Inflammation in Wound Repair: Molecular and Cellular Mechanisms

Sabine A. Eming[1], Thomas Krieg[1] and Jeffrey M. Davidson[2,3]

In post-natal life the inflammatory response is an inevitable consequence of tissue injury. Experimental studies established the dogma that inflammation is essential to the establishment of cutaneous homeostasis following injury, and in recent years information about specific subsets of inflammatory cell lineages and the cytokine network orchestrating inflammation associated with tissue repair has increased. Recently, this dogma has been challenged, and reports have raised questions on the validity of the essential prerequisite of inflammation for efficient tissue repair. Indeed, in experimental models of repair, inflammation has been shown to delay healing and to result in increased scarring. Furthermore, chronic inflammation, a hallmark of the non-healing wound, predisposes tissue to cancer development. Thus, a more detailed understanding in mechanisms controlling the inflammatory response during repair and how inflammation directs the outcome of the healing process will serve as a significant milestone in the therapy of pathological tissue repair. In this paper, we review cellular and molecular mechanisms controlling inflammation in cutaneous tissue repair and provide a rationale for targeting the inflammatory phase in order to modulate the outcome of the healing response.

Journal of Investigative Dermatology (2007) **127,** 514–525. doi:10.1038/sj.jid.5700701

Introduction

Wound healing is a highly dynamic process and involves complex interactions of extracellular matrix molecules, soluble mediators, various resident cells, and infiltrating leukocyte subtypes. The immediate goal in repair is to achieve tissue integrity and homeostasis (Martin 1997; Singer and Clark, 1999). To achieve this goal, the healing process involves three phases that overlap in time and space: inflammation, tissue formation, and tissue remodeling. During the inflammatory phase, platelet aggregation is followed by

Editor's Note

Wound healing has been recognized as important to health since the time of Hammurabi. A Sumerian clay tablet (c 2150 BC) described early wound care that included washing the wound in beer and hot water, using poultices from substances such as wine dregs and lizard dung and bandaging the wound. Hippocrates (c 400 BC) detailed the importance of draining pus from the wound, and Galen (c 130–200 AD) described the principle of first and second intention healing (Broughton G *et al.* (2006) A Brief history of wound care. *Plast Reconstr Surg* 117:6s). Wound healing advanced slowly over the centuries, with major advances in the 19th century in the importance of controlling infection, hemostasis and necrotic tissue. The discovery of cytokines and growth factors in the 1950s opened a new age in wound healing research and led to many important breakthroughs concerning the basic biology of healing wounds in the skin. In this issue of the JID, a new Perspective series focused on wound healing begins. These articles detail the role of inflammation in wound healing and fibrosis, the key involvement of fibroblasts, myofibroblasts, and keratinocytes in the healing wound, and the great opportunities that tissue engineering provides to improve wound healing. Hippocrates recognized that ''Healing is a matter of time, but it is sometimes also a matter of opportunity.'' These Perspectives show the great new opportunities that we now have for understanding and improving the process of healing wounds of the skin.

Russell P. Hall, III
Deputy Editor

[1]*Department of Dermatology, University of Cologne, Cologne, Germany;* [2]*Department of Pathology, Vanderbilt University School of Medicine, Tennessee, USA and* [3]*VA Medical Center, Nashville, Tennessee, USA*

Correspondence: Dr Sabine A. Eming, Department of Dermatology, University of Cologne, Joseph-Stelzmann Str. 9, 50931 Köln, Germany. E-mail: sabine.eming@uni-koeln.de

Abbreviations: MCP, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; SCC, squamous cell carcinoma; TGF, transforming growth factor; TNF, tumor necrosis factor

Received 30 June 2006; revised 21 September 2006; accepted 23 September 2006

infiltration of leukocytes into the wound site. In tissue formation, epithelialization and newly formed granulation tissue, consisting of endothelial cells, macrophages and fibroblasts, begin to cover and fill the wound area to restore tissue integrity. Synthesis, remodeling, and deposition of structural extracellular matrix molecules, are indispensable for initiating repair and progression into the healing state. Cellular responses to injury involve direct cell–cell and cell–matrix interactions, as well as the indirect crosstalk between different cell populations by soluble mediators. Indeed, complex interactions between the epidermal and dermal compartment are essential. During the past decade numerous factors have been identified that are engaged in a complex reciprocal dialogue between epidermal and dermal cells to facilitate wound repair (Werner and Grose, 2003). The sensitive balance between stimulating and inhibitory mediators during diverse stages of repair is crucial to achieving tissue homeostasis following injury.

The inflammatory response is regarded as the first of a number of overlapping processes that constitute wound healing. In skin repair, the infiltrating leukocytes are the principal cellular components of the inflammatory response. They are not only effector cells combating invading pathogens but are also involved in tissue degradation and tissue formation. As such, an excessive or reduced influx or activation of infiltrating leukocytes into the damaged tissue may have profound effects on downstream cell migration, proliferation, differentiation, and ultimately the quality of the healing response. Continuing progress in understanding the essential and complex role of the inflammatory response in wound repair will provide strategies to modulate diseases with pathologic tissue remodeling, such as healing disorders, various chronic inflammatory disease states, and cancer.

Inflammation in physiological wound repair: cell lineages, functions, and mediators

Tissue injury causes the immediate onset of acute inflammation. It has long been considered that the inflammatory response is instrumental to supplying growth factor and cytokine signals that orchestrate the cell and tissue movements necessary for repair (Simpson and Ross, 1972; Leibovich and Ross, 1975). In various experimental animal models and human skin wounds, it has been demonstrated that the inflammatory response during normal healing is characterized by spatially and temporally changing patterns of various leukocyte subsets (Martin 1997; Singer and Clark, 1999). The well-defined chronology of these events is essential for optimal repair.

PMNs. Immediately after injury extravasated blood constituents form a hemostatic plug. Platelets and polymorphonuclear leukocytes (neutrophils, PMN) entrapped and aggregated in the blood clot release a wide variety of factors that amplify the aggregation response, initiate a coagulation cascade, and/or act as chemoattractants for cells involved in the inflammatory phase (Szpaderska *et al.*, 2003). Within a few hours post-injury the bulk of neutrophils in the wound transmigrate across the endothelial cell wall of blood capillaries, which have been activated by proinflammatory cytokines IL-1β, tumor necrosis factor-α (TNF-α), and IFN-γ at the wound site, leading to expression of various classes of adhesion molecules essential for leukocyte adhesion and diapedesis. Adhesion molecules which are crucial for neutrophil diapedesis include endothelial P- and E-selectins as well as the ICAM-1, -2. These adhesins interact with integrins present at the cells surface of neutrophils including CD11a/CD18 (LFA-1), CD11b/CD18 (MAC-1), CD11c/CD18 (gp150, 95), and CD11d/CD18 (Kulidjian *et al.*, 1999). Chemokines and their receptors are most likely crucial mediators for neutrophil recruitment during repair (Gillitzer and Goebeler 2001; Esche *et al.*, 2005). These include IL-8, growth-related oncogene-α, and monocyte chemoattractant protein-1 (MCP-1) (Engelhardt *et al.*, 1998). In addition, bacterial products, such as lipopolysaccharides and formyl-methionyl peptides, which accumulate in the bacterially infected wound, can accelerate the directed neutrophil locomotion.

Recruited neutrophils begin the debridement of devitalized tissue and phagocytosis of infectious agents. To perform this task, neutrophils release a large variety of highly active antimicrobial substances (reactive oxygen species (ROS), cationic peptides, eicosanoids) and proteases (elastase, cathepsin G, proteinase 3, urokinase-type plasminogen activator) (Weiss 1989) (Table 1). Microarray technology has recently revealed that migration of PMNs to skin lesions induces a large transcriptional activation program, which may regulate cellular fate and function and promote wound healing (Theilgaard-Mönch *et al.*, 2004). Despite detailed knowledge on the synthesis of mediators released by PMNs and mechanisms involved in their recruitment and their role in host defense, these cells can be beneficial or detrimental to healing. Experiments in the 1970s showed that depletion of neutrophils by antiserum from guinea pigs did not significantly perturb tissue repair of incisional wounds under sterile conditions (Simpson and Ross, 1972). A recent study by Dovi *et al.* (2003), using a similar approach of neutrophil depletion, partially confirmed these early studies. Although dermal repair parameters were not affected by neutropenia, reepithelialization was significantly accelerated (Dovi *et al.*, 2003). However, in this study it remains unclear whether the lack of PMNs has a direct, beneficial effect on reepithelialization or whether the relative increase in other subsets of inflammatory cells, such as the macrophages, might be responsible for accelerated epithelialization. Recent *in vitro* studies demonstrated that neutrophils isolated from sites of repair can modulate the phenotype and cytokine profile expression of macrophages, thereby regulating the innate immune response during healing (Daley *et al.*, 2005). In addition, a recent report shows that closure of excisional wounds in CD18-depleted mice was significantly delayed, most likely due to impaired myofibroblast differentiation and reduced wound contraction (Peters *et al.*, 2005). The authors speculated

that in CD18-deficient wounds, which are devoid of neutrophils, the lack of apoptotic neutrophils at the wound site deprives macrophages of their main stimulus to secrete transforming growth factor-β1 (TGF-β1), a key mediator involved in myofibroblast differentiation. Furthermore, impaired wound healing is a cardinal feature of human diseases that are characterized by a deficiency in PMN function, such as the leukocyte-adhesion deficiency syndrome 1 (Kuijpers *et al.*, 1997) or disease states of PMN actin polymerization (Roos *et al.*, 1993).

Monocytes/macrophages. Unless stimuli for neutrophil recruitment persist at the wound site, the neutrophil infiltration ceases after few days, and expended neutrophils are themselves phagocytosed by macrophages, which are present at the wound side within 2 days after injury. Beside resident macrophages, the major portion of macrophages at the wound site is recruited from the blood. Whereas the extravasation of blood PMN is primarily regulated by the CD11/CD18 complex and ICAMs, emigration of blood monocytes into the wound is in addition regulated by the interaction of the very late antigen-4(α4β1 integrin) and endothelial vascular cell adhesion molecule-1 (Issekutz *et al.*, 1995; Cotran and Mayadas-Norton, 1998). Macrophage infiltration into the wound site is highly regulated by gradients of different chemotactic factors, including growth factors, proinflammatory cytokines, and chemokines macrophage inflammatory protein 1α, MCP-1, RANTES) (DiPietro *et al.*, 1998; Frank *et al.*, 2000; Wetzler *et al.*, 2000; Werner and Grose 2003). Major sources of these chemoattractants at the wound site include platelets trapped in the fibrin clot at the wound surface, hyperproliferative keratinocytes at the wound edge, fibroblasts, and leukocytes subsets themselves. As monocytes extravasate from the blood vessel they become activated and differentiate into mature tissue macrophages. This transformation implies a major change in the activation phenotype of the macrophages resulting in significant alterations in gene expression. There is evidence that the activation process is directed by mediators present in the microenvironment, and this pathway would be crucial for the proper adaptation of macrophage function to the specific metabolic requirements of the wound site (Gordon, 2003; Pinhal-Enfield *et al.*, 2003; Ramanathan *et al.*, 2003). Numerous cell surface receptors have been described through which macrophages sense and respond to their microenvironment, including Toll-like receptors, complement receptors, and Fc receptors (Gordon 2003; Karin *et al.*, 2006). It remains a challenge to characterize and better understand the mutual interplay and mechanisms that regulate the activation state of inflammatory cells in specific wound environmental conditions. Beside their immunological functions as antigen-presenting cells and phagocytes during wound repair, macrophages are thought to play an integral role in a successful outcome of the healing response through the synthesis of numerous potent growth factors, such as TGF-β, TGF-α, basic fibroblast growth factor, platelet-derived growth factor, and vascular endothelial growth factor, which promote cell proliferation and the synthesis of extracellular matrix molecules by resident skin cells (DiPietro and Polverini, 1993). A crucial role for macrophages in repair was established over 30 years ago. Macrophage depletion using antisera resulted in a significant delay of healing (Leibovich and Ross, 1975). More recent studies have supported and extended these observations. Double P- and E-selectin-deficient mice (Subramaniam *et al.*, 1997), mice deficient in β-1,4-galactosyltransferase, which glycosylates the P- and E-selectins (Mori *et al.*, 2004), and ICAM-1-deficient mice (Nagaoka

Table 1. Inflammatory cells, their functions and mediators released in tissue repair

Cell type	Functions	Mediators
PMN	Phagocytosis of infectious agents	ROS, cationic peptides, eicosanoids, proteases (elastase, cathepsinG, PR-3, and uPA)
	Mϕ activation through phagocytosis	
	Amplify inflammatory response	TNF-α, IL-1β, IL-6
	Stimulate repair response	VEGF, IL-8
MΦ	Phagocytosis of PMN and fragments of tissue degradation	
	Amplify inflammatory response	TNF-α, IL-1β, and IL-6
	Anti-inflammatory function	IL-10, TGF-β1
	Stimulate repair response: angiogenesis, fibroplasia	PDGF, VEGF, bFGF, TGF-α, and TGF-β
	Fibrolysis	t-PA, uPA, u-PAR, and PAI-1/-2
MC	Control vascular permeability	Histamine
	Control influx of PMN	Chymase, tryptase
	Regulate tissue remodeling	
T cell; Th1/Th2	Regulate tissue remodeling	CD40 ligand; IL-2, TNF-α/ IL-4, -5,-10
$\gamma\delta$T cells	Keratinocyte proliferation, differentiation, hyaluronan synthesis in Kc	FGF-7, FGF-10, and IGF-1,

bFGF, basic fibroblast growth factor; FGF, fibroblast growth factor; MC, mast cell; PDGF, platelet-derived growth factor; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

et al., 2000) have all shown a dramatic delay in wound closure that was associated with a significantly reduced infiltration of neutrophils and macrophages.

Mast cells. Mast cells are an additional leukocyte subset present in the skin and they are an important source of a variety of proinflammatory mediators and cytokines that can promote inflammation and vascular changes. Therefore, they are considered to be involved in tissue repair. Following injury residential mast cells degranulate within hours and thus may become less apparent. Mast cell levels return to normal around 48 hours post-injury, and then increase in number as tissue repair proceeds (Trautmann *et al.*, 2000). Analysis on the impact of mast cell deficiency in mice has been contradictory. Egozi *et al.* (2003) reported that mast cell-deficient mice (WBB6F1/J-kit^{W}/Kit^{W-V}) showed a decreased number of neutrophils at the wound site, whereas macrophage and T-cell infiltration was normal. In these studies, mast cell deficiency had no significant effect on epithelialization, collagen synthesis, or angiogenesis. These results suggest that mast cells modulate the recruitment of neutrophils into the site of injury. However, in normal uncomplicated conditions mast cells are unlikely to exert functions that are rate limiting for repair in mice. Iba *et al.* (2004) partially confirmed these results by demonstrating a significant effect of mast cell deficiency (W/Wv mice) on collagen deposition and tissue remodeling in the late phase of repair, however wound closure rate was not effected by mast cell deficiency. Recently, both studies have been challenged by a report describing a significant impact of mast cell deficiency on vascular permeability, PMN influx, and ultimately wound closure rate (Weller *et al.*, 2006). Further studies are required to better understand the impact of mast cells on the healing response and to clarify whether differences in model systems used may account for the diverse effects observed.

T cells. During the phase of tissue remodeling, when wound closure has been completed, and local infections are already overcome, cells of the adaptive immune response, in particular T cells constitute the most frequent leukocyte subset in human skin wounds (Fishel *et al.*,. 1987; Engelhardt *et al.*, 1998). Chemokines are crucial mediators for lymphocyte chemotaxis and function (Baggiolini 1998; Luster *et al.*, 1998; Rossi and Zlotnik, 2000). Lymphocyte accumulation is associated with the initial appearance of MCP-1 4 days after injury by the chemokines IFN-γ-inducible protein-10 and monokine induced by IFN-γ. Macrophages appear to be a major source for these cytokines. IFN-γ is a major inducer of inducible protein-10 and monokine induced by IFN-γ, which could reflect a major shift in cytokine expression profile from proinflammatory mediators to IFN-γ. IFN-γ gene deficiency leads to an accelerated healing response, most likely mediated by enhanced TGF-β1 levels at the wound site, consequent augmented TGF-β1-mediated signaling pathway and accelerated collagen deposition (Ishida *et al.*, 2004). The number of neutrophils, macrophages, and T cells were significantly reduced at the wound site of IFN-γ-deficient mice, most likely due to the lack of endothelial activation. These data may support a role for T cells in tissue remodeling. Definitive understanding of T-cell function, in particular the role of particular T-cell subsets during repair requires further investigation. It is likely that Th1- and Th2-cell subsets differentially regulate the wound microenvironment by secreting distinct cytokine profiles (Azouz *et al.*, 2004; Park and Barbul 2004). Along these lines Th1 cells are characterized by the release of IFN-γ, IL-2, and TNF-α, whereas Th2 cells classically release IL-4, -5, and -10. The expression pattern of both cytokine profiles has been associated with diverse processes of tissue remodeling. T cells might also influence the healing response by direct cell–cell interactions with resident and non-resident cells at the wound site. Interactions between the membrane-bound glycoprotein CD40 and CD40 ligand are reported to play an important role in these interactions. CD40 ligand-expressing T cells interact with CD40-expressing keratinocytes, fibroblasts, platelets, and macrophages, altering their expression profile of inflammatory mediators, and consequent repair functions (Yellin *et al.*, 1995; Henn *et al.*, 1998; Kaufman *et al.*, 2001).

Havran and co-workers recently reported on the role of a unique population of $\gamma\delta$ T lymphocytes in epidermal repair in the skin (Jameson *et al.*, 2002). Skin $\gamma\delta$ T cells are designated dendritic epidermal T cells (DETC), and they are strictly limited in their distribution to the epidermis ($\gamma\delta$DETC). They recognize an, as yet unidentified antigen, expressed by damaged stressed, or transformed keratinocytes in the epidermis. The role of $\gamma\delta\Delta$ETC in tissue repair is based on wound healing studies in $\gamma\delta$DETC-deficient mice, which showed a delayed healing response following mechanical injury. $\gamma\delta$ DETCs have been identified as important source of key growth factors such as fibroblast growth factor-7, -10, as well as IGF-1 thereby regulating keratinocyte proliferation and differentiation (Jameson *et al.*, 2002; Sharp *et al.*, 2005). In addition, this population of T cells was recently shown to be involved in keratinocyte-mediated hyaluronan deposition in the extracellular matrix, and inducing subsequent macrophage infiltration into the wound site (Jameson *et al.*, 2005). Taken together, these results demonstrate a novel function for skin $\gamma\delta$ T cells and provide a new perspective on T-cell regulation in tissue repair.

Mechanisms of inflammatory resolution. Successful repair after tissue injury requires *resolution of the inflammatory response*. However, whereas the knowledge about mechanisms and molecules inducing and perpetuating the inflammatory response is constantly increasing, mechanisms, that limit and down regulate this activity are less appreciated. Such mechanisms might include: downregulation of chemokine expression by anti-inflammatory cytokines such as IL-10 (Sato *et al.*, 1999) or TGF-β1 (Ashcroft *et al.*, 1999a, 1999b; Werner *et al.*, 2000), or upregulation of anti-inflammatory molecules like IL-1 receptor antagonist or soluble TNF receptor; resolution of the inflammatory response mediated by the cell surface

receptor for hyaluronan CD44 (Teder *et al.*, 2002; Jiang *et al.*, 2005); apoptosis (Greenhalgh 1998), receptor unresponsiveness or downregulation by high concentrations of ligands (Figure 1). Interestingly, recent *in vitro* data suggested that matrix metalloproteinases (MMPs) can downregulate inflammation via cleavage of chemokines, which then act as antagonists (McQuibban *et al.*, 2000, 2002). However, the relevance of these mechanisms for cutaneous tissue repair has to be further investigated. In recent studies, Nrf-2, a target of the keratinocyte growth factor-1 was identified as novel transcription factor regulating the inflammatory response during repair. The wound healing response in Nrf-2 knockout mice was characterized by a prolonged inflammatory response following wound closure, which was most likely mediated by prolonged expression of IL-1β and TNF-α (Braun *et al.*, 2002).

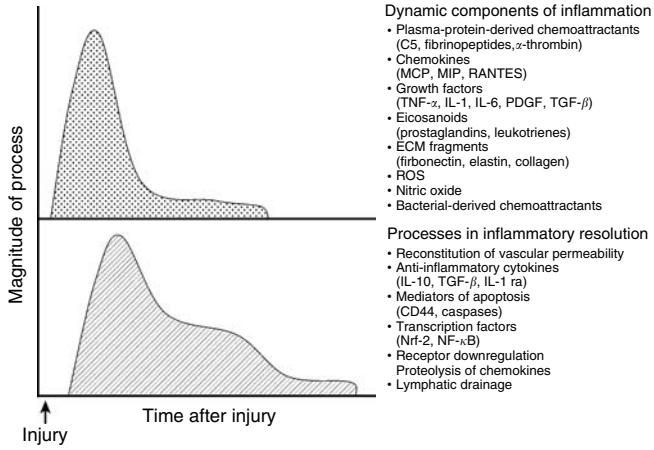

Figure 1. Mediators and mechanisms of inflammation and inflammatory resolution in repair. Tissue injury causes the immediate onset of acute inflammation mediated by chemoattractants derived from plasma proteins, resident and recruited hematopoetic cells, extracellular matrix and bacteria. Progression to complete wound healing is accompanied by resolution of the inflammatory response, which is essential for successful repair. Resolution of inflammation is directed by downregulation of proinflammatory mediators and the reconstitution of normal microvascular permeability, which contributes to the cessation of local chemoattractants, synthesis of anti-inflammatory mediators, apoptosis, and lymphatic drainage. An excessive or prolonged inflammatory response results in increased tissue injury and poor healing. Successful wound repair requires the coordinate expression of both inflammation and resolution of inflammation.

Inflammation directs the quality of tissue repair

Scar formation is the physiological and inevitable end point of mammalian wound repair and there is substantial evidence that inflammation is an essential prerequisite for scarring. Although, normal scar tissue provides a stable restoration of the skin barrier, the new tissue is inferior with respect to important structural, aesthetic and functional aspects. The notion has emerged that in the mammal, wound repair is evolutionary optimized for speed of healing under dirty conditions, where a multiply redundant, compensating, rapid inflammatory response, allows the wound to heal quickly without infection. Thus, a scar may therefore be the price mammalians have to pay for evolutionary survival after wounding (Bayat *et al.*, 2003).

There is considerable quantitative and qualitative variation in scarring potential between species and even among various body sites and organs. In the human pathological conditions and genetic disorders can lead to excessive scarring such as in hypertrophic scars or keloids, which might become severe health problems. Although the underling mechanisms for the differences in the outcome of scarring is not well understood so far, there is substantial experimental and clinical evidence that differences in scarring reflect an altered inflammatory and/or cytokine profile between individuals or in a disease state (Harty *et al.*, 2003; Martin and Leibovich, 2005). Thus, a better understanding in cellular and molecular mechanisms in inflammatory processes controlling scarring will serve as a significant milestone in the studies of tissue repair. The implications for therapeutic applications in wound management and in diseases where scarring is the basic pathogenic mechanism would be significant.

There are several situations that provide clear evidence that the inflammatory phase during repair is intimately linked to the extent of scar formation. First, in contrast to post-natal repair, wound healing in early fetal skin exhibits scarless regeneration of the dermal architecture. Although there are numerous intrinsic and extrinsic differences between the fetus and adult that may influence tissue repair, the hallmark of fetal repair is the lack of a typical inflammatory response (Bullard *et al.*, 2003; Reed *et al.*, 2004). This minimal fetal inflammatory response may play a pivotal role in the unique fetal repair process (Adolph *et al.*, 1993; Dillon *et al.*, 1994). In support of this concept, scar formation appears to be accelerated when inflammation is provoked in fetal wounds (Whitby and Ferguson, 1991). Liechty *et al.* (2000) wounded embryonic skin from IL-10-deficient mice that had been transplanted to strain-matched adult mice. IL-10 is a central immunosuppressive cytokine regulating innate and adaptive immune responses. Whereas control embryonic skin grafts showed little inflammation and scarless healing, wounded IL-10 (−/−) grafts were characterized by a significantly higher inflammatory cell infiltration and collagen deposition and an adult-like scarring response (Liechty *et al.*, 2000).

A second example indicating that inflammation plays a major role in the etiology of scarring is evident from studies investigating the influence of reproductive hormones on this process. These studies demonstrated that reduced systemic estrogen levels in ovariectomized mice results in a markedly impaired rate of healing associated with excessive inflammation and scarring (Ashcroft *et al.*, 1997, 1999a, 1999c, 2003; Margolis *et al.*, 2002). Evidence suggests that a crucial mechanism underlying the responses to estrogen involves a dampening of the inflammatory response, resulting in reduced proteolytic activity and en-

hanced matrix deposition (Ashcroft *et al.*, 1999a, 1999b). Recent evidence indicates that macrophage migration inhibitory factor, an important regulator of inflammation, is a crucial mediator of excessive inflammation in the absence of estrogens (Ashcroft *et al.*, 2003). Interestingly, no significant differences in the rate of healing were observed between intact wild-type and migration inhibitory factor null mice, suggesting that in the presence of estrogens the absence of migration inhibitory factor has no effect on the wound healing response (Ashcroft *et al.*, 2003; Hardman *et al.*, 2005).

Increased influx of neutrophils and macrophages does not inevitably result in an increased scarring response. Studies examining wound healing in the elderly revealed that neutrophil invasion and particularly the number of mature macrophages at the wound site is significantly increased in aged animals and humans (Ashcroft *et al.*, 1998, 2002; Swift *et al.*, 2001). However, acute wounds in aged human and animal models, although delayed, heal with a better quality of scarring compared to their young counterparts. In these models healing occurred with regeneration of the dermal architecture, and in an improved macroscopic and microscopic scar appearance resembling more closely that of normal skin (Ashcroft *et al.*, 2002). The regenerative appearance of dermal collagen, elastin, and fibrillin arcades may reflect activation of the mechanisms responsible for the developmental formation of such fiber frameworks. Similar to fetal repair, levels of TGF-β in wounds of the elderly are markedly reduced, and this phenomenon might represent the underlying mechanism of reduced scarring observed with age. Further studies are required to identify mechanisms that spare the aged skin from scarring despite an increased inflammation.

Genetic mouse models for inflammation and healing

Genetically modified mouse models have proven to be of immense help to dissect the contribution of specific immune modulators in tissue repair (Table 2). Proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α have been shown to be pivotal mediators during different cutaneous inflammatory processes (Hübner *et al.*, 1996; Werner and Grose, 2003). Proinflammatory cytokines are strongly upregulated during the inflammatory phase of healing and beside several resident cell types of the skin, neutrophils, and macrophages are both major sources and targets of proinflammatory cytokines. A critical role of proinflammatory mediators in leukocyte function is evident in skin wounds of mice deficient for the TNF receptor TNF-Rp55. Despite a significant reduction in leukocyte infiltration at the wound site, wounds showed an increase in angiogenesis, collagen content, reepithelialization, and expression of various growth factors and their receptors. (Mori *et al.*, 2002). Ultimately, TNF-Rp55 deficiency, and presumably lack of TNF-α signaling, resulted in accelerated wound closure. Along these lines, wound repair in mice deficient for the antagonist for IL-1 was delayed reflected by reduced angiogenesis and collagen deposition (Ishida *et al.*, 2006). Influx of neutrophils was increased during repair in IL-1 receptor antagonist-deficient mice, implicating an inhibitory effect of PMNs on wound closure. In contrast, wound repair in IL-6-deficient mice was significantly delayed (Gallucci *et al.*, 2000). In addition to a decreased inflammatory response, granulation tissue formation and reepithelialization was dramatically reduced in these mice. These studies highlight the importance of proinflammatory mediators and leukocytes in the healing response, whereas

Table 2. Genetic mouse models of inflammation and healing

Gened	KO/TG	Inflammatory response			Wound phenotype				Reference
		PMN	MΦ	T cells (CD3+)	Epithelialization	Angiogenesis	Collagen deposition	Wound closure	
TNF-Rp55	KO	↓	↓	n	↑	↑	↑	↑	Mori *et al.*, 2002
IL-1ra	KO	↑	NA	NA	NA	↓	↓	↓	Ishida *et al.*, 2006
IL-6	KO	Inflammation		↓	↓	↓		↓	Gallucci *et al.*, 2000
MCP-1	KO	NA	n	NA	↓	↓	↓	↓	Low *et al.*, 2001
MIP-1α	KO	n	n	n	n	n	n	n	Low *et al.*, 2001
CXCR2	KO	↓	↓	NA	↓	↓	NA	↓	Devalaraja *et al.*, 2000
IP-10	TG	↑	↑	n	↓	↓	NA	↓	Luster *et al.*, 1998
IFN-γ	KO	↓	↓	↓	↑	↑	↑	↑	Ishida *et al.*, 2004
PU.1	KO	↓	↓	NA	n	NA	n*	n	Martin *et al.*, 2003
Smad3	KO	↓	↓	NA	↑	NA	↓	↑	Ashcroft *et al.*, 1999a
SLPI	KO	↑	↑	NA	↓	NA	NA	↓	Ashcroft *et al.*, 2000
MIF	KO	N	n	n	n	n	n	n	Ashcroft *et al.*, 2003
IL-10	KO	N	↑	NA	↑	↑	↑	↑	Eming *et al.* (in press)

KO, knockout; n, normal; NA, not assessed; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PMN, polymorphonuclear leukocyte; SLPI, secretory leukocyte protease inhibitor; TG, transgenic; TNF, tumor necrosis factor.
*Reduced scar.

pointing to the involvement of non-leukocyte cell subsets that can direct the outcome of the repair response.

Beside the ''classical'' mediators of inflammation such as growth factors and proinflammatory cytokines, chemokines are also critically involved (Lusier, 1998). Engelhardt *et al*. (1998) had demonstrated the expression of distinct repertoires of chemokines in normal healing of adult skin wounds, that correlated spatially and temporally with the phase-specific recruitment of leukocyte subsets. In recent years, genetically modified mouse models with altered expression of specific chemokines or their receptors have allowed the elucidation of specific roles in repair, in particular during the inflammatory phase. In MCP-1 knockout animals, which lack one of the major macrophage chemoattractants, the total number of macrophages at the wound site was not altered, although wound reepithelialization, angiogenesis, and collagen synthesis were significantly delayed in these mice (Low *et al*., 2001). Deficiency of another important regulator of macrophage chemotaxis, macrophage inflammatory protein 1α, did not result in any healing defect in mice (Low *et al*., 2001). These data clearly demonstrate the importance of MCP-1α during wound repair and suggests that the role played by MCP-1α in healing wounds consists most likely in influencing the effector phase of macrophages (and possibly of other cells types) rather than simply modulating their number. A recent study indicates a compensatory upregulation of redundant chemokines in the MCP-1α knockout mouse, that might account for the normal macrophage infiltration at the wound site (Ferreira *et al*., 2005). Mice lacking CXCR2, the receptor for the potent neutrophil chemoattractant macrophage inflammatory protein 1α (mouse homolog for growth-related oncogene-α), exhibited defective neutrophil and macrophage recruitment into the wound site and a reduced angiogenic response. These alterations resulted in a significant delay in wound closure (Devalaraja *et al*., 2000). This chemokine-receptor knockout model supports a crucial function for neutrophils and macrophages in wound healing and highlight the role of CXCR2 in leukocyte chemotaxis during repair.

In the late inflammatory phase of wound repair, T lymphocytes appear in the wound bed. The IFN-γ-inducible chemokines inducible protein-10 and monokine induced by IFN-γ are strong chemoattractants for T cells and might regulate their infiltration into the wound site. Constitutive, transgenic expression of inducible protein-10 in keratinocytes caused an intense inflammatory phase, primarily consisting of macrophages and neutrophils. This, a prolonged, disorganized granulation phase resulted in impaired angiogenesis with decreased reepithelialization and ultimately delayed wound closure (Luster *et al*., 1998). Surprisingly, the number of T cells was not significantly different in comparison to wounds in wild-type mice. This data suggests that inducible protein-10 is able to disrupt and impair the normal healing response. Consistent with the concept that an exaggerated inflammatory response might be harmful for repair is the observation in recent wound healing studies in the IFN-γ knockout mouse. This mouse model revealed a decreased inflammatory response at the wound site, however granulation tissue formation, angiogenesis and ultimately wound closure was significantly improved in IFN-γ-deficient mice (Ishida *et al*., 2004). This data intend that IFN-γ and some of its downstream targets are negative regulators of the healing response.

In contrast to the generation of mouse models to identify the role of a single mediator coordinating inflammation during repair, Martin and coworkers posed the more complex question whether the influx of neutrophils and macrophages is essential for cutaneous healing. These studies investigated perinatal repair in the PU.1 null mouse, which lacks cells of the myeloid lineage (macrophages, neutrophils, mast cells, eosinophils) and B cells (McKercher *et al*., 1996; Martin *et al*., 2003). In newborn mutants, little inflammation occurred at the wound site. In contrast to the prevailing dogma, the healing occurred with a similar time course to wild-type siblings, and repair appeared scar free, resembling fetal wound repair. This study demonstrates the crucial impact of the inflammatory response on healing. The authors conclude that inflammation is not an essential prerequisite for efficient tissue repair. However, in these studies one has to consider that besides the reduced inflammatory response, factors intrinsic to the neonatal microenvironment are likely to contribute to the superior healing phenotype. Furthermore, these studies come with the caveat that in the model used, wounds were significantly smaller in size (2 μm) when compared to other murine healing models (4–6 μm) and might not heal by contraction. In line with the notion that inflammation is not essential for healing or even retards the healing process, are recent knockout studies that examined the role of TGF-β1 in repair. TGF-β1 is a pivotal, pluripotent mediator in tissue repair, which contributes to both the influx and activation of inflammatory cells, as well as immune suppressive effects (Ashcroft 1999b). Intracellular signaling of TGF-β1 is mediated primarily by the Smad family of proteins. Skin wound healing in mice deficient in Smad3 was significantly accelerated despite reduced leukocyte infiltration (Ashcroft *et al*., 1999a).

Secretory leukocyte protease inhibitor is a serine proteinase inhibitor predominantly found in the airways but also expressed in skin. Mice genetically deficient for secretory leukocyte protease inhibitor showed delayed cutaneous wound healing. Secretory leukocyte protease inhibitor, which is expressed during cutaneous repair, has an anti-inflammatory activity and also antagonizes the lipopolysaccharide-induced synthesis of proinflammatory mediators by macrophages. The deficiency of secretory leukocyte protease inhibitor in these mice enhanced the inflammatory response and the elastase activity at the wound site, which ultimately led to delayed healing (Ashcroft *et al*., 2000).

Taken together, current transgenic and knockout mouse models generated to address the role of the inflammatory response during repair highlight the view that inflammation is crucial during repair and suggest that the inflammatory response might provide a central target to modulate the outcome of the healing response. The network of

mediators which orchestrates the inflammatory response is complex and likely redundant. Mediators originally thought to be primarily involved in the regulation of leukocyte cell function have been shown to be pluripotent and they target non-leukocyte cell compartments during cutaneous wound healing. Thus, further studies are required to understand in more detail the inflammatory response during repair at its molecular and cellular level.

Excess inflammation associated with impaired wound healing

Wound healing disorders in the clinic present as hypertrophic scars or as non-healing chronic wounds (ulcers), the latter presenting the most prevalent wound healing problems in man. Most chronic wounds are associated with a small number of well defined, clinical entities, in particular venous insufficiency, diabetes mellitus, pressure necrosis, and vasculitis (Singer and Clark, 1999; Eming *et al.*, 2002, 2006; Scharffetter-Kochanek *et al.*, 2003). Despite this heterogeneity most non-healing wounds fail to progress through the normal phases of wound repair, but instead remain in a chronic inflammatory state (Loots *et al.*, 1998). Both underlying systemic defects and local stimuli might contribute to prolong the inflammatory phase of the local healing response, thereby generating a cascade of tissue responses that generate and amplify the hostile microenvironment (Figure 2). One of the most severe systemic defects affecting the individual's inflammatory reaction and the wound healing response is pyoderma gangrenosum. Effective treatment of this disease is only achieved by immune suppression. In addition, factors intrinsic to underlying metabolic disease, such as diabetic hyperglycemia, or increased hydrostatic pressure associated with venous disease, can enhance and perpetuate the inflammatory response. Tissue hypoxia, bacterial components, foreign bodies and fragments of necrotic tissue are powerful local stimuli that are capable sustaining a continued influx of neutrophils and macrophages (Figure 2) (Singer and Clark, 1999; Eming *et al.*, 2002). As recently shown, bacterial components may contribute to impaired repair mechanisms of the host by interference with cell–matrix interactions or attenuating the inflammatory response (Chavakis *et al.*, 2002; Athanasopoulos *et al.*, 2006).

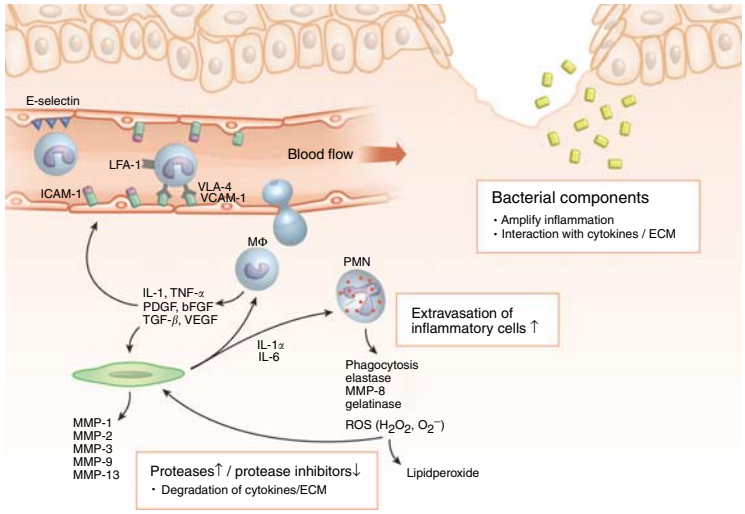

Figure 2. Model of multifactorial molecular and cellular mechanisms deleterious in tissue repair. Chronic wounds fail to progress through the normal pattern of wound repair, but instead remain in a state of chronic inflammation predominantly characterized by abundant PMN and MΦ infiltration. Persisting inflammatory cells play a major role in the generation of proinflammatory cytokines (IL-1, TNF-α, and IL-6) and a protease rich and pro-oxidant hostile microenvironment. Increased proteolytic activity (neutrophil elastase, MMP-8, and gelatinase) leads to degradation of growth factors and structural proteins of the extracellular matrix crucial for repair. Increased ROS (H_2O_2, O_2^-) can lead to direct damage of cells or extracellular matrix molecules, or contribute to increased expression of MMPs (MMP-1, -2, -3, -9, and 13). Bacterial components (extracellular adherence protein (Eap), formyl methionyl peptides, *N*-acetylmuramyl-L-alanyl-D-isoglutamine) may contribute to impaired repair mechanisms of the host by interference with cell–matrix interactions or promoting the inflammatory response.

A cardinal feature of non-healing wounds and a major consequence of the persistent inflammatory response at the wound site is the unbalanced proteolytic activity, which overwhelms local tissue protective mechanisms. Indeed, there is compelling evidence that the unrestrained protease activity is one of the major underlying pathomechanisms of non-healing wounds (Palolathi *et al.*, 1993; Harris *et al.*, 1995; Saarialho-Kere, 1998; Barrick *et al.*, 1999). In addition to cells in the wound site, such as activated keratinocytes at the wound edge, fibroblasts, and endothelial cells, all of which increase their expression of different protease classes, invading neutrophils and macrophages are considered to be the major source of numerous proteases. The expression and the activity of various MMP classes, including collagenases (MMP-1, MMP-8), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), as well as the membrane type MMP (MT1-MMP) have been shown to be highly upregulated in chronic venous stasis ulcers (Saarialho-Kere, 1998; Norgauer *et al.*, 2002). Proinflammatory cytokines are considered as potent inducers of MMP expression in chronic wounds, and they have been shown to down regulate the expression of tissue inhibitor of metalloproteinases, thus creating an environment with a relative excess of MMP activity. In addition, elevated levels of various serine proteinases have been found at the chronic wound site, particularly of neutrophil origin. These include cathepsin G, urokinase-type plasminogen activator, and particularly neutrophil elastase (Grinnell *et al.*, 1992; Grinnell and Zhu, 1996; Barrick *et al.*, 1999). As a consequence of the highly proteolytic microenvironment, mediators crucial for repair become targets of wound proteases. Indeed, the major protease inhibitors α1-proteinase inhibitor and α2-macroglobulin, as well as components of the provisional wound matrix such as fibronectin and vitronectin, have been shown to be degraded and inactivated within the chronic wound

environment (Grinnell *et al.*, 1992; Grinnell and Zhu, 1996). Growth factors pivotal for repair such as platelet-derived growth factor or vascular endothelial growth factor are targets of wound proteases, and they are inactivated by proteolytic cleavage (Wlaschek *et al.*, 1997; Lauer *et al.*, 2000, 2002; Roth *et al.*, 2006).

The chronic wound is a highly prooxidant microenvironment (Mendez *et al.*, 1998; Wenk *et al.*, 2001; James *et al.*, 2003; Wlaschek and Scharffetter-Kochanek, 2005). Leukocytes, especially neutrophils are a rich source of various ROS (superoxide anion, hydroxyl radicals, singlet oxygen, hydrogen peroxide), which are released into the wound environment (Weiss, 1989). Endothelial cells and fibroblasts, in particular senescent fibroblasts, which are prominent in chronic wounds, are also potential sources for ROS (Campisi, 1996; Mendez *et al.*, 1998). Evidence for a prominent role of ROS in multiple steps of the pathogenesis of non-healing wounds comes from several *in vitro* and *in vivo* studies (Wlaschek and Scharffetter-Kochanek, 2005). In addition to direct damage of cell membranes and structural proteins of the extracelluar matrix, ROS can selectively affect signaling pathways leading to the activation of transcription factors that control the expression of proinflammatory cytokines (IL-1, -6, and TNF-α), chemokines and proteolytic enzymes including MMPs and serine proteinases (Wenk *et al.*, 2001). Thus, the disturbed oxidant/antioxidant balance within the chronic wound microenvironment is considered a major factor, which amplifies the unrestrained and persistent inflammatory state of non-healing wounds.

Based on these observations it is a well-accepted concept that effective therapy toward healing of the chronic wound must disrupt this proinflammatory cycle. Unraveling pro- and anti-inflammatory pathways in tissue repair might be an important avenue to develop protective strategies, which shield the regenerative tissue from damage caused by the chronically inflamed microenvironment of the non-healing wound. The excessive and unbalanced inflammation characterizing the chronic wound suggests a promising target for future therapeutic interventions.

Chronic inflammation associated with malignant progression in chronic wounds

It has long been known that chronic wounds are at risk for neoplastic progression (Baldursson *et al.*, 1993, 1995, 1999). The risk of squamous cell carcinoma (SCC) is markedly increased, suggesting that keratinocytes are especially vulnerable to malignant transformation. Very little is known about the molecular inducers of tumorgenesis in chronic wounds. The main cause of cutaneous SCC, UV irradiation, is an unlikely explanation. A recent study investigating cell cycle regulators in SCCs associated with venous leg ulcers suggests differing mechanisms of carcinogenesis in venous leg ulcers and UV-induced SCC (Baldursson *et al.*, 2000a, 2000b). Furthermore, SCC of the skin has also been associated with human papillomavirus infection. Interestingly, human papillomavirus has been detected in verrucous carcinomas of the leg and foot (Garven *et al.*, 1991; Noel *et al.*, 1993) and human papillomavirus infection could play a role in SCC arising from chronic leg ulcers. So far this topic has been poorly investigated.

The tumor cell microenvironment, in particular the tumor stroma, appears to play an important role in the control of neoplastic progression through dynamic reciprocity between tumor cells and their surrounding tissue (Bissell and Radisky, 2001; Mueller and Fusenig, 2004). The inflammatory response within the tumor stroma, which is closely linked to fibroplasia and angiogenesis, has been considered to play a critical role in carcinogenesis (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; de Visser and Coussens, 2005; Impola *et al.*, 2005). Inflammation, fibroplasia, and angiogenesis are cardinal events that are intimately linked to wound repair. Thus the chronic inflammatory microenvironment of the non-healing wound could be a risk factor for malignant transformation. Two proposed mechanisms relate the increased inflammatory response within the stroma to cancerogenesis: first, the highly vascularized and growth factor rich tumor stroma provides an environment rich in nutrients that supports tumor growth; second, the tumor stroma might directly stimulate malignant transformation. For example, so-called carcinoma-associated fibroblasts mediate oncogenic signals (directly or indirectly) that stimulate the progression of a non-tumorigenic cell population to a tumorigenic one (Phillips *et al.*, 2001; Tlsty, 2001). Subcutaneous injection of benign tumorigenic SCC cells demonstrated an enhanced malignant phenotype upon re-cultivation, that was associated with the altered expression of various growth factors, such as G-CSF and GM-CSF (Mueller *et al.*, 2001). As a related example, treatment of cleared mammary fad pads with the carcinogen *N*-nitrosomethyl urea, generated a stromal environment that was characterized by the acquisition of genetic mutations and allowed the development of epithelial tumors from transplanted normal mammary epithelial cells (Maffini *et al.*, 2004). Thus the tumor stroma should be considered as a therapeutic target in malignant transformation and tumor growth, and by analogy that ''the stroma of the chronic wound'' might provide a microenvironment that sets wound keratinocytes at risk for malignant transformation. Experimental proof for this concept will be challenging, because until today animal models for chronic wound repair are lacking. Nevertheless, the identification of inflammatory mediators responsible for malignant transformation will be important in the management of non-healing wounds, in particular to predict the development of SCC from pseudoepitheliomatous hyperplasia of chronic wounds and potentially in other chronic inflammatory states associated with malignant transformation.

Recently, Ortiz-Urda *et al.* (2005) provided molecular evidence for the development of SCC only in a certain portion of patients suffering from recessive dystrophic epidermolysis bullosa. This group showed in an elegant analysis of recessive dystrophic epidermolysis bullosa keratinocytes that only those keratinocytes that retained a specific collagen VII fragment (FNC1 sequence

within the NC1 domain) were tumorigenic (Ortiz-Urda *et al.*, 2005). The invasiveness of epithelial cells required interaction of the FNC1 domain with laminin 5. Thus, these studies suggest that an intact adhesion complex between the FNC1 domain of collagen VII and laminin 5 is required for epidermal cells to receive the stromal signals that they need to migrate to and invade the dermal layer. Expression of structural molecules, generating the adhesion complex are regulated by dermal mediators and it is possible that the tumor–stroma interactions mediated by collagen VII might be relevant for tumor development in chronic wounds.

Concluding remarks

In summary, numerous clinical and experimental studies confirm the pivotal role of inflammation during repair. To achieve proper tissue homeostasis during healing, the fine tuned balance between a complex network of various leukocyte cell subsets and numerous pro- and anti-inflammatory mediators is crucial. Dysregulation of critical parameters of these interactions results in pathologic and chronic inflammatory disease states that impair the quality of healing. Further information is required on molecular pathways that disrupt the physiological inflammatory phase during repair, including underlying systemic diseases (diabetes mellitus, venous insufficiency) and/or microbial components, which contribute to the pathology of wound healing. Experimental mouse models will be particular useful to answer these open questions, because of the ability to manipulate the genetic, systemic, and wound environment. Although genetic eradication of specific mediators and leukocyte lineages may partially be compensated by redundant mechanisms, the chronic wound, through proteolysis, oxidative stress, and accumulation of toxic substances, may lead to depletion of critical resources.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SAE and TK, SFB 589), the European Community (SAE, LSHB-CT-2005-512102), and NIH and the Department of Veterans Affairs (Grants AG-06528 and AR-44104 to JMD).

REFERENCES

Adolph VR, DiSanto SK, Bleacher JC, Dillon PW, Krummel TM (1993) The potential role of the lymphocyte in fetal wound healing. *J Pediatr Surg* 28:1316–20

Ashcroft GS, Dodsworth J, Boxtel E, Horan M, Ferguson MWJ (1997) Estrogen accelerates cutaneous wound healing associated with an increase in TGF-b1 levels. *Nat Med* 3:1209–15

Ashcroft GS, Horan MA, Ferguson MWJ (1998) Ageing alters the inflammatory and endothelial adhesion molecule profiles during human cutaneous wound healing. *Lab Invest* 78:47–58

Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JJ, Mizel DE *et al.* (1999a) Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol* 1:260–6

Ashcroft GS (1999b) Bidirectional regulation of macrophage function by TGF-b. *Microbes and Infection* 1:1275–82

Ashcroft GS, Greenwell-Wild T, Horan M, Wahl SM, Ferguson MWJ (1999c) Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. *Am J Pathol* 155:1137–46

Ashcroft GS, Lei K, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze *et al.* (2000) SLPI mediates non-redundnat functions necessary for neonatal wound healing. *Nat Med* 6:1147–53

Ashcroft GS, Mills SJ, Ashworth JJ (2002) Ageing and wound healing. *Biogerontology* 3:337–45

Ashcroft GS, Mills SJ, Lei K, Gibbons L, Jeong MJ, Taniguchi M *et al.* (2003) Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. *J Clin Invest* 111:1309–18

Athanasopoulos A, Economopoulou M, Orlova V, Song K, Sobke A, Schneider D *et al.* (2006) The extracellular adherence protein (Eap) of Staphylococcus aureus inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* 107:2720–7

Azouz A, Razzaque MS, El-Hallak M, Taguchi T (2004) Immunoinflammatory responses and fibrogenesis. *Med Electron Microsc* 37:141–8

Baggiolini M (1998) Chemokines and leukocyte traffic. *Nature* 392:565–8

Baldursson B, Sigurgeirsson B, Lindelof B (1993) Leg ulcers and squamous cell carcinoma. An epidemiological study and a review of the literature. *Acta Derm Venerol* 73:171–4

Baldursson B, Sigurgeirsson B, Lindelof B (1995) Venous leg ulcers and squamous cell carcinoma: a large-scale epidemiological study. *Br J Dermatol* 133:571–4

Baldursson B, Hedblad MA, Beitner H, Lindelof B (1999) Squamous cell carcinoma complicating chronic venous leg ulceration: a study of the histopathology, course and survival in 25 patients. *Br J Dermatol* 140:1148–52

Baldursson B, Beitner H, Syrjänen S (2000a) Human papillomaviros in venous ulcers with and without squamous cell carcinoma. *Arch Dermatol Res* 292:275–8

Baldursson B, Syrjänen S, Beitner H (2000b) Expression of $p21^{WAF1/CIP1}$, p53, bcl-2 and Ki-67 in venous leg ulcers with and without squamous cell carcinoma. *Acta Derm Venerol* 80:251–5

Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? *Lancet* 357:539–45

Barrick B, Campbell EJ, Owen CA (1999) Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes. *Wound Rep Reg* 7:410–22

Bayat A, McGrouther DA, Ferguson MWJ (2003) Skin scarring. *BMJ* 326:88–92

Bissell MJ, Radisky D (2001) Putting tumours in context. *Nat Rev Cancer* 1:46–54

Braun S, Hanselmann C, Gassmann MG, Auf dem Keller U, Born-Berclaz C, Chan K *et al.* (2002) Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. *Mol Cell Biol* 22:5492–505

Bullard KM, Longaker MT, Lorenz HP (2003) Fetal wound healing. *World J Surg* 27:54–61

Campisi J (1996) Replicative senescence : an old lives tale? *Cell* 84:497–500

Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, Flock J *et al.* (2002) Staphyloccocus aureus extrcellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat Med* 8:687–93

Coussens LM, Werb Z (2002) Inflammation and acncer. *Nature* 420:860–7

Cotran RS, Mayadas-Norton T (1998) Endothelial adhesion molecules in health and disease. *Pathol Biol* 46:164–70

Daley JM, Reichner JS, Mahoney EJ, Manfield L, Henry WL, Mastrofrancesco B *et al.* (2005) Modulation of macrophage phenotype by soluble products released form neutrophils. *J Immunol* 174:2265–72

Devalaraja RM, Nanney LB, Qian Q, Du J, Yu Y, Devalaraja MN *et al.* (2000) Delayed wound healing in CXCR2 knockout mice. *J Invest Dermatol* 115:234–44

de Visser KE, Coussens LM (2005) The interplay between innate and adaptive immunity regulates cancer development. *Cancer Immunol Immunother* 54:1143–52

Dillon PW, Keefer K, Blackburn JH, Houghton PE, Krummel TM (1994) The extracellular matrix of the fetal wound: hyaluronic acid controls lymphocyte adhesion. *J Surg Res* 57:170–3

DiPietro LA, Polverini PJ (1993) Role of the macrophage in the positive and negative regulation of wound neovascularisation. *Am J Pathol* 143:678–84

DiPietro LA, Burdick M, Low Q, Kunkel SL, Strieter RM (1998) MIP-1a as critical macrophage chemoattractant in murine wound repair. *J Clin Invest* 101:1693–8

Dovi JV, He K, DiPletro LA (2003) Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol* 73:448–55

Egozi EI, Ferreira AM, Burns AL, Gamelli RL, DiPietro LA (2003) Mast cells modulate the inflammatory but not the proliferative response in healing wounds. *Wound Rep Reg* 11:46–54

Eming SA, Smola H, Krieg T (2002) The treatment of chronic wounds: current concepts and future aspects. *Cells Tissues Organ* 172:105–17

Eming SA, Smola-Hess S, Kurschat P, Hirche D, Krieg T, Smola HA (2006) novel property of Povidon-iodine: inhibition of excessive protease levels in chronic non-healing wounds. *J Invest Dermatol* 126:2731–3

Eming SA, Werner S, Bugnon P, Wickenhauser C, Siewe L, Utermöhlen O *et al.* (2007) Accelerated wound closure in mice deficient for interleukin-10. *Am J Pathol* 170: 188–202

Engelhardt E, Toksoy A, Goebeler M, Debus S, Bröcker E, Gillitzer R (1998) Chemokines IL-8, GROa, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am J Pathol* 153:1849–60

Esche C, Stellato C, Beck LA (2005) Chemokines: key players in innate and adaptive immunity. *J Invest Dermatol* 125:615–28

Ferreira AM, Rollins BJ, Faunce DE, Burns AL, Zhu X, DiPietro LA (2005) The effect of MCP-1 depletion on chemokine and chemokine-related gene expression: evidence for a complex network in acute inflammation. *Cytokine* 30:64–71

Fishel RS, Barbul A, Beschorner WE, Wasserkrug HL, Efron G (1987) Lymphocyte participation in wound healing: morphologic assessment using monoclonal antibodies. *Ann Surg* 206:25–9

Frank S, Kampfer H, Wetzler C, Stallmeyer B, Pfeilschifter J (2000) Large induction of the chemotactic cytokine RANTES during cutaneous wound repair: a regulatory role for nitric oxide in keratinocyte-derived RANTES expression. *Biochem J* 1:265–73

Gallucci RM, Someonova PP, Matheson JM, Kommineni C, Guriel JL, Sugawara T *et al.* (2000) Impaired cutaneous wound healing in IL-6-deficient and immunosuppressed mice. *FASEB J* 14:2525–31

Garven TC, Thelmo WL, Victor J, Pertschuk L (1991) Verrucous carcinoma of the leg positive for human papillomavirus DNA 11 and 18: a case report. *Hum Pathol* 22:1170–3

Gillitzer R, Goebeler M (2001) Chemokines in cutaneous wound healing. *J Leukoc Biol* 69:513–21

Gordon S (2003) Alternative activation of macrophages. *Nature* 3:23–35

Greenhalgh DG (1998) The role of apoptosis in wound healing. *Int J Biochem Cell Biol* 30:1019–30

Grinnell F, Ho C, Wysocki A (1992) Degradation of fibronectin and vitronectin in chronic wound fluid: analysis by cell blotting, immunoblotting and, cell adhesion assays. *J Invest Dermatol* 98:410–6

Grinnell F, Zhu M (1996) Fibronectin degradation in chronic wounds depends on the relative levels of elastase, a1—proteinase inhibitor, and a2-macroglobulin. *J Invest Dermatol* 106:335–41

Hardman MJ, Waite A, Zeef L, Burow M, Nakayama T, Ashcroft GS (2005) Macrophage migration inhibitory factor: a central regulator of wound healing. *Am J Pathol* 167:1561–74

Harris IR, Yee KC, Walters CE, Cunliffe WJ, Kearney JN, Wood EJ *et al.* (1995) Cytokine and protease levels in healing and non-healing chronic venous ulcers. *Exp Dermatol* 4:342–9

Harty M, Neff AW, King MW, Mescher AL (2003) Regeneration or scarring: an immunologic perspective. *Dev Dyn* 226:268–79

Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G (1998) CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 391:591–4

Hübner G, Brauchle M, Smola H, Madlener M, Fässler R, Werner S (1996) Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine* 8:548–56

Iba Y, Shibata A, Kato M, Masukawa T (2004) Possible involvement of mast cells in collagen remodelling in the late phase of cutaneous wound healing in mice. *Int Immunopharmacol* 4:1873–80

Impola U, Jeskanen L, Ravanti L, Syrjänen S, Baldusson B, Kähäri V *et al.* (2005) Expression of MMP-7 and MMP-13 and loss of MMP-19 and p16 are associated with malignant progression in chronic wounds. *Br J Dermatol* 152:720–6

Ishida Y, Kondo T, Takayasu T, Iwakura Y, Mukaida N (2004) The essential involvement of cross-talk between IFN-g and TGF-b in the skin wound healing process. *J Immunol* 172:1848–55

Ishida Y, Kondo T, Kimura A, Matsushima K, Mukaida N (2006) Absence of IL-1 receptor antagonist impaired wound healing along with aberrant NF-kappaB activation and a reciprocal suppression of TGF-beta signal pathway. *J Immunol* 176:5598–606

Issekutz AC, Chuluyan HE, Lopes N (1995) CD11/CD18-independent transendothelial migration of human polymorphonuclear leukocytes and monocytes: involvement of distinct and unique mechanisms. *J Leukoc Biol* 57:553–61

James TJ, Hughes MA, Cherry GW, Taylor RP (2003) Evidence of oxidative stress in chronic venous ulcers. *Wound Rep Reg* 11:172–6

Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R *et al.* (2002) A role for skin $\gamma\delta$T cells in wound repair. *Science* 296: 747–9

Jameson J, Cauvi G, Sharp LL, Witherdden DA, Havran WL (2005) Gammadelta T cell-induced hyaluronan production by epithelial cells regulates inflammation. *J Exp Med* 201:1269–79

Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y *et al.* (2005) Regulation of lunh injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 11:1173–9

Karin M, Lawrence T, Nizet V (2006) Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* 124:823–35

Kaufman J, Graf BA, Leung EC, Pollock SJ, Koumas L, Reddy SY *et al.* (2001) Fibroblasts as sentinel cells: role of the CD40-CD40 ligand system in fibroblast activation and lung inflammation and fibrosis. *Chest* 120:S53–5

Kuijpers TW, Van Lier RA, Hamann D, De Boer M, Thung LY, Weening RS *et al.* (1997) LAD1/variant. A novel immunodefiency syndrome characterized by dysfunctional beta2 integrins. *J Clin Invest* 100:1725–33

Kulidjian AA, Inman R, Issekutz TB (1999) Rodent models of lymphocyte migration. *Semin Immunol* 11:85–93

Lauer G, Sollberg S, Cole M, Flamme I, Mann K, Krieg T *et al.* (2000) Expression and proteolysis of VEGF is increased in chronic wounds. *J Invest Dermatol* 115:12–8

Lauer G, Sollberg S, Cole M, Krieg T, Eming SA (2002) Generation of a novel proteolytic resistant VEGF165 variant by a site directed mutation at the plasmin sensitive cleavage site. *FEBS Lett* 531:309–13

Leibovich SJ, Ross R (1975) The role of the macrophage in wound repair. *Am J Pathol* 78:71–100

Liechty KW, Kim HB, Adzick NS, Crombleholme TM (2000) Fetal wound repair results in scar formation in IL-10-deficient mice in a syngeneic murine model of scarless fetal wound repair. *J Pediatric Surg* 35:866–73

Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E (1998) Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. *J Invest Deramtol* 111:850–7

Low QEH, Drugea IA, Duffner LA, Quinn DG, Cook DN, Rollins BJ *et al.* (2001) Wound healing in MIP-1a (−/−) and MCP-1 (−/−) mice. *Am J Pathol* 159:457–63

Lusier AD (1998) Chemokines-chemotactic cytokines that mediate inflammation. *N Engl J Med* 338:436–45

Luster AD, Cardiff RD, MacLean JA, Crowe K, Granstein RD (1998) Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. *Proc Ass Am Phys* 110:183–96

Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C (2004) The stroma as a crucial target in rat mammary gland carcinogenesis. *J Cell Sci* 117:1495–502

Margolis DJ, Knauss J, Bilker W (2002) Hormone replacement therapy and prevention of pressure ulcers and venous leg ulcers. *Lancet* 23:675–7

Martin P (1997) Wound healing – aiming for perfect skin regeneration. *Science* 276:75–81

Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R *et al.* (2003) Wound healing in the PU.1

null mouse – tissue repair is not dependent on inflammatory cells. *Curr Biol* 13:1122–8

Martin P, Leibovich SJ (2005) Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol* 15:599–607

McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Feeney AJ *et al.* (1996) Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J* 15:5647–58

McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM (2000) Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* 289:1202–6

McQuibban GA, Gong JH, Wong JP, Wallace JL, Clark-Lewis I, Overall CM (2002) Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties *in vivo*. *Blood* 100:1160–7

Mendez MV, Stanley A, Parker HY, Shon K, Phillips T, Menzian JO (1998) Fibroblasts cultured from venous ulcers display cellular characteristics of senescence. *J Vasc Surg* 28:876–83

Mori R, Kondo T, Nishie T, Ohshima T, Asano M (2004) Impairment of skin wound healing in b-1,4-galactosyltransferase-deficient mice with reduced leukocyte recruitment. *Am J Pathol* 164:1303–14

Mori R, Kondo T, Ohshima T, Ishida Y, Mukaida N (2002) Accelerated wound healing in TNF receptor p55-deficient mice with reduced leukocyte infiltration. *FASEB J* 16:963–74

Mueller MM, Peter W, Mappes M, Huelsen A, Steinbauer H, Boukamp P *et al.* (2001) Tumor progression of skin carcinoma cells *in vivo* promoted by clonal selection, mutagenesis, and autocrine growth regulation by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. *Am J Pathol* 159:1567–79

Mueller MM, Fusenig NE (2004) Friends or foes-Bipolar effects of the tumor stroma in cancer. *Nat Rev* 4:839–49

Nagaoka T, Kaburagi Y, Hamaguchi Y, Hasegawa M, Takehara K, Steeber DA *et al.* (2000) Delayed wound healing in the absence of intercellular adhesion molecule-1 or L-selectin expression. *Am J Pathol* 157:237–47

Noel JC, Peny MO, Detremmerie O, Verhest A, Heenen M, Thiry L *et al.* (1993) Demonstration of human papillomavirus type 2 in a verrucous carcinoma of the foot. *Dermatology* 187:58–61

Norgauer J, Hildenbrand T, Idzko M, Panther E, Bandemir E, Hartmann M *et al.* (2002) Elevated expression of extracellular matrix metalloproteinase inducer (CD147) and membrane-type matrix metalloproteinases in venous leg ulcers. *Br J Dermatol* 147:1180–6

Ortiz-Urda S, Garcia J, Green CL, Chen L, Lin Q, Veitch DP *et al.* (2005) Type VII collagen is required for ras-driven human epidermal tumorgenesis. *Science* 307:1773–6

Palolathi M, Lauharanta J, Stephens RW, Kuusela P, Vaheri A (1993) Proteolytic activity in leg ulcer exudates. *Exp Dermatol* 2:29–37

Park JE, Barbul A (2004) Understanding of the role of immune regulation in wound healing. *Am J Surg* 187(Suppl):11S–6S

Peters T, Sindrilaru A, Hinz B, Hinrichs R, Menke A, Al-azzeh E *et al.* (2005) Wound healing defect of CD18−/− mice due to a decrease in TGF-b1 and myofibroblast differentiation. *EMBO J* 24:3400–10

Phillips JL, Hayward SW, Wang Y, Vasselli J, Pavlovich C, Padilla-Nash H *et al.* (2001) The consequences of chromosomal aneuploidy on gene expression profiles in a cell line model for prostate carcinogenesis. *Cancer Res* 61:8143–9

Pinhal-Enfield G, Ramanathan M, Hasko G, Vogel S, Salzman A, Boons G *et al.* (2003) An angiogenid switch in macrophages involving synergy between TLR 2, 4, 7, and 9 and adenosine A_{2A} receptors. *Am J Pathol* 163:711–21

Ramanathan M, Giladi A, Leibovich SJ (2003) Regulation of VEGF gene expression in murine macrophages vy nitric oxide and hypoxia. *Exp Biol Med* 228:697–705

Reed MJ, Cooper L, Wood W, Stramer B, Martin P (2004) Wound healing and inflammation: embryos reveal the way to perfect repair. *Phil Trans R Soc Lond* 359:777–84

Roos D, Kuijpers TW, Mascart-Lemone F, Koenderman L, De Boer M, Zwieten R *et al.* (1993) A novel Syndrome of severe neutrophil dysfunction: unresponsiveness confined to chemotaxis-induced functions. *Blood* 81:2735–43

Rossi D, Zlotnik A (2000) The biology of chemokines and their receptors. *Annu Rev Immunol* 18:217–42

Roth D, Piekarek M, Christ H, Paulsson M, Krieg T, Davidson J *et al.* (2006) Plasmin modulates VEGF-A mediated angiogenesis during wound repair. *Am J Pathol* 168:670–84

Saarialho-Kere UK (1998) Patterns of MMPs and TIMP expression in chronic wounds. *Arch Dermatol Res* 290(Suppl):S47–54

Sato Y, Ohshima T, Kondo T (1999) Regulatory role of endogenous IL-10 in cutaneous inflammatory response of murine wound healing. *Biochem Biophys Res Comm* 265:194–9

Scharffetter-Kochanek K, Schüller J, Meewes C, Hinrichs R, Eich D, Eming SA *et al.* (2003) Das chronisch venöse Ulcus cruris – Pathogenese und Bedeutung des ''aggresiven Mikromilieus''. *J Dtsch Dermatol Ges* 1:58–67

Sharp LL, Jameson JM, Cauvi G, Havran WL (2005) Dendritic epidermal T cells regulate skin homeostasis through local production of IGF1. *Nat Immunol* 6:73–9

Simpson DM, Ross R (1972) The neutrophilic leukocyte in wound repair. A study with antineutrophil serum. *J Clin Invest* 51: 2009–23

Singer AJ, Clark RAF (1999) Cutaneous wound healing. *N Engl J Med* 341:738–46

Subramaniam M, Saffaripour S, Van De Water L, Frenette PS, Mayades TN, Hynes RO *et al.* (1997) Role of endothelial selectins in wound repair. *Am J Pathol* 150:1701–9

Swift ME, Burns AL, Gray KL, DiPietro LA (2001) Age-related alterations in the inflammatory response to dermal injury. *J Invest Dermatol* 117:1027–35

Szpaderska A, Egozi EI, Gamelli RL, DiPietro LA (2003) The effect of thrombocytopenia on dermal wound healing. *J Invest Dermatol* 120:1130–7

Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Pure E *et al.* (2002) Resolution of lung inflammation by CD44. *Science* 296:155–8

Theilgaard-Mönch K, Knudsen S, Follin P, Borregaard N (2004) The transcriptional activation of program of human neutrophils in skin lesions supports their important role in wound healing. *J Immunol* 172:7684–93

Tlsty TD (2001) Stromal cells can contribute oncogenic signals. *Semin Cancer Biol* 11:97–104

Trautmann A, Toksoy A, Engelhardt E, Brocker EB, Gillitzer R (2000) Mast cell involvement in normal human skin wound healing: expression of MCP-1 is correlated with recruitment of mast cells which synthesize IL-4 *in vivo*. *J Pathol* 190:100–6

Weller K, Foitzik K, Paus R, Syska W, Maurer M (2006) Mast cells are required for normal wound healing of skin wound in mice. *FASEB J* 20:2366–8

Wenk J, Foitzik A, Achterberg V, Sabiwalsky A, Dissemond J, Meewes C *et al.* (2001) Selective pick-up of increased iron by deferoxamine-coupled cellulose abrogates the iron-derived induction of matrix-degrading MMP1 and lipid peroxidation in human dermal fibroblasts *in vitro*: a new dressing concept. *J Invest Dermatol* 116:833–9

Werner F, Jain MK, Feinberg MW, Sibinga NES, Pellacani A, Wiesel P *et al.* (2000) TGF-b1 inhibition of macrophage activation is mediated via Smad3. *J Biol Chem* 275:36653–8

Werner S., Grose R. (2003) Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 83:835–70

Wetzler C, Kämpfer H, Stallmeyer B, Pfeilschifter J, Frank S (2000) Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. *J Invest Dermatol* 115:245–53

Weiss SJ (1989) Tissue destruction by neutrophils. *N Engl J Med* 320:365–76

Whitby DJ, Ferguson MW (1991) Immunohistochemical localization of growth factors in fetal wound healing. *Dev Biol* 147:207–15

Wlaschek M, Peus D, Achterberg V, Meyer-Ingold W, Scharffetter-Kochanek (1997) Protease inhibitors protect growth factor activity in chronic wounds. *Br J Dermatol* 9:42

Wlaschek M, Scharffetter-Kochanek K (2005) Oxidative stress in chronic venous leg ulcers. *Wound Rep Reg* 13:452–61

Yellin MJ, Winikoff S, Fortune SM, Baum D, Crow MK, Lederman S. (1995) Ligation of CD40 on fibroblasts induces CD54 (ICAM-1) abd CD106 (VCAM-1) up-regulation and IL-6 production and proliferation. *J Leukoc Biol* 58:209–16