Wound Repair and Antimicrobial Peptides

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Abstract Wounding of protective barriers is a major insult to the organism and immediately sets in motion a complex cascade of cellular responses in order to re-establish tissue integrity. Antimicrobial proteins, AMPs, are important components in the innate immune system and play a vital role in this process. The defensin family of proteins and the human cathelicidin, hCAP18, are the most documented AMPs in human and the focus of this review. Still, many proteins display antimicrobial activity, suggesting that the capacity to defend against microbes has been a driving force during evolution. In addition to direct killing of microbes, AMPs are involved in the inflammatory reaction through chemotaxis and control of cytokine response. Furthermore, recent data also show that AMPs have growth-factor like effects and signal via receptors promoting angiogenesis and re-epithelialization. Thus, the role of AMPs in wound healing is only beginning to be understood and may be far-reaching.

Abbreviations

AMP	Antimicrobial protein
EGFR	Epidermal growth factor receptor
FPRL1	Formyl peptide receptor-like 1
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MAPK	Mitogen activated kinases
P2X7	Purinergic receptor
PI3K	Phosphoinositide 3-kinase
PDC	Plasmocytoid dendritic cell

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SLPI Secretory leukocyte protease inhibitor
TLR Toll-like receptor
TGF-β Transforming growth factor beta

1 Introduction

Epithelia are barriers that protect the organism from the hostile environment, shielding self from non-self. Wounding of such barriers represents a major threat and immediately evokes a plethora of responses to restore the integrity of the tissue. Wound healing in higher organisms is a fundamental and complex biological process that has been fine-tuned during evolution. It may seem like a paradox that this process in some aspects represents a compromise, such as higher organisms are unable to repair the tissue to completion. In this sense we have traded perfection for speed and the cost is scarring. That being said, wound healing is a beautifully and precisely orchestrated process that integrates fundamental pathways and recruits the most powerful players of the immune system including antimicrobial proteins.

Physiologic wound repair is characterized by sequential and partly overlapping phases that can be schematically divided into initial homeostasis tightly linked to the inflammatory phase which is followed by the proliferative phase during which new granulation tissue is formed and the epithelium is regenerated. The whole process is completed during an extended remodelling phase when excess tissue is removed and the mature scar is formed. Precisely how these phases are integrated and regulated at the molecular level is not yet completely understood (Li et al. 2007). Delayed or impaired wound healing is a significant cause of morbidity and a more detailed knowledge about the mechanisms involved would facilitate the development of effective treatments which today are largely lacking (Menke et al. 2007).

In the early 1990s Gallo et al. reported that the pig cathelicidin PR-39 was capable of inducing proteoglycans in wounds and speculated on a putative impact on wound healing (Gallo et al. 1994). Since then accumulating data from several groups do support the notion that antimicrobial proteins, AMPs, are important in this process. Their potential roles are many, and may vary during the different phases of wound repair and also according to the tissue affected. We will go through the temporal sequences of the repair process and discuss which AMPs might be involved and how. In the present paper we will concentrate on skin wound healing and the AMPs that are present in skin, but also briefly discuss wound-related APMs in other epithelia such as the lung and cornea.

2 AMPs in Skin

An increasing number of proteins with antimicrobial activity have been identified in human skin. The source of AMPs varies, they are produced by structural resident cells as well as by infiltrating migratory cells. In neutrophils, AMPs are stored in preformed granules and released upon demand and in other cells such as keratinocytes and other leukocytes there is de novo synthesis. In addition to the "classical" AMPs, such as the human cathelicidin, hCAP18 and the defensins, an impressing list of molecules with other established biological functions such as neuropeptides, chemokines and protease inhibitors have now also been coined as antimicrobial proteins. One might speculate that antimicrobial activity has been a critical driving force during evolution and that this property has conferred a survival advantage for proteins involved in microbial defense, which thus has lead to their enrichment in the human genome. Subsequently, such proteins may have evolved to function in more complex biological pathways and whether they are classified as AMPs or otherwise would then depend on which function was discovered first. Current findings that cathelicidins and defensins in addition to their direct antimicrobial activities, also have effects on host cells and participate in receptor mediated signalling support this hypothesis.

2.1 Cathelicidins

The cathelicidins belong to a family of mammalian proteins that share a conserved cathelin-like domain at the N-terminus and a highly variable cationic C terminal peptide, typically 20–40 amino acids, that is proteolytically released through the activity of serine proteinases (Sorensen et al. 2001; Yamasaki et al. 2006). For a detailed background, the reader is referred to comprehensive reviews (Zanetti et al. 2000; Radek and Gallo 2007). In humans there is a single cathelicidin gene, wheras in other species such as pigs and cattle, there are multiple. The human cathelicidin, hCAP18 releases a 37 a-a long mature peptide, LL-37, which has broad antimicrobial activity as well as other biological effects (Agerberth et al. 1995; Cowland et al. 1995; Zanetti et al. 1995). Cathelicidin derived peptides display features that are common among antimicrobial proteins such as net positive charge and an amphipathic structure to allow interaction and disruption of the microbial membrane. At high concentrations, the peptide exhibits in vitro toxicity also towards eukaryotic cells. However, multiple factors in the tissue such as serum components protect the host cells from direct exposure to the peptide and net toxicity in vivo is thus unclear (Sorensen et al. 1999). Mammalian cathelicidins were initially identified from neutrophils and are now known to be constitutively expressed in most leucocytes. In epithelia, there is induction upon demand, such as inflammation, injury and infection (Frohm et al. 1997).

2.2 Defensins

Defensins are cystein-rich AMPs that fall into three distinct categories: α-defensins, β-defensins and θ-defensins. The mature peptides share features such as disulfide bridges. α-defensins are constitutively produced by neutrophils (HPN1-4), Paneth cells of the gut (HD5 and HD6) and epithelial cells of the female reproductive tract (HD5). In humans, β-defensin, hBD1-4, have been primarily identified in epithelial cells including skin, whereas the circular θ-defensins are not produced in humans. For a detailed background please be referred to selected reviews (Ganz 2003; Selsted 2004). Most defensins show broad antimicrobial activity against bacteria, fungi and some viruses and act by permeabilization of microbial membranes. In addition, like cathelicidins, the defensins also have effects on host cells and are involved in diverse inflammatory responses linking the innate and the adaptive immune systems.

3 The Wound Repair Process

3.1 The Acute Inflammatory Phase

Upon injury, the physical barrier is disrupted exposing the organism to potential hazardous invasion of microorganisms. This sets off the innate immune system where the AMPs are key players. In intact uninjured skin epithelium there is only low constitutive expression of AMPs, but following injury, these are rapidly upregulated (Frohm et al. 1997; Dorschner et al. 2001; Heilborn et al. 2003). The efficient and timely production of AMPs in skin and other epithelia is likely of major importance in the protection against pathogens in vivo, even though it has proven challenging to provide conclusive in vivo evidence. Support for the notion that functional antimicrobial effectors are critical in acute wounding was suggested by experimental data from mice with a disrupted cathelicidin gene, that were unable to efficiently eliminate a bacterial pathogen and developed large and necrotic ulcerations upon microbial challenge (Nizet et al. 2001). In human, it was recently shown that patients with atopic dermatitis fail to upregulate hCAP18 upon wounding which may underlie the susceptibility to infections in their skin so often traumatized by scratching (Mallbris et al. 2010). Interestingly, several studies have shown that the different AMPs have slightly different antimicrobial profiles and may act in synergy (Nagaoka et al. 2000). Their mode of action differs, but involves non-specific direct interaction with the microbial cells membranes.

The acute inflammatory response following injury typically lasts up to 48 h, but may persist longer. Following the initial hemostasis, there is influx of leukocytes into the tissue, first the neutrophils which are a rich source of antimicrobial molecules, proteases and reactive oxygen species. Experimental data show that neutrophils migrating into an acute wound induce a transcriptional program that

orchestrates the repair process (Singer and Clark 1999). However, neutrophils may function as a double-edged sword, when exaggerated, the inflammatory response will be detrimental to healing. Animal studies actually suggest that depletion of neutrophils does not delay repair of sterile incisional wounds, but rather accelerates re-epithelialization. Following the neutrophils, monocytes, which differentiate into macrophages, become after a few days the dominating cell type in the wound tissue. Several factors in the wound environment, including fibrin degradation products, proteases, cytokines and notably also AMPs, contribute to the recruitment of these inflammatory cells. LL-37 as well as the defensins exhibit significant chemotactic activity (Agerberth et al. 2000; De Yang et al. 2000). To further facilitate the recruitment of migratory cells, AMPs also have the capacity to upregulate chemokines and chemokine receptors (Scott et al. 2002; Lai and Gallo 2009). AMPs also function as important modulators linking the innate immediate response and the adaptive immune system (Davidson et al. 2004). In this way AMPs may modulate and amplify the inflammatory response following injury.

Ideally, the acute inflammatory reaction is self-limiting and should prepare the wound for healing. When the inflammatory phase is prolonged and becomes chronic, it will have a detrimental effect on the repair process There will be excessive tissue degradation through the sustained activation of matrix metalloenzymes (Wysocki et al. 1993) and a reduced concentration of factors to promote cell proliferation and formation of new tissue (Falanga 1992). The causes for failing to limit the inflammatory response in the chronic ulcer are manyfold and not entirely known but likely involve the presence of bacteria. Here, AMPS may play an important role not only through direct killing of microbes, but also in their capacity to neutralize microbial derived products such as lipopolysaccharide, LPS, and lipoteichoic acid, LTA, thus inhibiting subsequent cellular responses which may perpetuate the inflammatory reaction (Scott et al. 1999; Larrick et al. 1994). In line with these findings are recent data demonstrating that LL-37 actually suppresses the production of proinflammatory cytokines from dendritic cells exposed to microbial stimuli, such LPS, LTA and flagellin (Kandler et al. 2006; Mookherjee et al. 2006). Still, the net biologic effects exerted by AMPs are complex and vary between target cells. In lung epithelial cells, it was recently shown that the cathelicidin LL-37 rather augments the inflammatory response upon microbial exposure through promoting the uptake of LPS with subseqeuent production of proinflammatory cytokines (Shaykhiev et al. 2010). Clearly, in vitro findings are difficult to translate into physiologic effects in vivo and the overall biologic consequences will be defined by the microenvironment and the temporal and spatial context.

3.2 Regulation of AMPs and TLRs

Toll-like receptors, TLRs, are key molecules in the innate immune system mediating critical signals during injury. TLRs are typically activated in epithelial barriers upon microbial exposure and in turn induce the expression of AMPs.

Specifically, activation of epithelial cells with agonists for several TLRs: TLR2, TLR4 (Wang et al. 2003; Vora et al. 2004), TLR 5 and TLR9 (Miller et al. 2005) can induce the expression of human beta-defensins which is dependent on NF-kB signalling and activation of TLR9 stimulates the production of hBD-2 in airway epithelium (Platz et al. 2004).

The main transcriptional regulator of hCAP18/LL-37 in human skin (Weber et al. 2005) is 1,25-dihydroxyvitamin D3 that binds directly in the promoter region of the hCAP18 gene. It was recently shown that in addition to upregulating the expression of AMPs, injury potentiates the function of TLR2 through a vitamin D dependent mechanism. (Schauber et al. 2007). The importance of vitamin D was also stressed by recent data showing that vitamin D treatment even further enhances the expression of hCAP18 in acute wounds (Heilborn et al. 2010). Thus, vitamin D and AMPs produced in the same epithelial cells constitute a powerful biological unit serving to protect and repair the skin barrier if needed.

In contrast to skin, the expression of hCAP18 is differentially regulated in the gastrointestinal epithelium where short fatty acids but also microbial DNA control the production of hCAP18/LL-37. This emphasizes the vital role of the microenvironment in controlling AMP production and how AMPs have evolved to make optimal use of the specific conditions in different biological niches (Schauber et al. 2003, 2004; Islam et al. 2001).

Recently, it was shown that plasmocytoid dendritic cells (PDCs) rapidly infiltrate the skin upon injury (Gregorio et al. 2010). PDCs are a rare population of circulating cells not found in normal intact/non-inflammatory skin and which are involved in the antiviral defense through production of large amounts of type 1 interferons. Now it was shown that PDCs may be important in mediating early inflammatory responses following injury and also to promote re-epithelialization of skin wounds. LL-37 peptide is key in activating and recruiting PDCs into the skin and the production of hCAP18/LL-37 at the wound site may thus further support wound healing through this mechanism (Lande et al. 2007).

3.3 Angiogenesis

Formation of new tissue requires new blood vessel formation and angiogenesis is an integral component of wound healing. Relevant to this process were findings that hCAP18/LL-37 directly stimulated the proliferation of endothelial cells and supported functional neoangiogenesis in an animal model (Koczulla et al. 2003). The mechanism that was proposed for mediating this effect involved binding of LL-37 to the G protein-coupled receptor formyl peptide receptor-like 1, FPRL1, expressed on endothelial cells, since blocking the receptor through neutralizing anti-FPRL antiserum and by pertussis toxin abolished the effect. Downstream signalling seemed to involve the mitogen activated kinase pathway, MAPK and to some extent also the phosphoinositide 3-kinase, PI3K, pathway (Koczulla et al. 2003).

Angiogenetic capacity was recently demonstrated also for HBD-2,that was shown to stimulate migration and proliferation as well as tube formation of endothelial cells (Baroni et al. 2009). The in vivo relevance and relative importance for AMPs in supporting the outgrowth of new blood vessels during wound healing however, remains to be established. Considerable biologic redundancy is likely at play, and as multifunctional proteins, AMPs may be team-players participating in this process.

3.4 Re-epithelialization

Re-epithelialization begins within hours after wounding and is a critical feature of the repair process since formation of a new protective interface re-instates the integrity of the organism. The epithelial cells undergo a profound phenotypic change, loose their attachment to the surrounding tissue and neighbouring cells and move laterally into the wound. Initial migration is followed by proliferation. The precise molecular signals regulating these processes are not completely known but involve local release of growth factors from infiltrating cells, fibroblast and keratinocytes. In order to allow efficient migration through the wound, the production of tissue degrading enzymes such as matrix metalloproteinases is important. A balance between degradation and tissue formation is needed to avoid excessive degradation, which is thought to be at play in non-healing ulcers.

During recent years several studies have reported on the involvement of AMPs in re-epithelialization and wound closure, stimulating the migration as well as the proliferation of the epithelial cells. Wounding of mucous membranes show accelerated healing rate as compared to skin and histatins in human saliva, which are known antifungal agents, have been shown to stimulate wound closure through a receptor mediated mechanism and downstream activation of the ERK pathway (Oudhoff et al. 2008).

In human skin, it was shown that hCAP18 is produced in the migrating epithelial cells in sterile ex-vivo wounds that heal in cell culture which demonstrated that inflammation is not a prerequisite for the induction of hCAP18 in skin (Heilborn et al. 2003) Furthermore it was shown that re-epithelialization was blocked in a concentration dependant manner when adding anti-hCAP18 antiserum to the tissue (Fig. 1). The lack of hCAP18 immunoreactivity in the epithelium of non healing leg ulcers further suggested that hCAP18 may be important in the wound healing process (Heilborn et al. 2003).

In a diabetic mouse model simulating impaired wound healing, it was shown that adding LL-37 peptide to an experimental ulcer accelerated its closure, lending further support to the potential in vivo significance of hCAP18/LL-37 in the repair process (Carretero et al. 2008). Subsequently it has been shown that cathelicidins are capable of stimulating wound healing also in other systems such as lung epithelium (Shaykhiev et al. 2005), intestinal epithelium (Otte et al. 2009), cornea (Huang et al. 2006; Yin and Yu 2010) and also in rat gastric ulcers

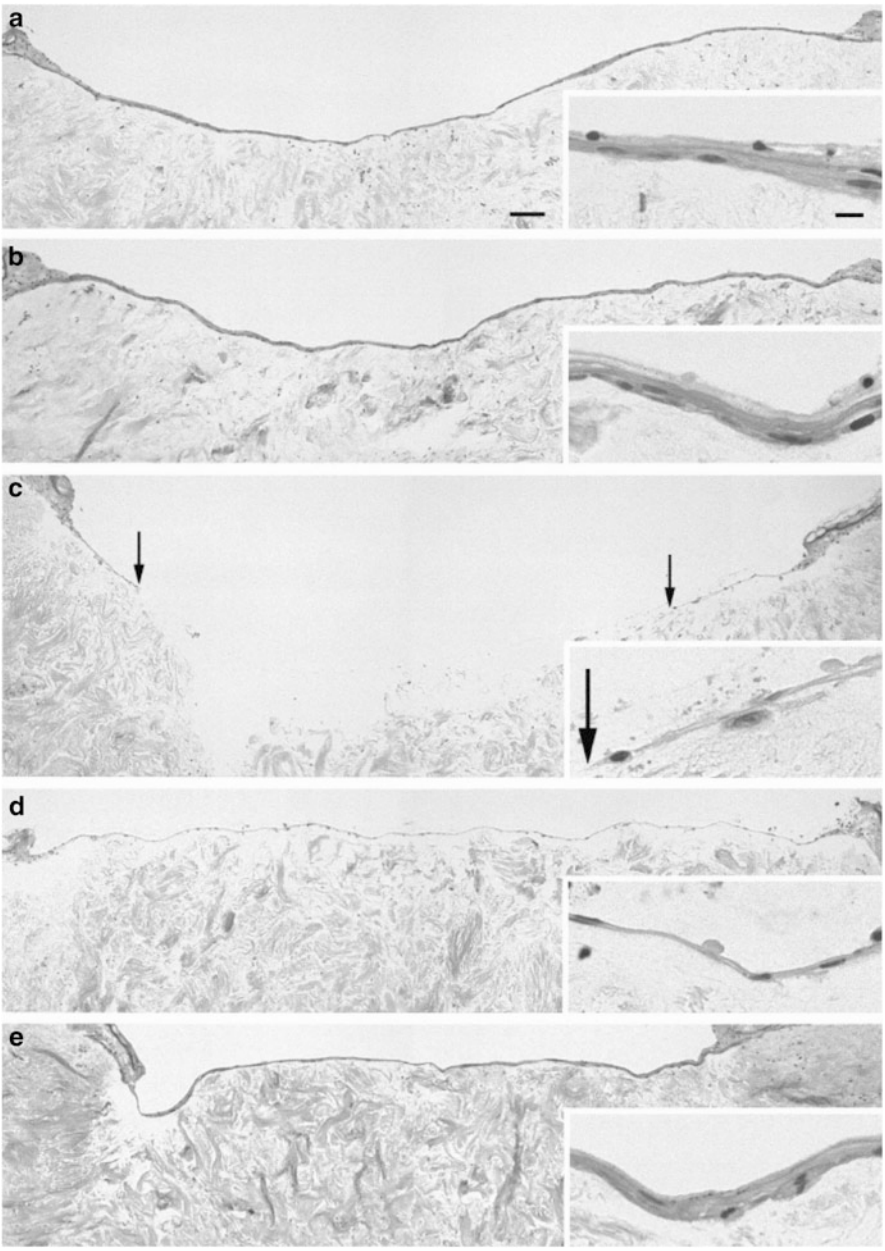

Fig. 1 *LL-37 antibody inhibited re-epithelialization in a concentration-dependent manner in the organ cultured full-thickness ex vivo wound model*. (**a**) Normal control wound completely re-epithelialized at 7 days. *Inset* shows higher magnification of the epithelium of the right wound margin comprising two to three cell layers. (**b**) *Preimmune* serum, at a final IgG concentration equal to the 1:10 dilution of the LL-37 anti-serum, did not affect re-epithelialization or the

(Yang et al. 2006). The effects seem to be mediated via activation of signalling pathways involving transactivation of the epidermal growth factor receptor, EGFR leading to activation of MAP kinases (Tjabringa et al. 2003; Shaykhiev et al. 2005) and via the G protein-coupled receptor FPRL1. The involvement of other receptors cannot be excluded and novel data show that LL-37 is a partial agonist for the type I insulin-like growth factor receptor, IGF1R, inducing downstream signaling confined to the ERK pathway and not affecting phosphatidylinositol 3 kinase/Akt signaling (Girnita et al. 2012). Furthermore, functional experiments demonstrated that LL-37 activating IGF1R signalling increased the migratory capacity of cells consistent with a potential role in epithelial wound closure (Girnita et al. 2012). In this context it was recently reported that growth factors such as insuling-like growth factor and transforming growth factor-alpha in turn stimulate the expression of cathelicidins and defensins in keratinocytes, demonstrating cooperation of multiple forces to optimize healing (Sorensen et al. 2003).

A mitogenic effect for neutrophil defensins on epithelial cells was shown already in the early 1990s (Murphy et al. 1993). This has later been substantiated reporting a direct stimulatory effect for HNP1-3 on lung epithelial cells and in vitro wound closure (Aarbiou et al. 2002; Aarbiou et al. 2004). Like cathelicidins, beta-defensins are also upregulated during re-epithelialization of the cornea, further substantiating the notion that induction of AMPs is a physiologic and general response to epithelial injury (McDermott et al. 2001). In an in vivo pig model for infected diabetic wounds, application of human beta-defensin -3 peptide was recently shown to significantly enhance wound closure in addition to reducing the bacterial load, lending further support for a role for defensins in wound healing (Hirsch et al. 2009).

In addition to effects on the epithelial cells in promoting wound closure, exciting new data suggest that AMPs may also be involved in osteogenesis (Zhang 2010). Thus, it was shown that blood monocytes, when treated with physiologic concentrations of LL-37 peptide, underwent differentiation towards a novel cell type called monoosteophils, capable of forming new bone. A potential implication for repair and healing of fractures was proposed. These results point to a growth-factor like role for cathelicidins in cell biology and are in line with data from several reports exploring a potential role for LL-37 in cancer development (Weber et al. 2009; Coffelt et al. 2009).

Fig. 1 (continued) appearance of the epithelium in control wounds at 7 days or the appearance of the epithelium. (**c**–**e**) Adding polyclonal anti-LL-37 IgG affected re-epithelialization in a concentration-dependent manner. (**c**) The highest concentration of LL-37 anti-serum (1:10) severely impaired re-epithelialization and inhibited wound closure. Higher magnification demonstrates a thin, profoundly affected epithelium. *Arrows* indicate the epithelial edges. (**d**) At 1:100 dilution of LL-37 anti-serum, the wound bed was covered with a single layer of keratinocytes with a fragile appearance at day 7. (**e**) At 1:1,000 antibody dilution re-epithelialization occurred at a level equal to that of the controls. *Scale bars*: (**a**–**e**) 50 μm; *insets* 10 μm. Used with permission from the publisher

3.5 *Tissue Remodeling*

Following the acute phases of wound healing, the tissue undergoes extensive remodelling during a prolonged period to produce a mature scar. In this process the activity of fibroblasts is crucial in fine-tuning the balance between matrix production and degradation to optimize tissue structure and function. The potential involvement of AMPs in this process has not been studied in detail, but an interesting observation was recently reported demonstrating that LL-37 has the capacity to suppress collagen synthesis in human fibroblast induced by transforming growth factor beta, TGF-β (Park et al. 2009). This finding opens up for the notion that AMPs may participate during all phases of wound healing and also for a putative function for cathelicidin peptides in the regulation of fibrosis and control of scar formation.

4 Wound Healing and Cancer

Wound healing and cancer share fundamental features and tumors have been called wounds that do not heal (Dvorak 1986). The molecular machinery that directs wound healing is also activated during tumor development and it is plausible that mechanisms driving the controlled repair of tissue are at work also in abnormal and uncontrolled tissue growth and spread. However, unlike in the wound microenvironment, the cancer milieu is abnormal and characterized by cellular mutations and epigenetic alterations resulting in a different outcome compared with physiologic tissue repair.

Cellular mechanisms implicated in mediating non-antimicrobial effects of cathelicidins include signalling through G protein coupled receptors such as FPRL1 and the purigernic ion channel P2X7 as well as transactivation of the EGFR (Tjabringa et al. 2003). These and other pathways are likely involved in mediating the wound healing promoting effects of AMPs (Yin and Yu 2010) and are also involved in the biology of cancer. Several recent reports suggest a role for the human cathelicidin LL-37 in tumor development demonstrating growth-factor like effects and implicating signalling through oncogenic pathways (Heilborn et al. 2005; von Haussen et al. 2008; Coffelt et al. 2009). The defensins have not been as extensively studied in this respect, but there are reports showing overexpression in transformed lung epithelium (Aarbiou et al. 2004) and in tumor-associated blood vessels (Kawsar et al. 2010).

Current thinking stresses a strong link between inflammation and cancer and here AMPs may be positioned in the crossfire between the two being involved in the physiologic inflammatory response and tissue repair as well as possibly being utilized by transformed cells to promote their growth and spread.

Another relevant aspect is apoptosis, where LL-37 was recently shown to inhibit apoptosis in keratinocytes in vitro (Chamorro et al. 2009). Suppression of apoptosis

is a fundamental aspect of cancer and important also for wound healing (Xue et al. 2004). Still, the picture is complex since LL-37 has been shown to mediate contrasting effects on apoptosis in different cellular systems (Barlow et al. 2006; Nagaoka et al. 2006). Thus, the net in vivo effects are difficult to predict and dependent on multiple factors such as cell type, tissue environment as well as the spatial and temporal exposure to the peptide. Current findings (see above) linking LL-37 to IGF1R signaling further strengthens its link to tumor growth (Girnita et al. 2012).

5 Other AMPs

A number of proteins detected in skin and other barrier organs display antimicrobial activity when tested in vitro. In relation to wound healing, there is limited data so far, but worth mentioning is secretory leukocyte protease inhibitor, SLPI, a cationic serine protease inhibitor. SLPI is constitutively expressed in glandular epithelia and induced in keratinocytes in hyperproliferative conditions such as psoriasis and wound healing. In mice lacking SLPI, experimental mucosal wounds healed significantly slower compared with wild type, suggesting that SLPI may play a role in tissue repair (Angelov et al. 2004). Interestingly, deletion of SLPI was also associated with an enhanced cellular inflammatory infiltrate in the wound bed, which is in line with the notion that sustained inflammation is detrimental for repair.

Neutrophil gelatinase–associated lipocalin, NGAL, is an antibacterial protein packaged in neutrophils, but also induced in hyperproliferating keratinocyte in psoriasis and in squamous cell carcinoma (Mallbris et al. 2002). Like hCAP18, its expression is upregulated by growth factors, IGF-1 and TGF-α, suggesting that it may play a role in tissue regeneration even though experimental evidence is lacking. Psoriasin is another AMP that is overexpressed in keratinocytes and is responsive to keratinocyte derived growth factors (Gläser et al. 2004). Interestingly it is implicated in cancer development, and is upregulated in skin wounds (Kesting et al. 2010) but has not been studied specifically in wound healing.

Overall it is to be expected that many molecules that function in the innate immune system in barrier organs will show activity towards microorganisms and possibly also help repair the epithelium if needed. Thus, the most likely scenario is one of redundancy and cooperation and it will be a real challenge to develop experimental systems to evaluate the relative contribution and importance of individual molecules.

6 Future Perspectives

Following their discovery in the 1980s, our perception of mammalian AMPs has undergone profound changes. From being regarded as purely antimicrobial effector molecules, i.e. endogenous peptide antibiotics, they are now emerging as versatile

players providing a vital link between the innate and the adaptive immune systems. The full functional repertoire of the most studied mammalian AMPs, the defensins and the cathelicidins, is still unclear and their potential involvement in diverse biological processes including wound healing opens up new perspectives and possibilities.

In view of the rapidly developing resistance to existing antibiotics, much hope and resources are invested in trying to develop AMPs into pharmaceutical drugs. It is believed that microbial resistance to AMPs is less likely to become a clinical problem compared with conventional antibiotics, which would be a huge benefit. However, one could only speculate and the consequences of widespread clinical use of AMPs as drugs are difficult to predict. Especially, extensive use over prolonged periods may create a different scenario compared with the endogenous controlled usage of AMPs which has been fine-tuned during evolution.

Regarding non-antimicrobial activities induced by AMPs, new pieces of this fascinating puzzle are steadily emerging. Still, it seems that we are only in the beginning to grasp the full functions of AMPs in normal physiology let alone disease. So far most studies have reported on expression patterns in different tissues and the majority of functional studies are performed in vitro. The few animal models, e.g. the cathelicidin deficient mouse (Nizet 2001), have generated valuable information and we expect to see an increase in such more complex system to test the in vivo relevance and potential of primary observations and hypotheses.

In wound healing it seems obvious that induction of AMPs represents a consistent and physiologic response to injury. We are beginning to understand the mechanisms for their induction at the transcriptional level, but much less of posttranscriptional regulation. LL-37 is perhaps the most documented AMP in skin injury. The complete lack of immunoreactive hCAP18/LL-37 in chronic ulcer epithelium is puzzling and in sharp contrast to the pronounced induction in acute skin wounds. What are the mechanisms to suppress translation of hCAP18 protein in chronic ulcers? What is the role of micro-organisms in this process? Is there a rational for using LL-37 in the treatment of chronic ulcers? Even with support from animal models, the hypothesis could only be ultimately tested in human. In the complex process of wound repair, it is difficult to predict the respective contribution and importance of individual molecules. Most likely there is redundancy and cooperation between the AMPs.

AMPs are fascinating molecules with a long history in biology, but with a short history in biomedical science. The initial discovery of AMPs represented a paradigm shift in biology and the start of a new era in understanding innate immunity. The research field is growing and the number of researchers engaged in studying AMPs from different perspectives is increasing rapidly. We can only hope that their combined efforts will help us understand and make use of the full potential offered by AMPs.

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