concentration of defensins in the tumour tissue was about 2–12 times higher than in the nontumour tissue.<sup>23</sup>

## $\beta$ -Defensins

Beta defensins have been identified in many cell types including epithelial cells and neutrophils.<sup>24</sup> H $\beta$ D 1–4 have been identified in humans. H $\beta$ D-1 (MW 3.9 kDa) is constitutively produced by various epithelial tissues including the urogenital and respiratory tracts.<sup>25</sup> H $\beta$ D-2, a 4.3 kDa peptide has been isolated from extracts of lesional scales of psoriatic skin.<sup>8</sup> Expression of H $\beta$ D-1 is constitutive while H $\beta$ D-2 expression is increased by injury or inflammation of the skin. H $\beta$ D-3 (MW 5.1 kDa) peptide has recently been identified in epithelial and nonepithelial tissue.<sup>26</sup> It has significant bactericidal activity against gram-positive *S. aureus* at physiological salt

concentrations.<sup>27</sup> H $\beta$ D-2 and H $\beta$ D-3 expression is inducible by external stimuli, including IFN-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$  and gram positive and negative bacteria. Numerous studies have demonstrated that defensins, when used at concentrations above 2  $\mu$ M, have the capacity to kill a vast spectrum of microorganisms under low salt and serum free conditions. It has also been demonstrated that defensins also participate in stimulating the host adaptive immunity.<sup>28</sup> Studies have been undertaken to identify and localise expression of H $\beta$ D-1.<sup>29</sup> RT-PCR and *in situ* hybridisation demonstrated that H $\beta$ D-1 mRNA and peptide were expressed in skin samples from various body sites.<sup>6,29</sup>

### $\theta$ -Defensins

This is a novel class of defensins isolated from rhesus monkey neutrophils and named  $\theta$ -defensins for their circular molecular structure.<sup>30</sup> This class is not discussed in this review as they have not been identified in humans.<sup>5</sup>

Defensins have been synthesised and are commercially available.<sup>31</sup> Their production in potatoes has been achieved, providing supplies for preclinical studies.<sup>32</sup> Several derivatives of AMPs for topical use have been in human phase I–III studies, primarily in the management of acne. Concerns regarding production costs, lability to proteases *in vivo* and unknown toxicity must be addressed to allow exploitation of these peptides as therapeutic agents.<sup>33,34</sup>

### Granulysin

Granulysin is an AMP produced by activated T cells and natural killer cells.<sup>35</sup> It is distinct from AMPs such as cathelicidins and defensins as it is not produced by epithelia, but rather is brought to the skin by T cells.<sup>5</sup> Expression of granulysin has been investigated in various cutaneous inflammatory diseases. Immunoperoxidase studies have been undertaken on skin biopsy specimens from patients with psoriasis, lichen planus, nummular eczema and AD and compared to skin obtained from healthy individuals. Strong expression of granulysin was observed in the lymphomononuclear infiltrates and dermal dendritic cells in psoriasis plaques. Very few granulysin-positive T cells were seen in AD and in nummular eczema specimens. Also it is important to note that increased numbers of granulysin-positive T cells

( $p < 0.01$ ) were observed in psoriasis, where secondary bacterial infection is extremely rare. These observations thus suggest that lesional granulysin levels may determine relative risk of secondary infection in certain inflammatory conditions of the skin. Increased levels of granulysin can provide some evidence to the resistance of psoriatic plaques against both gram-positive and gram-negative bacterial infections.<sup>36</sup>

### Psoriasin

Another peptide discovered in psoriatic skin lesions is psoriasin. Psoriasin is an AMP (MW 11 kDa) which is constitutively expressed in healthy skin keratinocytes and is a member of the S100 gene family.<sup>37</sup> Although the physiological function of S100 proteins is not fully understood, a few studies have indicated involvement of S100 proteins in innate host defence.<sup>38</sup> Over-expression of psoriasin may be linked to inflammation which is common in psoriasis. Glaser et al.<sup>39</sup> also identified psoriasin as the principal *Escherichia coli*-killing AMP in healthy human skin, which is present on the skin surface and focally expressed in healthy skin keratinocytes. Psoriasin was induced *in vitro* and *in vivo* in keratinocytes by *E. coli*, indicating that its focal expression in skin may be derived from local microbial induction. Zn<sup>2+</sup>-saturated psoriasin showed diminished antimicrobial activity, suggesting that Zn<sup>2+</sup> sequestration could be a possible antimicrobial mechanism.<sup>39</sup>

### RNase 7

RNase 7 (MW 14.5 kDa) is a basic protein that is abundant in healthy skin and is believed to be one of the principal cationic proteins of healthy human skin.<sup>40</sup> The RNase A superfamily has been extensively researched over the last few decades and among the better characterised RNases are RNase 1 and RNase 5. Others include eosinophil cationic protein (ECP) or RNase 3, and RNases 4, 6, 7 and 8.<sup>41</sup> These were identified by Harder and Schröder<sup>40</sup> who showed that crude extracts from stratum corneum contained antimicrobial activity against *E. coli* and *S. aureus*. RNase 7 may be an inducible peptide since the levels are high in psoriatic skin and gene expression is increased on contact of keratinocytes with bacteria.<sup>40</sup>

### Adrenomedullin

AM (MW 6 kDa) is a 52 amino acid peptide which is expressed in keratinocytes of the epidermis and hair follicles, cells of the eccrine, apocrine sweat glands and secretory ducts of normal skin, and in skin tumours of different histologies. AM is reported to have numerous physiological roles including vasodilation, renal homeostasis, hormone regulation, neurotransmission and growth modulation.<sup>7,42</sup> It is also thought to be involved in the wound repair process. Martinez et al.<sup>42</sup> characterised adrenomedullin receptor (AM and AM-R) expression in human skin in order to understand its potential functions in the skin. The presence of AM and its receptor in normal and neoplastic skin were confirmed by RT-PCR and Western blot analysis performed on cell extracts from human skin cell lines. Immunoreactivity for AM and *in situ* hybridisation signal for AM-R was found in all epithelial components of the skin. Many specimens corresponding to the major histological types of skin cancer showed a positive staining for AM and AM-R.<sup>42</sup>

### Antileukoprotease

Antileukoprotease (ALP), also called mucous protease inhibitor or secretory leukoprotease inhibitor, is present in human callus and detected in supernatants of cultured human primary keratinocytes<sup>43</sup> and in various human body fluids.<sup>44</sup> It is a very potent human serine protease able to inhibit the neutrophil derived serine proteases polymorphonuclear leukocyte elastase (HLE) and Cathepsin G (CG). Wiedow et al.<sup>43</sup> described the constitutive production and release of ALP by human keratinocytes and epithelial carcinoma cell lines (KB grown in Dulbecco's Modified Eagle and A431 grown in minimum essential medium). When the antimicrobial activity of ALP was compared with H $\beta$ D-2, ALP was seen to be constitutively produced and expressed by the keratinocytes whereas H $\beta$ D-2 is only expressed after stimulation of keratinocytes with bacteria. Recombinant ALP exhibited microbicidal activity in a dose-dependent manner.<sup>43</sup>

### Dermicidin 1L

DCD-1L is a novel AMP family with a broad spectrum of activity and no similarities to other known peptides. It is constitutively expressed in

eccrine sweat glands and transported to the epidermal surface.<sup>45</sup> DCD expression was not observed in epidermal keratinocytes of healthy human skin.<sup>46</sup> Immunohistochemistry and RT-PCR studies showed that DCD-1L expression was not induced in keratinocytes in inflammatory conditions such as psoriasis, AD and lichen planus. DCD-1L thus functions by modulating the colonisation of the skin rather than responding to inflammation.<sup>47</sup> Lai et al.<sup>48</sup> further expressed DCD-1L in *E. coli* as a fusion protein to understand its mechanism and investigate its antimicrobial spectrum. DCD-1L displayed antimicrobicidal activity against nosocomial pathogens, but no haemolytic activity against human erythrocytes.<sup>48</sup> The antimicrobial activity of DCD was not affected by the low pH and high salt concentrations of human sweat. This finding suggests that sweat glands may have a function in the innate immune responses of the skin by secreting these antimicrobial agents.<sup>45</sup>

## PEPTIDES INVOLVED IN WOUND HEALING

Wound healing is a localised process which involves a series of specific and coordinated events such as inflammation, wound cell migration and mitosis, neovascularisation, and regeneration of the extracellular matrix (ECM).<sup>49,50</sup>

### Growth Factors

Polypeptide growth factors act as regulators in wound healing and on exogenous application can modify the process.<sup>51</sup> Two peptide growth factors which play a pivotal role in normal wound healing in tissues such as skin, cornea and the gastrointestinal (GI) tract are the structurally related peptides epidermal growth factor (EGF) and transforming growth factor alpha (TGF- $\alpha$ ). Other peptides such as basic and acidic fibroblast growth factors ( $\beta$ FGF and  $\alpha$ FGF), platelet-derived growth factors (PDGF-AA, -AB and -BB) and insulin-like growth factor (IGF-I) have been identified as potential wound-healing agents.<sup>49,50</sup> EGF/TGF- $\alpha$  receptors are expressed by many types of cells including skin keratinocytes, fibroblasts, vascular endothelial cells and epithelial cells of the GI tract. Healing of a variety of wounds in animals and patients has been enhanced by treatment with EGF or TGF- $\alpha$ .<sup>49</sup> EGF also increased the tensile strength of skin

incisions in rats and corneal incisions in rabbits, cats and primates. Sorensen et al.<sup>52</sup> demonstrated that two of the important growth factors in wound healing, IGF-I and TGF- $\alpha$ , induce the expression of the AMPs/polypeptides hCAP-18, h $\beta$ D-3, NGAL and SLPI in human keratinocytes.

### RGD Peptides

Most cells are attached to the ECM through integrins that link the intracellular cytoskeleton with the ECM. Many of the integrins recognise a tripeptide (arginine-glycine-aspartate) or RGD in target proteins of the ECM.<sup>53</sup> Vigor et al.<sup>54</sup> hypothesised that during the proteolytic remodeling of the ECM, small soluble RGD-containing peptides were released into the matrix. Soluble RGD-peptides have previously been found to be internalised into a cell in an integrin-independent manner and are able to directly bind to and activate caspase-3, thus inducing apoptosis.<sup>55</sup> To investigate this hypothesis dermal fibroblasts were embedded into collagen type I or fibrin matrices and viability was assessed by *in situ* haematoxylin staining. Results indicate that apoptosis was induced specifically in response to collagen matrix remodelling. Small soluble RGD containing peptides were produced by enzymatic cleavage of collagen, which induces apoptosis of dermal fibroblasts through specific caspase-3 cleavage.<sup>54</sup>

### Copper Peptides

Copper peptides have been used in skin care in a similar way to vitamin C, alpha lipoic acid and green tree extracts. Copper functions as a part of cytochrome *c* oxidase and superoxide dismutase which are used in energy production and as antioxidants. It is also essential to the normal growth, development and function of the human body. Copper is bound to glycine, histidine and lysine which are used to synthesise a copper based peptide. Copper peptide has a positive influence on the growth and regulation of hair follicles and when used on wounds increases collagen disposition, tensile strength and angiogenesis in healing tissues.<sup>56</sup> A method for stimulating hair-growth by topically administering or injecting an effective amount of a peptide copper complex has been described and patented.<sup>57</sup> Buffoni et al.<sup>58</sup> examined the effects of Gly-His-Lys-Cu and of three synthetic analogues (I, II and III) on wound

healing of guinea-pig dorsal skin and on cultured fibroblasts. Hydroxyproline, proteins, DNA and semicarbazide-sensitive amine oxidase were measured and it was found that both the peptides caused a decrease in the activity of semicarbazide-sensitive amine oxidase but there was no significant difference between the two peptides. The main effects of these peptide-copper complexes were slower reorganisation of the skin and a delayed activation of fibroblasts.<sup>58</sup>

### Interleukins (IL)

Interleukins are a class of cytokines identified for their role in mediating immunological functions. IL-1 is produced by keratinocytes in two forms: IL-1 $\alpha$  and IL-1 $\beta$ . IL-6 (MW 26 kDa) is secreted from the cells in multiple glycosylated forms. Like TGF- $\alpha$ , the levels of IL-6 are elevated in psoriasis, cultured keratinocytes and skin tumour cell lines. IL-8, also known as neutrophil activating protein (NAP), mediates both growth stimulatory and inflammatory processes. It is produced by cultured human dermal fibroblasts and keratinocytes in response to IL-1 or TNF- $\alpha$ .<sup>59</sup> Rennekampff et al.<sup>60</sup> hypothesised that IL-8 was released from an intracellular preformed pool in keratinocytes in the presence of psoriasis. Sticherling et al.<sup>61</sup> showed that IL-8 is produced *de novo* by wound cells leading to increased reepithelialisation *in vitro* and *in vivo* by stimulating keratinocyte proliferation and migration. They investigated *in vitro* whether IL-8 upregulates the underlying phenotype of keratinocytes with respect to the expression of integrin subunits  $\alpha$ 2,  $\alpha$ 3,  $\alpha$ 5 and  $\alpha$ 6. An inverse relationship between IL-8 immunoreactivity and expression of the  $\alpha$ 6 integrin was found and loss of intracellular IL-8 immunoreactivity was accompanied by an increase in  $\alpha$ 6 expression. Flow cytometry analysis revealed strong expression of integrin subunits  $\alpha$ 2 and  $\alpha$ 3 and weaker expression of  $\alpha$ 5 and  $\alpha$ 6 on cultured, unstimulated keratinocytes. IL-8 was shown to be the major bioactive chemoattractant for PMNs in human blister and skin graft donor site wound fluids.<sup>60</sup> *In vitro* experiments on the effect of recombinant human (rh) IL-8 on keratinocytes proliferation revealed a rise in cell number accompanied by an increase in cells in S phase and over-expression of the integrin. Topical application of IL-8 on human skin grafts in a chimeric mouse model showed enhanced reepithelialisation in IL-8 treated animals over controls.<sup>60</sup>



## GROWTH HORMONE AND RELATED PEPTIDES

Growth hormone (GH) and prolactin (PRL) are produced in the anterior pituitary gland and skin is one of the target organs for GH and prolactin bioregulation. Dermal fibroblasts in cell culture have been shown to produce PRL and GH mRNA.<sup>62,63</sup> Slominski et al.<sup>64</sup> investigated whether the epidermis expresses the genes for GH and PRL. Detectable levels of GH and PRL were not found in human immortalised keratinocytes or in malignant melanocytes (basal cell carcinoma) but IGF-1 was expressed in malignant specimens. GH mRNA was detected in normal human skin but not in cultured human epidermal keratinocytes.<sup>64</sup>

### Transforming Growth Factor- $\beta$

Transforming growth factor- $\beta$  (TGF- $\beta$ ) can act as a multi-functional regulator of both cell growth and differentiation. Three isoforms of TGF- $\beta$ s namely TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3, have been found in human tissues. TGF- $\beta$ 2 is usually expressed in the intercellular space of all the layers of the epidermis and TGF- $\beta$ 3 is present in the subepidermal area of the dermis.<sup>65</sup> Falanga et al.<sup>66</sup> could not detect TGF- $\beta$ 1 or TGF- $\beta$ 2 in the epidermis or epithelial structures of forearm skin from healthy human volunteers. The dermal matrix contained minimally detectable amounts of the two isoforms. In all cases, the dermal matrix and cells contained greater amounts of TGF- $\beta$ 1 than TGF- $\beta$ 2.

## PROOPIOMELANOCORTIN AND MELANOCORTIN PEPTIDES (POMC)

Melanocytes are the key elements of the skin pigmentary system. They are associated with the hair follicles and are present in the basal layer of the intermolecular epidermis.<sup>67</sup> Melanocortins are a group of structurally related peptides comprised of adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormone (MSH),  $\beta$ -lipotropic hormone ( $\beta$ -LPH) and  $\beta$ -endorphin.<sup>68</sup> They are derived from the precursor protein POMC, which is synthesised by the pituitary gland.<sup>69</sup> Melanocortin peptides exert their effects through interaction with the melanocortin receptors. For example, on binding to the melanocortin-1 receptor (MC1-R),  $\alpha$ -MSH activates adenylate cyclase increasing intracellular

cAMP which is believed to mediate its melanogenic effects on melanocytes.<sup>67</sup> Although the mechanism of  $\alpha$ -MSH action is unclear, it is known that it antagonises proinflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  which are significant in HIV infection. Consequently the ability of melanocortin peptides to inhibit production of inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in whole blood samples of HIV-infected patients and normal subjects has been investigated.<sup>70</sup> TNF- $\alpha$  and IL-1 $\beta$  were not detectable in the plasma of control subjects and levels were near the limit of detection of the assay in the majority of HIV-infected patients. This suggested that melanocortin peptides were active in inhibiting cytokine production and hence could be used therapeutically.

The effect of ultraviolet irradiation on expression of both  $\alpha$ -MSH and the MC1-R in human epidermis *in vivo* has been determined.  $\alpha$ -MSH and IL-10 protein levels in blister fluids were significantly increased 24 h after ultraviolet irradiation, an effect that could be abolished by application of the broad-spectrum sunscreen Anthelios XL prior to ultraviolet (solar-simulating) radiation exposure. This suggests that POMC-derived peptides, such as  $\alpha$ -MSH may therefore play an important part in modulating ultraviolet-induced inflammation.<sup>71</sup>

## ANTI-INFLAMMATORY PEPTIDES

Peptide T (ASTTTNYT) is a ligand for the CD3/T4 receptor and is a fragment of the gp120 envelope protein of the human immunodeficiency virus (HIV). Synthetic peptide T and its analogous, D-Ala-Peptide T amide (DAPTA) inhibit the binding of the HIV envelope to brain membranes as well as the HIV infection of human T cells *in vitro*. It was thus tested as a therapeutic agent for the treatment of HIV infection and for the treatment of psoriasis.<sup>72,73</sup>

Peptide hormones derived from the proopiomelanocortin gene (POMC) are produced by epidermal cells (keratinocytes and melanocytes) and are known for their immunomodulating properties. POMC peptides in particular  $\alpha$ -MSH, ACTH and  $\beta$ -endorphin have been demonstrated to affect inflammatory responses in different ways. Bhargava et al.<sup>74</sup> investigated whether  $\alpha$ -MSH affects the production of IL-10, a cytokine known to inhibit the production of proinflammatory cytokines. Their results indicated that  $\alpha$ -MSH preferentially induced the production of IL-10 by human monocytes in a dose-dependent manner.

Also additional evidence from other studies indicate that  $\alpha$ -MSH and related hormones ACTH or endorphins alter monocyte functions. This could be of major importance in states of immunosuppression and investigating and understanding the mechanisms involved may help to develop new therapeutic strategies.<sup>74</sup>

Antiflammin-1 and antiflammin-2 are synthetic nonapeptides and are inhibitors of phospholipase A<sub>2</sub>. Antiflammins show potent anti-inflammatory effects. These peptide can inhibit PMN adhesion when using a leukocyte stimulus and can exert inhibitory actions as glucocorticoids on activation induced changes in adhesion molecule expression by PMNs. The effects of antiflammins on the expression of adhesion molecules on human leukocytes have been studied. It was observed that antiflammins mimic the actions of glucocorticoids on adhesion molecule expression on human leukocytes but not on endothelial cells and attenuate PMN adhesion to HCAEC. Thus, they may be useful agents to prevent or attenuate the neutrophil mediated tissue injury that accompanies chronic disease states like rheumatoid arthritis.<sup>75</sup> Psoriasis is characterised by inflammation and dilated capillaries in the papillary dermis. The anti-inflammatory compounds discussed above may have potential in modifying the inflammatory pathways in psoriasis.

#### HUMAN NEUTROPHIL ELASTASE (HNE)

HNE (MW 30 kDa; also known as polymorphonuclear or human leukocyte elastase: HLE) is a constituent of the azurophilic granules of human neutrophils and has physiological functions in host defence and ECM remodelling. HNE is detected in psoriatic lesions. Under normal conditions its proteolytic activity is controlled by endogenous inhibitors but upon inflammation an imbalance between HNE and its natural inhibitors leads to abnormal tissue destruction.<sup>76</sup> During acute inflammatory disorders the local concentration of HNE exceeds that of its natural inhibitors, such as  $\alpha_1$  proteinase inhibitor, leading to degradation of the ECM.<sup>76</sup> HNE is found in psoriatic lesions<sup>76</sup> and therefore HNE inhibitors may be used as a potential treatment option in psoriasis.

#### ELAFIN

Elafin, also called skin-derived antileucoprotease, is an elastase inhibitor produced by the

keratinocytes. Over-expression of elafin in the subcorneal region of the epidermis has been reported in wound healing and in skin disorders such as psoriasis and epidermal tumours (squamous cell carcinoma, actinic keratosis and keratoacanthoma).<sup>77</sup> Protein purification and the sequencing of the NH<sub>2</sub>-terminal of SKALP/elafin from cultured human keratinocytes as well as the cloning of its cDNA, revealed the existence of a mature protein. Western blots demonstrated that immunoreactive elafin is present in high molecular weight proteins extracted from psoriatic skin.<sup>78</sup> Tanaka et al.<sup>77</sup> showed that elafin expression in the upper epidermis was also enhanced in lesional skin of Behcet's syndrome, Sweet's syndrome, pyoderma gangrenosum, allergic vasculitis and acute infectious disease. These results suggested that skin disorders with dermal neutrophil infiltration may show over-expression of elafin due to IL-1beta and TNF-alpha released by dermal neutrophils. Elafin may function as a protective agent against damage of the epidermis caused by neutrophil elastase.<sup>77</sup>

#### SKIN PEPTIDE TYROSINE-TYROSINE (SPYY)

Neuropeptide Y (NPY) and polypeptide YY (PYY) are two ubiquitous neuropeptides, found in brain and intestines, where they exert important regulatory functions. Skin-PYY (SPYY) which is closely related to NPY and PYY has been isolated from amphibian skin. SPYY has antifungal activity, broad spectrum antibiotic activity and is a potent inhibitor of  $\alpha$ -MSH secretion *in vitro*.<sup>79,80</sup> It was proposed that SPYY and related YY peptides may act by mild perturbation of the membrane structure causing crossmembrane leakage of ions and other small molecules from high to low concentration.<sup>80</sup> SPYY has been shown to permeate phospholipid membranes and inhibit *C. neoformans*, *C. albicans* and *A. fumigatus* growth with MIC values of 20, 15 and 80  $\mu$ g/mL respectively. Analogs of SPYY may provide novel antifungal peptide based products.<sup>81</sup>

#### HUMAN SKIN DISEASES AND RELEVANT THERAPEUTIC PEPTIDES

*In vivo* evidence in humans and mice supports the role of AMPs in immunity. For example, LL-37 and human  $\beta$ -defensin (h $\beta$ D-2 and h $\beta$ D-3) are poorly expressed in skin lesions caused by AD

which increases susceptibility to skin infections, while expression of h $\beta$ D-2 and h $\beta$ D-3 is enhanced in psoriasis.<sup>2,7,82</sup>

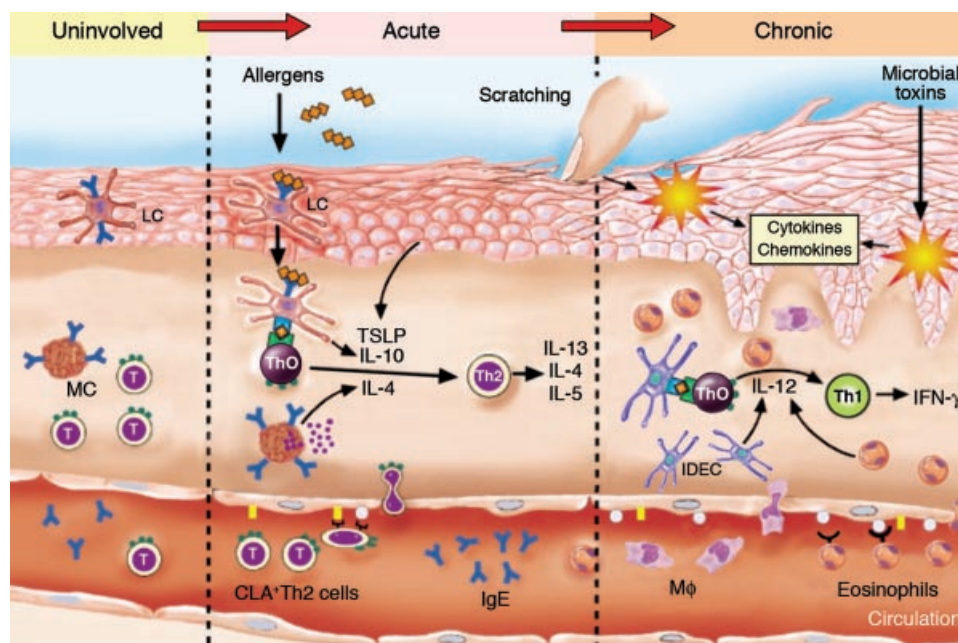
### Atopic Dermatitis (AD)

AD is a chronic inflammatory skin disease with abnormal expression or processing of AMPs.<sup>83</sup> It is characterised by dry skin and involves non-lesional skin and increased transepidermal water loss. *S. aureus* can be isolated from skin lesions. The bacterium gains access to underlying viable skin layers due to skin barrier dysfunction, reduced skin lipid content, alkaline pH of the skin and increased deposition of fibrinectin and fibrinogen. About 30% of patients with AD have bacterial or viral infections of the skin as compared to 7% with psoriasis.<sup>84</sup> LL-37 and H $\beta$ D-2 expression is greatly increased in patients with inflammatory skin conditions.<sup>9,85</sup> Furthermore, the AMPs that are normally induced in keratinocytes by inflammation show good antimicrobial activity against *S. aureus*, HSV and vaccinia virus.<sup>86</sup>

This suggests that lack of AMP expression in AD might contribute to bacterial and viral infections associated with the disease. Keratinocytes in lesional AD exhibit evidence of cytokine and lymphokine modulation.<sup>87</sup> In addition AD

patients show reduced expression of the AMP DCD in their sweat. A marked reduction in the viable bacterial cells on the skin surface of healthy individuals was observed after sweating, but not in patients with AD. Thus decreased DCD expression correlated with infections and therefore may contribute to the propensity of AD skin to recurrent bacterial and viral skin infections, and altered skin colonisation.<sup>45</sup>

Clinically unaffected skin of AD patients demonstrates an increased number of Type 2 helper (Th2) cells expressing IL-4 and IL-13 but not IFN $\alpha$  mRNA. In addition the development of skin lesions is accompanied by local tissue expression of proinflammatory cytokines and chemokines (Fig. 2). Thus early treatment with microbial probiotics may be beneficial by boosting Th1 immune responses in AD. Other approaches include cytokine modulation (e.g. TNF- $\alpha$  inhibitors), blocking inflammatory cell recruitment by using chemokine receptor antagonist and inhibition of T cell activation and the use of synthetic AMPs. Recombinant IFN- $\gamma$  was administered to AD patients in a double blind placebo controlled study. Significantly improved clinical outcome was accompanied by a reduction in eosinophil count although no reduction in serum IgE was observed. Reduction in the overall white blood cell count was also seen, suggesting that AD patients responded well to IFN $\gamma$  therapy.<sup>87</sup> Neuropeptides



**Figure 2.** Immunologic pathways in atopic dermatitis (reproduced from Ref. 83 with permission).

(NP), which are known to play a crucial role in neurogenic inflammation, could be involved in the development of skin inflammation.<sup>88</sup> In particular, substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) have been shown to be the main mediators of flare and weal reactions.<sup>89</sup> Acute local pretreatment with capsaicin which actually releases CGRP, was found to mimic the antiedema effects of exogenous CGRP in human skin and cheek pouch, thus indicating that CGRP from endogenous sources may inhibit inflammatory plasma leakage.<sup>90</sup> These effects of capsaicin and CGRP may provide new approaches for drug development.

### Psoriasis

Psoriasis is a condition of unknown aetiology characterised by epidermal hyperplasia, vascular alterations and inflammation.<sup>37</sup> Almost 2% of the population in western countries is affected by this disease and genetic factors are thought to be of fundamental importance in the expression of the disease. Many studies have been directed towards understanding the involvement of cytokines, growth factors and arachidonic acid derived mediators as potential candidates with a pathogenic role in cutaneous inflammation.<sup>37,91</sup> The protein psoriasin is highly upregulated in psoriatic epidermis and is most likely linked to the inflammatory stimuli.<sup>37</sup> Identification of the receptor or cellular target for this protein facilitate the development of new strategies to block the local epidermal inflammatory response that characterises psoriasis.<sup>92</sup>

Expression of the peptide elafin is also elevated in the spinous layers of psoriatic skin.<sup>93</sup> IL-1 $\beta$  and TNF- $\alpha$  are thought to be major inflammatory cytokines released from PMN in the early stages of acute inflammation<sup>94</sup> and it is suggested that over-expression of elafin may be caused by IL-1 $\beta$  and TNF- $\alpha$  released by dermal neutrophils. In psoriatic skin this peptide functions as a protective agent against damage of the epidermis caused by neutrophil elastase.<sup>77</sup> Antileukoprotease like elafin, a potent serine protease inhibitor, is also present in psoriatic scales hence protecting the psoriatic epidermis against proteolytic degradation.

Peptide T analogue, DAPTA has been shown to clear psoriasis lesions in clinical trials.<sup>72,95</sup> In this study it was seen that DAPTA significantly inhibited the monocyte and lymphocyte chemo-

tactic properties of RANTES (a beta chemokine found in increased amount in psoriatic lesions).<sup>72</sup>

ALP is present in psoriatic scales in concentrations sufficient for inhibition of neutrophil derived serine proteases HLE and CG.<sup>44</sup> Chronic inflammatory skin disorders could benefit from the development of serine protease inhibitors for use in therapy. The restitution of the HLE-HLE inhibitory balance in psoriasis may be of therapeutic value given that bathing in hypertonic solutions is known to remove HLE from the surface of psoriatic lesions.<sup>44</sup> In microbiocidal assays recombinant ALP exhibited antimicrobial activity against several human skin associated microorganisms like *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis* and *Candida albicans* indicating that ALP may actively participate in mechanisms allowing homeostasis of bacterial and yeast colonisation on human skin.<sup>43</sup>

IL-1 is of particular interest in psoriasis because of its ability to stimulate production of chemotactic cytokines. IL-6 and IL-8 may directly contribute to the epidermal hyperplasia seen in psoriatic lesions. TGF- $\alpha$ , which is required for the normal growth of epithelial cells, is over-expressed in psoriasis.<sup>96</sup> In addition EGF/TGF- $\alpha$  receptor expression is also increased in lesional epidermis.<sup>97</sup> LTB<sub>4</sub> levels are also seen to be increased in lesional psoriatic skin. Neutrophil activating peptide-1/IL-8, which is responsible for the release of LTB<sub>4</sub> has been isolated from psoriatic skin. This peptide shares homology with a number of peptides which have proinflammatory or growth stimulating activities, such as beta thromboglobulin, platelet factor 4, gamma IFN and a peptide with melanoma growth stimulatory activity.<sup>61</sup>

H $\beta$ D-2 and H $\beta$ D-3 have also been isolated from extracts of lesional scales of psoriatic skin.<sup>8</sup> In keratinocytes the expression of H $\beta$ D-2 is induced by IL-1 $\alpha$ , IL-1 $\beta$  or *P. aeruginosa*.<sup>8</sup> IL-1 and H $\beta$ D-2 have a key role in epidermal defence during inflammation.

It is known that secondary infection is rare in psoriasis. This has been attributed to the presence of a number of AMPs including granulysin which is known to be expressed in psoriatic plaques.<sup>2,98</sup> In addition, antimicrobial activity of lesional cytokines,<sup>99</sup> defensin expression by keratinocytes and increased numbers of NK-T cells in lesions is likely to contribute to the bacterial resistance in psoriasis plaques. RNase 7 is another broad spectrum AMP present in normal skin and in

elevated levels in psoriatic skin. Support for its antimicrobial defence action is strengthened by the observation that contact of keratinocytes with bacteria induced RNase 7 gene expression. In addition, peptide T analogue DAPTA, which inhibits the lymphocyte/monocyte chemotactic activities of RANTES, is expressed in high amounts in the keratinocytes of psoriatic tissue.<sup>72,95</sup>

A better understanding of the role of these peptides may provide new strategies for the treatment of diseases like psoriasis and to prevent secondary infection in a number of skin conditions.<sup>40</sup> For example, the development of peptides structurally related to AMPs and/or with a similar mode of action as psoriasin could be utilised. AMPs can also be artificially induced to fight skin infections. Another strategy could be to apply substances known to induce AMP synthesis or activity such as L-isoleucine and some structurally related molecules.<sup>32</sup>

### Wound Healing

The wound healing process involves four stages: inflammation which involves the release of chemotactic agents; migration where keratinocytes migrate from the wound edge towards the centre of the wound; deposition when the matrix components are deposited in the dermis by fibroblasts and; maturation which involves contraction of the dermis and squamous differentiation of the keratinocytes on the wound surface. Cytokines act as mediators in all the four phases of wound healing.<sup>59</sup> EGF, TGF and several other growth factors have been isolated from wounds and are known to promote keratinocyte growth and migration both in culture and in wounds.<sup>100</sup> In a murine model, the EGF-receptor-mediated signalling systems played an active part in epidermal wound repair: an increase in the number of EGF receptors precedes the hypertrophic response, and a decrease precedes the attenuation of this response.<sup>50</sup> Combinations of platelet derived growth factor (PDGF) with either IGF-1 or TGF- $\alpha$  have been shown to enhance connective tissue deposition, angiogenesis and collagen content and maturity.<sup>101</sup> Potent activators of keratinocytes such as IL-1 have an important role to play in the wound healing process. IGF-1 and TGF- $\alpha$  also induce or enhance the expression of the AMPs/polypeptides hCAP-18, h $\beta$ D-3, NGAL and SLPI in human keratino-

cytes thereby fighting potential secondary infection. TGF- $\alpha$  has also been shown to induce the expression of the same number of AMPs/polypeptides as the proinflammatory cytokine IL-1.<sup>52</sup> PMN-specific  $\alpha$ -chemokine IL-8 is a major biologically active PMN attractant in wound fluid but prolonged IL-8 levels may also contribute to additional healing activities in the later phases of wound healing. A strong correlation between TNF- $\alpha$  levels and IL-8 levels in wound fluid has been found.<sup>60</sup>

During the proteolytic remodelling of the extracellular matrix, small soluble RGD-containing peptides are released into the matrix milieu.<sup>54</sup> As these peptides are known to directly activate apoptosis they are likely to be especially helpful in situations in which leukocyte accumulation and resistance to apoptosis contribute to disease pathology.<sup>55</sup> They are useful in wound repair as they are internalised into a cell and are able to bind and activate caspase-3 inducing apoptosis.<sup>54</sup>

Copper peptides have also been used as wound healing agents.<sup>58,102</sup> For example, glycyl-L-histidyl-L-lysine-Cu (GHK) accelerates wound healing and stimulates biological events important in tissue repair such as angiogenesis, nerve outgrowth and chemoattraction of cells critical to healing (e.g. macrophages, monocytes, mast cells, capillary endothelial cells). A stimulating effect of GHK-Cu on collagen synthesis by fibroblasts has been reported.<sup>102</sup> Copper peptides have also been used in combination with Retin A to reduce inflammation.<sup>56</sup> They can improve healing after cosmetic procedures such as chemical peels, laser and dermabrasion procedures, where they are reported to decrease skin inflammation by increasing skin nutrient density.<sup>56</sup>

### Viral Disease

Alterations in the skin barrier can lead to infection with bacteria and selected viruses, including HSV, varicella-zoster virus and vaccinia virus. Evidence of the protective effect of AMPs in skin is demonstrated by the induction of epidermal cathelicidin LL-37 during the development of verruca vulgaris and condyloma acuminatum, two human papilloma virus infections responsible for common cutaneous warts.<sup>103</sup> The effects of cathelicidin,  $\alpha$ -defensins,  $\beta$ -defensins and control peptides on vaccinia virus replication *in vitro* have been investigated. Cathelicidins but not

defensins demonstrated antibacterial activity which caused a reduction in the vaccinia viral plaque formation.<sup>86</sup> It was observed that mice deficient in the murine LL-37-equivalent showed less effective wound healing upon infection with group A Streptococci. This makes LL-37 an attractive candidate as a novel therapeutic agent. A cathelicidin based indolicidin like peptide has been developed for acne treatment and is currently in phase 2 clinical trials (Migenix, Inc., Vancouver, BC, Canada). LL-37-versions for topical treatment of AD are in preclinical research stages (Ansata Therapeutics, Inc., La Jolla, CA).<sup>32</sup>

### Human Epithelial Cancer

AM, a calcitonin gene related peptide is found to be present in normal and malignant skin. AM may be involved with the wound repair process, sustaining normal epidermal turnover and influencing tumour initiation and proliferation.<sup>42</sup> It plays an important role in skin homeostasis and carcinogenesis. Some metastatic melanomas showed strong expression of AM at the periphery of the metastasis, whereas the majority of melanoma cells at the centre showed little or no expression. Keratoacanthoma showed a similar pattern of AM distribution. Thus, AM is a possible growth regulatory factor of the skin and malignant tissue therefore targeted delivery to induce inhibition at malignant tissue could be a treatment option.

### PEPTIDES AS COSMECEUTICALS

Cosmeceuticals are topical cosmetic-pharmaceutical hybrids lying on the spectrum between drugs and cosmetics.<sup>104,105</sup> There is an increasing trend towards the use of these agents in skin care regimens. There are numerous cosmeceutically active products on the market which can be broadly classified into the following categories: antioxidants, amino-peptides, growth factors, anti-inflammatories, polysaccharides and pigment lightening agents.<sup>104</sup> In most cases the overall purpose is antiaging but the method by which this is achieved varies. With the advances in biotechnology various peptides are now being used as cosmeceuticals. Examples include copper peptides, amino-peptides, acetyl hexapeptide 3 and dimethylaminoethanol.<sup>104</sup> Copper peptides such as

GHK-copper complex are included in products to improve skin firmness and texture, fine lines and hyperpigmentation.<sup>106</sup> Pal-KTTS is a pentapeptide fragment that stimulates collagen I and III and fibronectin production *in vitro* and functions in wound healing. Another synthetic peptide, acetyl hexapeptide-3 when tested in an open label trial demonstrated improvement in periorbital rhytides (wrinkles on the face and near eyes).<sup>104,107</sup> It is claimed to have muscle-relaxing effects similar to Botox injections (www.centerchem.com). Palmitoyl pentapeptide-3 (Matrixyl) is a fatty acid mixed with amino acids (www.sederma.fr). Sederma claim that their palmitoyl pentapeptide-3 product has superior antiwrinkle effects than a retinol or vitamin C product based on visual changes in 'half-face' studies. Similar claims have been made for Syn-Ake, a synthetic tri-peptide based on Waglerin 1, a peptide derived from the venom of the Temple Viper (www.pentapharm.com). It reduces the contraction frequency of muscle cells in the face thereby decreasing expression lines to provide an antiwrinkle/antiaging effect.

Antioxidative collagen derived peptides are present in human placenta where they protect the embryo from oxidative stresses. The antioxidant activity of these collagen peptides accounted for approximately 15% of the total antioxidant activity of human placenta extract (PLx).<sup>108</sup> A number of patents have been filed on the use of peptides for cosmetic products. These include the tripeptide lysine-proline-valine for stimulating or inducing hair growth.<sup>109</sup> Another patent describes topical compositions containing the tetrapeptide Arg-Ser-Arg-Lys for reducing wrinkles.<sup>110</sup> This tetrapeptide is a fibroblast growth factor derived peptide which slows down or decreases cellular activity. Carnosine and related naturally occurring histidine containing dipeptides such as anserine, exhibit antioxidative properties by quenching free radicals.<sup>111-113</sup> Carnosine also accelerates healing of wounds and ulcers and is reported to act as an antiaging peptide.<sup>111,113</sup> Fibroblast collagen production has been reported to be stimulated by a pentapeptide fragment (Lys-Thr-Thr-Lys-Ser) of the collagen molecule. It is a potent stimulator of collagen and fibronectin synthesis, which are both important components of the interstitial matrix<sup>114</sup> and if enhanced can decrease signs of ageing in the skin.

Growth factors act as regulatory proteins to mediate inter- and intracellular signalling pathways. They play an active role in wound healing and are involved in the induction of collagen,

elastin and glycosaminoglycan formation. A study conducted by Fitzpatrick et al.<sup>115</sup> showed their efficacy in improving wrinkles through stimulation of epidermal thickening.

The use of chemically modified peptides for healing, hydrating and improving skin appearance has stimulated substantial patent activity. A combination of peptides with the general sequence X-Thr-Thr-Lys-Y was reported to have activity against the formation or deterioration of wrinkles.<sup>116</sup> Cosmetic compositions have been formulated with at least one ceramide compound and one peptide attached to a fatty acid chain. Ceramides and their analogues have been known to protect and repair skin or hair fibres from damage caused by various agents and hair treatments. They provide a barrier effect which minimises the leakage of proteins.<sup>117</sup> Protein hydrolysates such as wool keratin hydrolysate were included in the formulation to improve ceramide binding to the skin or hair fibre.

## CONCLUSIONS

It is clear that many peptides play a wide variety of roles in normal skin and many are present or increased in skin disease. As we further develop our understanding of the role of these peptides in normal function and disease, the potential to exploit them for therapeutic or cosmetic purposes becomes clearer. This will require the synthesis of these peptides, or in some cases chemicals capable of inhibiting their action. In addition an efficient means of delivering them in active form to their target sites within the skin is required. As many of these peptides are greater than 500 Da and hydrophilic in character they will not diffuse passively across intact skin, therefore their delivery will require an enhancement system. Research into delivery of peptides and proteins to and across the skin is well advanced and is discussed in a separate review.<sup>118</sup> The potential for new treatment options, particularly for chronic skin diseases that are often poorly controlled by available therapeutics, is a priority in dermatological research.

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